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Systematics of Juncaceae

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2008

Submitted as partial fulfilment of the requirements of the degree of Doctor of Philosophy

Declaration

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Summary

The systematics of Juncaceae is investigated using morphological and molecular data. A study of the anatomy of Juncaceae is presented as anatomical descriptions of the leaves, including observations of previously undescribed taxonomically informative characters, particularly in the leaf sheaths.

The DNA regions ITS, *rps*16, *trn*L intron and *trn*L-F intergenic spacer are sequenced. Molecular, morphological and combined cladistic analyses are carried out, resulting in a phylogenetic hypothesis of Juncaceae. Juncaceae is shown to be a sister group to Cyperaceae. Within Juncaceae, *Luzula* is shown to be monophyletic, but *Juncus* is paraphyletic with respect to *Distichia*, *Marsippospermum*, *Oxychloe*, *Patosia* and *Rostkovia*. Within *Juncus*, however, two well-supported clades are identified (Clades A and B), and a third clade (C) contains the remaining species of *Juncus* and all other genera except for *Luzula*.

The distribution of the morphological characters is investigated in relation to the phylogeny, and characters are identified which are unique to the three major clades of *Juncus* identified in the phylogeny.

The classification of *Juncus* is revised in the light of these results. *Juncus* is divided into three genera. *Juncus* L. consists of the Section *Juncus* (10 spp.) and some members of Section Graminifolii, but the placement of other members of Section Graminifolii is unknown. *Stygiopsis* (Dumort) E.Jones consists of the Sections *Iridifolii*, *Ozophyllum* and *Stygiopsis*, and it is defined by the presence of leaves with uni- or pluri-tubulose leaf blades, which contain transverse septae. *Tenageia* (Dumort.) Fourr. consists of the Sections *Juncotypus*, *Steirochloa* and *Tenageia*, and is defined by the presence of cymose inflorescences, the presence of floral bracteoles, and leaf blades which are more-or-less flat or canaliculated, or reduced to rudimentary blades on a well-developed sheath.

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Chapter 1 Introduction

1.1 Introduction to Juncaceae

The family Juncaceae is relatively small, containing seven genera and approximately 440 species (Kirschner 2002a), but it is very widespread; representatives of the family are to be found on all continents but Antarctica (Balslev 1996). Juncaceae are herbaceous plants, ranging from a few centimetres to about two metres in height. The leaves are variable in form: often, as in *Luzula* DC., they are flat and bifacial, but in other genera such as *Juncus* L., they may be cylindrical, sometimes filled with an aerenchymatous pith, sometimes hollow. They may also be flattened into an ensiform leaf similar to that seen in *Iris* L..

Juncaceae commonly possess a rhizome from which the short-lived aerial stems (culms) arise at intervals, each culm terminating in an inflorescence. In some species the rhizome is reduced or lost, so that the growth habit may be spreading, caespitose or solitary (Fig. 1.1A–B). The leaves may be clustered around the base of each culm or along the culm, and sometimes they are reduced to scale-like structures present only on the rhizome.

Distichia Nees & Meyen, *Oxychloe* Phil. and *Patosia* Buchenau are the most notable exceptions to this growth form, as they are cushion plants adapted to growth at high altitudes in the Andes. They possess only aerial stems, along which the leaves are arranged at regular intervals, the roots arising along the stems from the internodes. The oldest parts of the stems die back as the youngest parts continue growing and branching dichotomously. This results in the formation of dense mounds, which can be several metres in diameter. The surface of the mounds is formed by the tips of the short, stiff leaves, so that they are almost moss-like in appearance (Fig. 1.1C).

Juncus and *Luzula* bear cymose inflorescences, each with numerous (rarely solitary), small flowers, each usually less than one centimetre in diameter. The perianth is brown or green in colour, rarely white (e.g. *Luzula nivea* DC.). The remaining genera, *Marsippospermum* Desv., *Rostkovia* Desv., *Oxychloe*, *Patosia* and *Distichia*, bear a single flower per inflorescence. The flowers of Juncaceae are usually anemophilous, but a few species such as *Luzula nivea* DC. with bright white flowers may be entomophilous (Buchenau 1906).



Figure 1.1 Habit. A Juncus bufonius caespitose habit. B Juncus arcticus rhizomatous habit. C Distichia muscoides cushion-forming habit. A, B taken from Balslev (1996); originally published in Harling, G. & Sparre, B. (eds.) (1979), Flora of Ecuador 11: 1–45.

The flowers bear two whorls of three perianth segments, opposite which are two whorls of three stamens. The gynoecium consists of three fused carpels, each with one or more ovules, and the single style is divided part way along its length into three stylodial branches.

The two largest genera, *Juncus* (315 species) and *Luzula* (115 species) (Kirschner 2002a, b) have a cosmopolitan distribution. The other five genera are small, with between one and five species per genus, and most have very restricted geographic distributions. These genera occur only in the southern hemisphere, with the majority to be found in South America, in temperate regions or at high altitudes in the Andes; only *Distichia muscoides* Nees & Meyen extends into the northern hemisphere, with a distribution from Colombia to Argentina. *Marsippospermum* and *Rostkovia* are the most wide-spread of these genera, occurring in Patagonia, the Falkland Islands and New Zealand, and also on Tristan de Cunha, Gough Island, South Georgia and Campbell Island. *Rostkovia magellanica* Hook.f. is also present disjunctly in Ecuador. The cushion-forming genera, *Distichia, Patosia* and *Oxychloe*, occur at high altitudes (generally between about 3000 to 5000 m) in the Andes (Balslev 1996). In damp areas they may be the dominant part of the vegetation.

Although cosmopolitan in distribution, Juncaceae are mainly to be found in temperate regions, preferring a cool climate and often growing in open, damp habitats, although the genus *Luzula* is often found in drier woodland. Where they occur in tropical latitudes they remain in the highlands, rarely occurring at the warmer, lower altitudes; for example in Ecuador they do not occur below an altitude of about 1000 m (Balslev 1996).

Juncaceae are not of great economic value. In horticulture few species are commonly grown. *Juncus effusus* L. 'Spiralis', an ornamental form with twisted leaves, is sometimes grown, and some variegated or unusually coloured forms of *Luzula*, such as *Luzula sylvatica* (Huds.) Gaudin 'Aurea', are used as ground cover in moist shady areas.

The tough, sclerenchymatous leaves of some *Juncus* species have been of some use in areas where the larger species are common. *Juncus effusus* is sometimes used for weaving mats, for example in Costa Rica and Guatemala (Standley 1937; Standley & Steyermark 1952), and *Juncus balticus* Willd. ssp. *andicola* (Hook.) Snogerup for weaving baskets in Ecuador (Balslev 1996). The cushion plant *Distichia muscoides* has been used as a fuel by

highland peoples in Peru. Blocks are dug out, dried and then burnt (Ferreya 1979). Historically, species of *Juncus* with tall stems containing pith were used in Europe to make rush lamps, as a cheap alternative to candles. *Juncus conglomeratus* L. was probably the most common species used (White 1789).

All modern uses of Juncaceae remain localised to relatively small areas, and there is little widely recognised value to these plants. Juncaceae are, perhaps, best known in temperate countries as a familiar but inedible constituent of wet pasture-land.

1.2 A history of the systematics of Juncaceae

Among the wind-pollinated monocotyledons, it is not unusual to find considerable debate on the phylogenetic position of a plant group, and Juncaceae have been no exception. The floral parts of such plants are generally extremely reduced and the inflorescences may be highly condensed, leading to many different interpretations of the origins of the floral structures. This is further confused by convergent evolution, which seems to occur rather frequently between the families, so that the distribution of some characteristics appear to conflict with the distribution of others when they are used to determine relationships, and it can be difficult to decide which are homologies and which are parallelisms.

Such debates have affected the classification of Juncaceae at many taxonomic levels, from the relationships between the genera, to the phylogenetic position of Juncaceae among the monocotyledons. In particular, the determination of the exact relationship of Juncaceae with the other monocotyledonous families has been confused, with almost as many different classifications as there were authors.

In the past, the floral morphology of Juncaceae was most commonly, but inaccurately, interpreted as that of a much reduced Liliales flower. The flowers consist of two whorls of three, more-or-less equal, perianth segments; two whorls (rarely one) of three stamens; and three fused carpels, an arrangement very similar to that seen in the Liliales. The most obvious difference is in the size of the flowers. Thus, Juncaceae was initially considered to be a member of the Liliidae, and was frequently placed close to the order Liliales by most authors. It was rarely considered to be a member of the related Commelinidae.

Influential authors such as Bentham and Hooker (1883), Hutchinson (1964) and Takhtajan (1980) have all favoured an origin of Juncaceae from within the order Liliales, and others, such as Hallier (1912), have proposed a derivation of Juncaceae from the family Liliaceae itself, considering the two families closely related.

However, while studies centering on the floral morphology have long been fashionable in determining relationships between taxa, it is wrong to ignore other features. In fact, Cronquist (1968, 1988) realised that despite the similarities in the floral morphology, there are a great many differences between the Liliales and Juncaceae. It was on the evidence of

a much wider range of characters than those previously considered that he concluded the floral resemblance was purely superficial.

Such characters included the presence of a starchy endosperm in the seeds of Juncaceae (in Liliales the seeds do not have a starchy endosperm), and the distribution of vessels throughout the plant body (in Liliales vessels are confined mostly to the roots). In addition, the stomatal types imply only a remote connection of Juncaceae with Liliales. The stomata of the Juncaceae have two subsidiary cells, whereas the stomata of the Liliales do not have subsidiary cells. As observed by Stebbins & Khush (1961), stomatal types can be correlated with a number of other characters, including reproductive characteristics and geographic distributions, and are likely to be useful indicators of phylogenetic relationships. Rather than an origin of Juncaceae from within the Liliales, Stebbins & Khush (1961) suggested that the liliaceous type is relatively advanced and cannot, therefore, have given rise to the type seen in Juncales, instead believing that both stomatal types were derived independently from a more primitive form.

These characteristics, Cronquist noted, clearly setting Juncaceae apart from the Liliales, are all to be found within the Commelinidae, and in fact serve to define the group as a whole. Most authors, such as A.P.G. (2003), Chase *et al* (1993) and Dahlgren *et al* (1985) have since agreed with Cronquist in this respect, and it is now accepted that Juncaceae are members of the Commelinidae (Commelinids in A.P.G. 2003).

This change in opinion did little, however, to clarify the position of Juncaceae in relation to the other monocotyledon families. Among the authors who believed in a liliaceous origin of Juncaceae, it had not been uncommon for authors to ally Juncaceae with families now also placed in Commelinidae. In placing Juncaceae in Commelinidae, Cronquist affected the question of which families are allied with Juncaceae more by removing the liliaceous contenders from the running rather than by adding new Commelinidae ones. Of all the classifications published up until that point, some of the most frequent families to be allied with Juncaceae, excepting a few Liliaceous families, were the Flagellariaceae and Thurniaceae, with Restionaceae, Cyperaceae, Centrolepidaceae, Rapateaceae and Poaceae slightly less frequently. All of these are now placed within Commelinidae. But, apart from the clearly Juncaceous family Thurniaceae, there has been much disagreement over the inter-relationships of these families.

1.2 A history of the systematics of Juncaceae

This can be illustrated by the numerous different definitions of the order Juncales; a summary of these, invoking many different combinations of the above families, and too varied to illustrate in full here, was produced by Hamann (1961). In general, however, it was Flagellariaceae, Restionaceae and Centrolepidaceae which were the most frequently included with Juncaceae in Juncales. For example, Bentham & Hooker (1883), Hutchinson (1959) and Emberger (1960), among others, all included one or more of these three families within the Juncales, or equivalent grouping where this name was not used.

Certainly, it can be seen that these families do bear several features in common with Juncaceae, although the authors rarely stated the reasoning behind their classifications. The flowers of Juncaceae and Flagellariaceae are of a strikingly similar form, each having two whorls of three perianth segments, six stamens and a superior ovary of three fused carpels, forming three locules. The flowers of Restionaceae are a little more reduced, but nevertheless similar to those of Juncaceae. The stamens are reduced to three fused carpels with one to three locules. The flowers of Centrolepidaceae are more reduced still; this family was, perhaps, only considered to be allied to the Juncaceae through the Restionaceae, which they resemble more clearly. The growth habit and vegetative form of these families is also similar to the Juncaceae, particularly the Restionaceae, in which the leaves are often reduced to sheathing bases, a common occurrence in some species of *Juncus*. But, as before, these characters have since been shown to be misleading.

Among some of the families less frequently combined with Juncaceae, Cyperaceae was one which recurred only occasionally during this period, in works by Grisebach (1854), Hallier (1912) and Deyl (1955). For most authors, the differences between the floral morphologies of the families prevented such an association being made. Members of Cyperaceae typically lack perianths or they are reduced to scales, bristles or hairs; they have one to three stamens, a single ovule, and a bi- or tri-carpellate pistil that develops into a nutlet (Dahlgren *et al* 1985).

However, these differences do not preclude a close relationship between Juncaceae and Cyperaceae. It is easily conceivable that most of these differences were derived by the reduction of a morphology similar to that seen in Juncaceae. When other features are taken into account it can be seen that there are many similarities between Juncaceae and Cyperaceae, although most are, perhaps, not as immediately obvious as the floral characters.

The pollen development of Juncaceae and Cyperaceae has been shown to have an unusual feature in common. In both families microsporogenesis is simultaneous, and the four microspores are retained in tetrads and are released in this form (Schnarf 1929; Wulff 1939). In families such as Flagellariaceae, Restionaceae, Typhaceae and Eriocaulaceae microsporogenesis is successive, and the pollen is released as monads. Even so, the development of the pollen in Cyperaceae differs from that in Juncaceae. In Cyperaceae only one of the four microspores in each tetrad becomes functional: the other three degenerate, and are incorporated into the wall of the fourth (Juel 1900; Piech 1928; Maheshwari 1950), so that it appears to be a monad. Consequently the pollen grains of Cyperaceae are not referred to as tetrads; instead they have been described as 'cryptotetrads' (Cronquist 1968) or, more usually, 'pseudomonads' (Selling 1947; Dahlgren *et al* 1985). However, the Mapanioideae of Cyperaceae appear to have derived true monads secondarily from pseudomonads, referred to as *Mapania*-type pollen (Simpson *et al* 2003). These unusual features of the two families were first noted in the early part of the 1900's, but they have been well studied throughout the last century.

Similarities in the chromosome cytology were also recorded during the mid 1900s. Diffuse, or non-localised, centromeres have been found in *Carex L., Eleocharis R.Br.* and *Cyperus L.* of Cyperaceae, and in *Luzula* of Juncaceae (Godward 1985). Diffuse centromeres have been documented by many authors, but initially by Malheiros *et al* (1947) and Malheiros & Castro (1947). They occur rarely, not just among the angiosperms, but among all organisms. Among the monocotyledons they occur only in Cyperaceae and Juncaceae (the state for Thurniaceae is unknown), and in *Chionographis* Maxim. of Melanthiaceae (Tanaka & Tanaka 1977). Among the dicotyledons it occurs only among representatives of Myristicaceae and Cuscutaceae (Greilhuber 1995). It is generally considered to be a derived character in the angiosperms, and must have occurred at least four times independently, as a close relationship between most of these taxa is unlikely. Nevertheless, the occurrence of such a rare characteristic in both Juncaceae and Cyperaceae may be taken as an indication of their close relationship; noting its frequency

among all angiosperms, the chances of it having appeared independently in both families, where a close relationship is possible, seem very small.

It has also been noted that the pattern of meiosis in these taxa differs from the usual one, as a form which has been termed 'post-reductional' or 'inverted' meiosis (Battaglia 1955; Greilhuber 1995), in which the first meiotic division is equational and the second reductional. In most organisms, the first meiotic division is reductional, and the second equational. In angiosperms inverted meiosis appears to be linked to the presence of diffuse centromeres (Greilhuber 1995).

Chemical similarities between the two families were observed by Williams & Harborne (1977) and Harborne *et al* (1985). The presence of the secondary metabolite flavonoid luteolin 5-methyl ether, a compound which occurs quite rarely among the monocotyledons, links Cyperaceae and Juncaceae; it was found in 70% of Juncaceae species examined and 15% of Cyperaceae.

Thus, it can be seen that several characters are shared by Juncaceae and Cyperaceae, but although many of these have been recognised for several decades, it seems that they were not always taken into account in taxonomic treatments. Far more commonly, Cyperaceae were believed to be grouped with Poaceae and not with Juncaceae, as both share highly reduced flower morphologies which, if only superficially, bear much in common with one another. To give one example, Cronquist (1988) followed this tradition by placing Cyperaceae and Poaceae into Cyperales, whereas his Juncales consisted only of Juncaceae and Thurniaceae.

But it was at around this time that some authors began to look more closely at non-floral characteristics. Three other major classifications published at about this time by Takhtajan (1980), Thorne (1983) and Dahlgren *et al* (1985) did place Juncaceae and Cyperaceae in the same order. The weight of evidence provided by numerous characteristics made a close relationship between Juncaceae and Cyperaceae seem likely, despite the differences between their inflorescences. Their conclusions were that the similarities between the inflorescence of Juncaceae and the Restionales on the one hand, and between Cyperaceae and Poaceae on the other, were due to convergence. Even Cronquist (1968, 1988) admitted that Juncaceae and Cyperaceae showed many similarities, but he chose to

disregard them in his classification. It is hard to find suitable justification for this treatment, as, unlike his work regarding the re-classification of Juncaceae from Liliidae to Commelinidae, his classification is supported only by the reduced floral morphologies.

It is true that the various characteristics appear to support different classifications, depending upon which characteristics are invested with more importance; there is an interlocking network of shared characteristics that seems to deny a simple, heirarchical classification. In our current taxonomic treatments it has become usual to consider evolutionary relationships to be more important than superficial resemblances, so in order to decide which view reflects the true phylogenetic relationships, one must decide which of the similarities between taxa are homologies, and which due to convergence. Cronquist chose to view the similarities between Cyperaceae and Juncaceae as taxonomically less important than the floral characters, as did many other authors. The implications of continuing to follow Cronquist's classification are to interpret these shared characteristics as due to convergence. But the similarities between Juncaceae and Cyperaceae are hard to explain in this manner. Many of them are unusual features, occurring only rarely, if at all, among the other monocotyledons, and it is unlikely that they would have arisen independently in both families. The differences between the inflorescence structures are a different matter; they can be explained by reduction, the loss of an existing character rather than the acquisition of a new one. It is easily conceivable that such features could be derived more than once in different taxa, creating misleading similarities with other families. In light of this it was, perhaps, inevitable that the majority of later classifications would consider Juncaceae more closely related to Cyperaceae than to other families.

Further features shared by Juncaceae and Cyperaceae have also been noted. A number of embryological features, such as onograd embryo development, have been pointed out by Munro & Linder (1997) as occurring in both Juncaceae and Cyperaceae. Studies by Savile (1979; 1990) into the parasitology have also supported this classification, as the two families share highly evolved rusts and smuts.

Recently, the advent of computerised cladistic analyses, combined with the possibility of using DNA sequences to provide large additional datasets, has also produced strong evidence for this view. Analyses of morphological data (Simpson 1995; Stevenson & Loconte 1995), molecular data (A.P.G. 2003; Chase *et al* 1993, 1995; Muasya *et al* 1998;

Plunkett *et al* 1995), and combined data analyses (Linder & Kellogg 1995; Munro & Linder 1998) have consistently produced phylogenetic trees where Juncaceae, Thurniaceae and Cyperaceae are placed together, excluding other families from this group. A more recent study by Drábková *et al.* (2003), containing many more Juncaceae species than had previously been included in phylogenetic studies, also supports this phylogenetic position of Juncaceae.

Within the last ten years, the general consensus has been that Juncaceae and Cyperaceae are sister groups, with Thurniaceae sister to both. Dahlgren *et al* (1985) had placed three families in Juncales: Juncaceae, Cyperaceae and Thurniaceae, and as subsequent studies have supported this classification it is currently accepted that this is a monophyletic group.

The Angiosperm Phylogeny Group (A.P.G. 1998, 2003) have, however, proposed a broader definition of the order, and have included these families within Poales, thus including an additional fifteen families. However, they note that within Poales there is strong support for a 'cyperoid' clade consisting of Juncaceae, Cyperaceae and Thurniaceae (A.P.G. 2003), although they do not assign it a formal name or taxonomic rank.

This thesis is concerned with the family Juncaceae, and it must also be concerned with the families most closely related to Juncaceae, in part to determine the status of Juncaceae as a family, as well as to clarify the relationships of Juncaceae with other families. Thus, the families which will form the focus of this thesis are those shown to form a monophyletic group within Poales: Juncaceae, Cyperaceae and Thurniaceae.

1.3 Relationships among the 'Cyperoid' clade

There are either three or four families recognised in the 'Cyperoid' clade, depending on the authority. The A.P.G. (2003) recognises three: Cyperaceae, Juncaceae and Thurniaceae. The Species Plantarum Flora of the World (pub. A.B.R.S., Canberra), a series which is still incomplete, does however contain four volumes concerning the families in this clade (Kirschner 2002a, b, c; Munro *et al* 2001), and recognises the family Prioniaceae as distinct from Thurniaceae, with which it was combined by Chase *et al* (2000). It has not yet produced the volume containing Thurniaceae.

On a morphological basis, if four families are recognised, it is easy to distinguish each from another. Cyperaceae is recognised in part by a single, basal ovule per pistil, Thurniaceae by three ovules and seeds subulate at either end and a condensed inflorescence, and Juncaceae by at least three ovules per pistil, but seeds not subulate at both ends. Prioniaceae, otherwise similar to Juncaceae, has an unusual growth form, possessing a woody, upright stem, and distinctive anatomical features. There are no obvious morphological characteristics uniting Prioniaceae and Thurniaceae. However, until this has been studied more thoroughly, and as consistency is necessary in any discussion, for the purposes of this thesis the classification of the A.P.G. (2003) will be followed.

1.3.1 Thurniaceae

Thurniaceae consists of two monospecific genera, *Thurnia* Hook.f. and *Prionium* E. Mey., each with very restricted distributions. *Thurnia sphaerocephala* Hook.f. is a sedge-like herb, and is found only in Guyana and parts of the Amazon valley (Cutler 1965), growing in wet, sandy lowlands. In contrast *Prionium serratum* (Thunb.)Drege is quite distinctive and easily recognised as, unlike most species in Juncales, it is shrubby, possessing a tall, unbranched woody stem which can reach five feet in height. It is found only in South Africa's Cape region, growing along watercourses on sandstone.

Both *Thurnia* and *Prionium* have frequently been grouped with Juncaceae throughout their history, sometimes included within it, and sometimes recognised as distinct families,

1.3 Relationships among the 'Cyperoid' clade

Thurniaceae and Prioniaceae. They have been associated with Juncaceae in part because of a shared floral morphology. The inflorescences are of the same basic structure; *Prionium* differs mainly in that the stylodial branches are not fused at the base, and *Thurnia* in the shapes of the inflorescence and flower parts. Pollen studies have shown that both genera, along with Juncaceae, share simultaneous microsporogenesis, with pollen always released in tetrads. *Thurnia* was included within Juncaceae by Bentham & Hooker (1883), but *Prionium* has been included within Juncaceae far more frequently (Endlicher 1836; Bentham & Hooker 1883; Buchenau 1875, 1890; Baker 1897; Cutler 1969; Dahlgren & Clifford 1982; Dahlgren *et al* 1985).

However, among these shared characteristics are features in both genera which set them apart from one another and from Juncaceae, which lead some authors to recognise them as families in their own right. *Thurnia* differs the most markedly from Juncaceae. On a macromorphological level, the seeds bear subulate processes at either end, and the inflorescence is swollen at the base such that the flowers are arranged in a dense, spherical head subtended by a rosette of several long, leafy bracts, held on an otherwise leafless stem. *Thurnia* seeds also possess a free endosperm, whereas Juncaceae seeds do not.

Thurnia has long been recognised to differ from Juncaceae. Buchenau excluded it from Juncaceae in his 1906 monograph, holding it to be an anomoly within the family, and suggesting that it may be closer to Sparganiaceae. It was first given the status of family by Engler (1907).

Since this time further unusual features have been observed which support the exclusion of *Thurnia* from Juncaceae. The anatomy has been thoroughly studied by Cutler (1963; 1965), who drew attention to a number of features. Most striking are inverted vascular bundles in the leaves, of a type which appears to be unique to this genus; they have not been recorded for any other plant. These are small vascular bundles which occur next to the abaxial side of the bundle sheaths of the main vascular bundles, but inverted so that the phloem poles of the two vascular bundles are adjacent. Although inverted bundles occur in leaves of a few other genera (e.g. *Iris*), they are of a different orientation, with the xylem poles of the bundles facing towards one another. He also points out that silica is present in the tissues, a characteristic not shared by Juncaceae.

Since the erection of the family, Thurniaceae has been widely recognised; but due to its unusual combination of features it has often been allied with a variety of families rather than just Juncaceae. To provide some examples, Buchenau (1906) suggested a relationship with Sparganiaceae; Hutchinson (1959) placed it in an order which contained Juncaceae, Restionaceae and Centrolepidaceae; while Emberger (1960) placed it with Cyperaceae, Rapateaceae and Flagellariaceae, but not Juncaceae).

Cutler (1965; 1969) also had doubts about its relationship with Juncaceae. He observed that many of the anatomical features, while differing from Juncaceae, were similar to those found in various other families: the distribution of sclerenchyma in the leaves is similar to that in Rapateaceae, and the silica bodies present in the leaf epidermis resemble those found in Rapateaceae, Restionaceae and some Cyperaceae. Dahlgren *et al* (1985) have also suggested that Thurniaceae may be more closely related to Rapateaceae and Xyridaceae than to Juncaceae. But the total combination of features do not indicate a clear relationship with any one or another of these families, and when compared to the similarities *Thurnia* bears to Juncaceae, most authors continued to retain Thurniaceae within Cyperales.

Thurnia has rarely been included in cladistic analyses, in part due to a lack of knowledge of some characters. Two morphological studies have produced conflicting results, as Simpson (1995) places it within Juncaceae, due to a shared presence of dehiscent fruit, more than one ovule per locule and more than three stamens; while Munro & Linder (1998) place it as a sister group to all remaining families in Juncales, but this branch is poorly supported by bootstrap values. A third study by Givnish *et al* (1999), based on *rbcL* sequence data, also places *Thurnia* as outlying to Juncaceae and Cyperaceae, but few Juncaceae taxa were sampled, so the placement of *Thurnia* cannot be considered conclusive.

In contrast, *Prionium* bears many more features in common with Juncaceae than *Thurnia* does, and its relationship with Juncaceae has been called in to question far less frequently. Nevertheless, De Laharpe (1825) acknowledged the odd growth form of *Prionium* and suggested that an affinity with Restionaceae was possible, although he realised that the floral morphology made this unlikely. As well as the woody habit, otherwise unknown in Juncaceae, a few other features may be found to distinguish *Prionium* from the rest of

Juncaceae. In particular, Cutler (1969) noted some distinctive features in the anatomy: chlorenchymatous air canals present in the leaves and an unusual distribution of the sclerenchyma in the leaves and stem caused him to recommend that *Prionium* should no longer be placed within Juncaceae. Supporting these conclusions, Williams & Harborne (1975) also found flavone-c-glycosides in the tissues of *Prionium* but not in Juncaceae.

The geographical distribution of *Prionium* is also worth noting. *Prionium* occurs only in the Cape region of South Africa, whereas all the other genera have representatives in the New World, several of these (*Distichia, Oxychloe* and *Patosia*) being endemic to South America. But neither woodiness nor a South African distribution can rule out inclusion within Juncaceae. Although there are no other woody species in Juncaceae, woodiness does occur, if rarely, within the closely related Cyperaceae, as can be seen in the monotypic genus *Microdracoides* Hua and in some members of *Gahnia* J.R.Forst. & G.Forst. (Dahlgren *et al* 1985), implying that such variation within the family is not unreasonable. Thus *Prionium* was generally accepted to remain within Juncaceae, despite the anatomical differences (Dahlgren *et al* 1985).

Recent studies have changed this view. The majority of molecular studies have placed *Prionium* outlying to both Cyperaceae and the remainder of Juncaceae (Chase *et al* 1993; Muasya *et al* 1998; Munro & Linder 1998; Plunkett *et al* 1995). It is clear from these studies that Juncaceae *sensu lato* is paraphyletic, and it is probable that *Prionium* is not as closely related to Juncaceae as was previously thought. As a result of this Munro & Linder (1998) erected a new family Prioniaceae, which can be distinguished from Juncaceae by its woody habit and unusual chlorenchymata with air canals and usual distribution of sclerenchyma in the leaf and culm (Cutler 1969).

It is worth mentioning here that there has been less agreement among morphological studies regarding the status of *Prionium*. Although the morphological tree produced by Munro & Linder (1998) is in agreement with the molecular studies in this respect, the branch is poorly supported with a bootstrap value of 23, and the tree produced by Simpson (1995) places *Prionium* within Juncaceae (and sister to *Thurnia*). Thus, although it is frequently accepted that *Thurnia* and *Prionium* are not members of Juncaceae, there is, perhaps, justification for further investigation of this, particularly on a morphological level.

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1.3 Relationships among the 'Cyperoid' clade

The nature of the relationship between *Thurnia* and *Prionium* has also been subject to some confusion. *Thurnia* has rarely been included in phylogenetic studies, but where it has, in morphological studies it has appeared either as sister to *Prionium* and within Juncaceae (Simpson 1995), or as sister to a Cyperaceae and Juncaceae group (Munro & Linder 1998). As regards molecular studies, sequence data has only recently been obtained for *Thurnia*, in Givnish *et al* 1999 for the *rbcL* region. Here the phylogeny of 96 monocotyledon taxa was investigated. These results place *Thurnia* sister to *Prionium*, and is in agreement with Simpson (1995) in this respect, but differs in that this clade is sister to Cyperaceae and Juncaceae. This *Thurnia-Prionium* clade shows good bootstrap support in the tree, and these results are confirmed by Chase *et al* (2000), who chose to place both *Prionium* and *Thurnia* in Thurniaceae.

However, it is worth noting that as the number of taxa sampled from Cyperales is low in these studies (just four taxa are sampled in Givnish 1999), and as it is not entirely in agreement with any morphological studies, a more detailed study of the relationship between *Prionium* and *Thurnia* should be carried out.

1.3.2 Juncaceae and Cyperaceae

The advent of cladistic studies, both morphological and molecular, has shown that not only are Juncaceae and Cyperaceae closely related, but they may not be as distinct from one another as was previously thought. Recently, almost all cladistic studies have shown Juncaceae to be paraphyletic with respect to Cyperaceae. The case of *Prionium*, as discussed above, is one example, and this was accounted for by excluding it from Juncaceae and erecting a new family, Prioniaceae. But the monophyly of the remainder of Juncaceae has been unsupported by many molecular studies, and of the few morphological studies, Munro & Linder (1998) show Juncaceae *sensu stricto* to be paraphyletic.

Whereas in the past taxonomists may have had little trouble in distingushing Cyperaceae and Juncaceae, when the distinguishing characteristics are looked at more closely it can be seen that while Cyperaceae is defined by features which were uniquely acquired by that taxon (autapomorphies), Juncaceae is defined only by the absence of these. The likelihood that Cyperaceae was derived from primitive Juncaceae has been discussed by a number of authors, including Takhtajan (1980) and Dahlgren *et al* (1985), although it was not, at the time, considered to throw any doubt on the status of the families.

In floral construction Juncaceae are less derived than Cyperaceae, and the states found in Cyperaceae may have evolved from those in Juncaceae. This relationship is also reflected in the pollen tetrads, as those of Juncaceae are like those of Cyperaceae, except that three of the grains in the tetrad of Cyperaceae degenerate soon after their formation, whereas they do not in Juncaceae. Absence of silica bodies in Juncaceae is probably an ancestral state, because the silica bodies in Cyperaceae are generally unlike those in all other monocots and are likely to be independently derived. Further justification for the view that Cyperaceae are more derived than Juncaceae is given by the vessel features: Cyperales have more specialised vessel features than Juncaceae (Metcalfe 1971; Takhtajahn 1980).

Whereas all the character states which define Cyperaceae as a group seem to be advanced, and thus can be used to support the monophyly of this family, those defining Juncaceae appear to be plesiomorphic, and in the absence of a derived synapomorphy they should not be used to support a close relationship between the genera which excludes Cyperaceae.

Among these studies there is also little agreement as to the relationships between genera in Juncaceae, especially regarding the larger genera *Juncus* and *Luzula*. Most frequently *Juncus* or *Luzula* appear sister to the rest of Juncaceae and to Cyperaceae in cladistic analyses (Chase *et al* 1993; Duvall *et al* 1993; Givnish *et al* 1999; Muasya *et al* 1998), *Luzula* generally being sister to a group containing the remainder of Juncaceae. But a significant number of analyses do not show this arrangement. Of the genera with single-flowered inflorescences, most (with the occasional exception of *Oxychloe*) generally fall within one clade, but their arrangement within this clade may vary.

A further problem has been the placement of the genus *Oxychloe*. Several *rbcL* analyses (Plunkett *et al* 1995; Muasya *et al* 1998; Chase *et al* 1993; Givnish *et al* 1999; Duvall *et al* 1993) all place *Oxychloe* with Cyperaceae rather than with Juncaceae. In most analyses it has appeared within Cyperaceae, and in one (Muasya *et al* 1998) sister to Cyperaceae. Many of these analyses, however, used a sequence obtained from a single specimen by Plunkett *et al* (1995). When the voucher specimen was checked by Muasya *et al* (1998), the material of *Oxychloe* was found to be mixed with a similar plant of Cyperaceae, so that

1.3 Relationships among the 'Cyperoid' clade

the material sampled may have included material of Cyperaceae. Further work (Kristiansen *et al* 2005) also supports this theory. Starr, J. (In Press) presents evidence that the sequence used was a chimera, consisting of a section of Juncaceae DNA and a section of Cyperaceae DNA. However, it was also noted by Muasya *et al* (1998) that although *Oxychloe* shares some features with Juncaceae (for example many ovules and prominent tepals) it also has spiro- or orthodistichous leaves and an indehiscent fruit, unusual for Juncaceae but similar to some Cyperaceae, suggesting a possible relationship to Cyperaceae.

In summary, it can be seen that there are numerous problems within the systematics of Juncaceae. Among the studies carried out there has been little agreement in the placement of *Prionium* and *Thurnia*, so that the status of the families Prioniaceae and Thurniaceae is uncertain. The nature of the relationship between Juncaceae and Cyperaceae is also unclear, as are the relationships between Juncaceae genera.

Very few of the studies discussed above were carried out with the intention of solving these problems. Only Simpson (1995) was concentrating on the phylogeny of Juncales, Muasya *et al* (1998) on Cyperaceae and Munro & Linder (1998) on the position of *Prionium*. The other studies were focussing on a much wider range of families than those of Cyperales, and therefore the number of Juncaceae and Cyperaceae specimens included in many of these studies is very small (for example, two Juncaceae genera in Chase *et al* 1993 and Duvall *et al* 1993). Of those studies which do include species from most of the Juncaceae genera, some find Juncaceae to be paraphyletic and some do not. Where such disagreement, or lack of resolution, occurs, a larger dataset should produce trees with stronger branch support and more resolution, and a wider range of Juncaceae species should be sampled to represent the range of variation within the family.

1.4 Aims

- 1. The phylogenetic relationships of taxa within Juncaceae are investigated.
- 2. The relationship between Juncaceae and Cyperaceae is investigated, clarifying whether Juncaceae is monophyletic with respect to Cyperaceae, and clarifying the relationship of *Oxychloe* to other genera.
- 3. The separate status of Prioniaceae from Juncaceae is examined, and its relationship with Thurniaceae investigated.
- 4. Character evolution within Juncaceae is studied.
- 5. The classification of Juncaceae is reviewed in the light of the results of this study.

1.5 Selection of analytical techniques

The study of plant systematics has been greatly assisted in recent years by the development of techniques to utilise DNA, such as PCR. With the advent of PCR and sequencing techniques it has become possible to obtain large molecular datasets, greatly adding to the number of characters which can be used to determine relationships when compared to the use of morphological characters. In parallel to this, computerised cladistic analyses have become the generally accepted means of determining phylogenies. These allow for more detailed analyses, far more rapidly than can be carried out with pen and paper, and more easily able to utilise the large datasets.

The use of DNA sequences in systematic studies has a further advantage over the use of morphological characters, as determining the homologies of morphological characters is a necessary but difficult task; as is discussed throughout the previous sections, mistakes regarding the homologies are frequently made, leading to erroneous conclusions about the relationships of the taxa. There is no solution to this problem but to spend much time studying the characters in detail. In contrast, determining homologies of the characters of DNA sequences is not as difficult, as statements of homology of characters (nucleotides at nucleotide sites) are made via sequence alignment; furthermore, a variety of phylogenetic computer applications, utilising analysis methods such as maximum parsimony, are readily available to systematists.

As a result of this the majority of publications relating to plant systematics use DNA sequences as the raw data, sometimes exclusively, and the morphology of the group in question may on occasion be neglected. This is the case with both Juncaceae and Thurniaceae, as is described in Sections 1.3.1 *Thurniaceae* and 1.3.2 *Juncaceae and Cyperaceae*. The end result of a systematic study is to clarify the relationships between taxa, with implications for the taxonomy. The morphology is, therefore, important, as it is by using the morphology that plants are identified both in the field and in the laboratory. If a new taxon is proposed but no morphological characters are identified to support this group, identification of that taxon may become difficult, and the results of the work are, perhaps, likely to be of less use to other workers in the field of plant biology.

The main aims of this thesis are to study the phylogeny of Cyperales at family level, and of Juncaceae at genus level. Previous molecular cladistic studies (Muasya *et al* 1998; Munro & Linder 1998; Simpson 1995) have shown that good resolution can be obtained at family level in Cyperales. A DNA sequence cladistic study is carried out here (Chapter 5 *Phylogeny*) to study the phylogeny of Cyperales.

In addition to this, a detailed study of morphology is also carried out. The characters are mapped onto the phylogeny resulting from the molecular analyses, in order to achieve the aims of studying the evolutionary history of characters, and identifying characters which are diagnostic of taxa (Section 5.4 *Character distribution and evolution*).

It was decided that the morphological cladistic studies would concentrate only on the relationships within Juncaceae. Although this thesis is also concerned with relationships between the families of Cyperales, the difficulty of determining homologies in morphological data becomes greater at higher taxonomic levels, where a much greater degree of variation occurs. In addition, Givnish *et al* (1999) highlighted the difficulties of using morphological data to determine relationships between the families of the subclass Commlinidae, finding that a wide range of morphological characters showed repeated convergences or reversals when they were mapped onto molecular maximum parsimony analyses. As the molecular study is expected to provide good resolution at this level, it was not considered necessary to use the morphological data to examine the relationships at family level.

1.5.1 The structure of this thesis

The collection of the morphological data is covered in three chapters. The families in this study are all known to have much variation in the anatomy (Cutler 1969), as well as the macromorphology. A large part of the morphological study concentrates on anatomical data. In addition to this, one character which shows much variation within Juncaceae in particular is the form taken by the junction between the leaf sheath and leaf blade (Fig. 4.1). The presence or absence of ligules has been used previously in cladistic studies (Simpson 1995; Munro & Linder 1998). In Cyperaceae, a ligule may be either present or absent, but when present, it does not appear to take the same form as any structure seen in Juncaceae, so a study of the homology of these structures is carried out to elucidate

relationships between the families further. The collection of the macromorphology, anatomy, and ligule data are each dealt with in separate chapters as different techniques are used for each, and different approaches are used to present the data.

However, there is no reason to distinguish between the three sets of morphological data for the purposes of analysis; they differ only in terms of physical scale and the techniques required to observe them. Analyses of the molecular and morphological data are presented in Chapter 5 *Phylogeny*, and a discussion of the evolution of some the characters is also found here.

Chapter 2 Macromorphology

2.1 Introduction

2.1.1 The use of morphology in the taxonomy of Juncaceae

The difficulties of using morphological characters to determine relationships among the Cyperales were highlighted in Chapter 1 *Introduction*, particularly in the use of floral morphology. However, among publications on the taxonomy of Juncaceae it is usual to find that a very wide range of morphological characters have been considered, with classifications based on observations of the rhizomes, culms, leaves, inflorescences, flowers, fruits and seeds, as well as including many anatomical characters (Balslev 1996; Buchenau 1890, 1906; Chrtek & Krísa 1974, 1980; Grisebach 1844–1845; Kirschner 2002a, b, c).

The classification of Juncaceae has changed little at genus level since Buchenau's monograph in 1906, with the exception of *Prionium*, which was removed from Juncaceae by Munro & Linder (1998). There have been changes to the subgeneric classifications of *Juncus* and *Luzula*, however. These are summarised in brief here by a comparison between Kirschner (2002a, b, c), a recent complete revision of the family (Tables 2.1, 2.2), and Buchenau (1906), the most thorough previous monograph of Juncaceae.

Kirschner (2002b, c) recognises two subgenera and 10 sections in *Juncus* (Table 2.1). These subgenera are also recognised as distinct groups by Buchenau (1906), but he did not give them a formal taxonomic rank. Instead he gave them descriptive names ('Flores prophyllati' and 'Flores eprophyllati'), and then further divided these groups into subgenera. Buchenau's subgenera are broadly equivalent to Kirschner's sections, although most of the names used by Buchenau (1906) have been changed to reflect original usage. In addition, two of the subgenera recognised by Buchenau (1906) have been further divided, each into two groups, on the basis of whether they are annual or perennial, and a third split into two on the basis of whether the leaves are ensiform or not ensiform. One of Buchenau's subgenera (*J.* subgen. *Singulares*) is no longer recognised, as only one

Table 2.1 Subgeneric classifications of Juncus

Buchenau 1906	Kirschner 2002	Buchenau 1906	Kirschner 2002	Characters defining the taxa			
No taxonomic rank given	Subgenus	Subgenus	Section				
Flores Eprophyllati	Juncus	Thalassii	Juncus	Flowers not subtended by bracteoles.	Inflorescence pseudolateral. Leaves terete. Leaves with no central air canal and not sep Parenchymatous pith. Vascular bundles scattered throughout tran	septate. ransverse section.	
		Septati	Ozophyllum	Infloresc-	Leaves with one or more central air canals with transverse septae.	Annual or perennial. Leaves terete.	
				Iridifolii	racemoe.		Perennial. Leaves laterally flattened (ensiform).
		Alpini	Stygiopsis	_	Leaf blade flat, canaliculate, sub-terete to t Leaves with one or more central air canals Anthers sometimes distinctly exserted. Seeds with distinct appendages.	te to terete. canals with transverse septae. d.	
		Graminifolii Caespito	Caespitosi		Leaf blade dorsiventrally flattened. Leaves with no central canal; not septate.	Annual.	
			Graminifolii			Perennial.	
		Singulares	Not applicable		Distichous leaves. Only one specimen was (2002) considers that it may be a variant of <i>Graminifolii</i>).	s ever collected. Kirschner	

Tabl	e 2.1	Continued

Buchenau 1906	Kirschner 2002	Buchenau 1906	Kirschner 2002	Characters defining the taxa		
No taxonomic rank given	Subgenus	Subgenus	Section			
Flores Prophyllati	Agathryon	Subulati	Forskalina	Flowers subtended by bracteoles (small membranous bracts).	Leaves terete. Leaves contain aerenchymatous pith.	
		Poiophylli	Steirochloa		Leaves flat to canaliculate. Po Some adaxial epidermis cells enlarged. Margins with sclerenchymatous strands.	Perennial.
		Gen		Tenageia	Inflorescence cymose.	Leaves lack pith: chlorenchyma occurs in the inner part of the leaves, sometimes interrupted by air canals.
			Genuini	Juncotypus		Inflorescence pseudolateral. Basal leaves lack a blade, or blade rudiment 'Sterile shoots' are terete and stem-like, with aerenchymatous pith.
specimen was ever collected. Kirschner (2002b) considers that this specimen may represent a variant of a species of *J*. sect. *Graminifolii* Engelm.

The change in the taxonomic ranks of the subdivisions of *Juncus* may be due in part to the small number of formal taxonomic ranks used by Buchenau (1906), as between genus and species levels he only used the rank 'subgenus'. He appeared to consider the division of *Juncus* into two groups based upon the presence or absence of bracteoles subtending the flowers to be significant, and his subgenera formed further subdivisions of these two groups - but nevertheless, he did not assign them formal taxonomic ranks.

The classification of *Luzula* has undergone more significant changes, as two of the three subgenera recognised by Buchenau (1906) are not equivalent to any groupings recognised by Kirschner (2002a), although the third (*L.* subgen. *Pterodes* (Griseb.) Buchenau) is still recognised (Table 2.2). Buchenau's subgenera are based mainly upon the structure of the inflorescence, which varies in the branching patterns, relative lengths of the branches, and the arrangement of flowers on distinct pedicels or in dense clusters. The species which make up these two subgenera in Buchenau (1906) are split between two different subgenera in Kirschner (2002a), according to whether they are annual or perennial, and whether the inflorescence is racemose or cymose. *Luzula* subgen. *Pterodes*, recognised by both authors, is defined mainly by a distinct, apical seed appendage.

The differences between the classifications of Juncaceae are generally related to the different significance applied to each of the characters, rather than to the recognition of new characters not previously observed; the majority of the characters used to define taxa by Kirschner (2002a, b, c) were described by Buchenau (1906), even in the instances where Buchenau did not choose to use them to define his subgenera.

Relatively few publications have applied statistical analyses to morphological data for Juncaceae. Chase *et al* (1995), Linder & Kellogg (1995), Munro & Linder (1998), Simpson (1995) and Stevenson & Loconte (1995) have used morphological data for Juncaceae in cladistic analyses. Of these, only Simpson and Munro & Linder focussed on Cyperales; the other authors included data for families rather than genera (Juncaceae, Cyperaceae and *Thurnia* in Chase *et al* 1995 and Stevenson & Loconte 1995; Juncaceae and Cyperaceae in Linder & Kellogg 1995).

Buchenau 1906	Kirschner 2002		Morphological characters defining the taxa		
Subgenus	Subgen.	Section	Subgenera		Sections
Anthelaea	Marlenia	(No sections)	Leaf apex acute to subulate. Inflorescence	Annual. Leaf apex acute, extending to one or two hairs. Inflorescence cymose. Flowers arranged singly. No seed appendage.	
Gymnodes	Luzula	Anthelaea	anthelate or more-or- less corymbose. Seeds lack distinct caruncle; appendage often basal, short, fibrillate. Inflorescence anthelate or umbelloid, sometimes flowers arranged in dense heads. Seed often with distinct caruncle. When seed appendage is present, it is basal.	Perennial. Inflorescence racemose.	Inflorescence an anthela of anthelas. Flowers in clusters of 2–4. Seed appendage basal, short, fibrillate.
		Diprophyllatae			Inflorescence a panicle. Flowers arranged singly, or some in clusters of 2–5. Seed appendage absent or, when visible, basal, fibrillate.
		Nodulosae			Nodulose rhizomes. Leaf tips acuminate, subulate. Inflorescence umbelloid or anthelate. Flowers in dense, large clusters. Seed appendages absent.
		Alpinae			Leaf tips acute to acuminate. Inflorescence spike-like or paniculate, congested. Seed appendage indistinct, short or lacking.
		Thyrsano- chlamydeae			Leaf tips acute, acuminate or mucronate. Inflorescence subcongested or in glomerules. Seed appendages basal, short or lacking.
		Luzula			Leaf tips obtuse, usually slightly swollen. Inflorescence congested to umbelloid. Flowers in spike- or head-like clusters of 3–many. Seed appendage basal, usually conspicuous.

Table 2.2 Subgeneric classifications of Luzula

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2.1 Introduction

Tabl	e 2.2	Continued	

Buchenau 1906	Kirschner 2002		Morphological characters defining the taxa		
Subgenus	Subgen.	Section	Subgenera	Sections	
N/A (Species not known by Buchenau in 1906.)	<i>Luzula</i> continued	Atlanticae	See above for characteristics of subgenus Luzula	Leaf tips acute. Inflorescence 2 branches rare. Flowers in suglobose clusters. Seed appendage indistinct.	
Pterodes	Pterodes	(No sections)	Perennial. Leaf apex obtuse, sometimes swollen. Inflorescence simple or compound. Seed with large caruncle. Seed appendage is always apical.		

Simpson (1995) used data for all seven genera of Juncaceae, *Prionium*, *Thurnia*, 12 genera of Cyperaceae, and two outgroup genera. The data included characters of the habit, leaves, inflorescences, flowers, pollen, embryos, fruits, seeds, anatomy, parasitism and secondary metabolites. Munro & Linder (1998) also used the data of Simpson (1995) for Juncales, but added embryological data obtained in Munro & Linder (1997). They also reinterpreted a small number of the characters, for example the character states were changed for microsporogenesis, carpel fusion and placentation types. Munro & Linder (1998) found that the results from their morphological analysis differed from those of Simpson (1995), in terms of the placement of the genera *Thurnia* and *Prionium* relative to Juncaceae and Cyperaceae, and also in the monophyly of Juncaceae with respect to Cyperaceae, and believed that the differences were due to the reinterpretation of the characters, as well as the addition of the embryological characters.

An investigation of the morphological characters which have been used to define Commelinales and Commelinidae was carried out by Givnish *et al* (1999), who tested the existing morphology-based classification by using molecular data, and used the results to investigate the evolutionary trends in the characters. The morphological characters were mapped onto a phylogeny resulting from a molecular cladistic analysis. These characters included floral morphology, leaf morphology, anatomy, palynology, embryology, fruit morphology, cotyledon morphology, distribution and habitat. They found that many of the characters had undergone repeated convergences or reversals, and concluded that many of the characters were unreliable guides to the relationships between the families. They found only a relatively small proportion of morphological characters which supported their molecular phylogeny, and of these, many were anatomical characters which they considered to be relatively unaffected by phenotypic plasticity due to environmental pressures.

From these studies it can be seen that although a wide range of morphological characters have been used to study relationships in Cyperales, they have produced conflicting results. The cladistic analyses of Simpson (1995) and Munro & Linder (1998) give significantly different results, which may be due to differences in the interpretation of the characters, or to the inclusion of additional characters by Munro & Linder, or to a combination of these. The study of Givnish *et al* (1999) has shown that many morphological characters are

unreliable taxonomically, as there are many instances of convergence. It is interesting to note that the findings of Givnish *et al* (1999) show that anatomical characters may be more reliable guides to the evolutionary relationships than many of the macromorphological characters.

The anatomy of Juncaceae is dealt with in this thesis, but it was decided that due to the different techniques involved in collecting the data, and the different approach that is taken to presenting the data, it is dealt with here separately, in Chapter 3 *Anatomy*. In addition, the ligular structures are dealt with in Chapter 4 *Auricles or ligules?*, where anatomical work and a literature review are used to determine the homologies of the structures observed.

2.1.2 Aims

- A review of the literature is carried out to obtain descriptions of Juncaceae (Section 2.1.3).
- 2. A study of the macromorphology of Juncaceae specimens is carried out.
- 3. A list of characters which show variation within Juncaceae is created for use in phenetic and cladistic analyses, and character states are determined.
- 4. Aspects of the morphology which are found to differ from descriptions in the literature, and observations which have not previously been made, are described.
- 5. Data is collected (Table A2) for further analysis, and for discussion in terms of character evolution, in Chapter 5 *Phylogeny*.

2.1.3 Description of Juncaceae from the literature

There are seven genera in Juncaceae, and about 440 species, occurring on every continent except Antarctica, with centres of species diversity in temperate regions in Europe, North America, South America, the Far East, Australia and New Zealand (Kirschner 2002a, b). Their centre of generic diversity is, however, in South America, where all genera are found along the line of the Andes and on subantarctic islands. Juncaceae are most common in temperate to subarctic regions; in the tropics they are found only at high altitudes.

Habit

Most Juncaceae are perennial, but *Luzula* subgen. *Marlenia* Ebinger and *Juncus* sects. *Caespitosi* Cout. and *Tenageia* Dumort., and some species of *J*. sect *Ozophyllum* Dumort., are annual.

The genera *Juncus*, *Luzula*, *Marsippospermum* and *Rostkovia* usually have a stem which is differentiated into rhizomes or stolons, and an erect, short-lived culm which bears a terminal inflorescence. They are solitary, caespitose or mat-forming, or rarely procumbent (e.g. *Juncus bufonius* L.). The annual species usually lack a rhizome or stolons. *Distichia*, *Oxychloe* and *Patosia* are cushion-forming, with an undifferentiated ascending stem which branches dichotomously.

Roots

The roots are adventitious along the rhizome in perennial species (Balslev 1996). They are fibrous, or cylindrical from 0.25–2.5 mm in diameter, rarely to 3 mm. The root hairs are usually short-lived, but under some ecological conditions they may persist, forming the 'radices velutinae' of certain species descriptions (Buchenau 1906).

Rhizomes

The rhizome is well-developed in many species of Juncaceae, particularly in many perennial species of *Juncus* and *Luzula*, and also in *Marsippospermum* and *Rostkovia*. In these species it occurs as a long-lived, more-or-less horizontal, underground stem, bearing

scales, roots, and aerial culms. In *Luzula* the rhizome often does not occur underground, but in the litter layer on the ground (Balslev 1996). In the cushion-plant genera, *Distichia*, *Oxychloe* and *Patosia*, the rhizomes are entirely aerial, and do not bear scales, but leaves are inserted at regular intervals. The rhizome eventually dies back in the oldest parts, but continues growth at the tips. Some species of *Juncus* and *Luzula* have a rhizome which is so condensed that it is almost indistinguishable from the culm bases, with culm insertions occurring directly next to one another, and some species may lack a rhizome altogether.

The rhizome varies in diameter, with species growing in wet habitats usually ranging from 2–5 mm in diameter, rarely to 10 mm, but they tend to be thinner in species occurring in drier places and in woodland habitats, for example in many species of *Luzula* (Buchenau 1906). It also shows considerable variation in the internode lengths, with some having conspicuous internodes, while other rhizomes are more condensed, and the internodes may be difficult to distinguish (Balslev 1996). Culms occur at some nodes, and they may be densely arranged or widely spaced. The internode lengths and branching patterns of the rhizome result in variation in the arrangement of the aerial parts of the plant, which may be caespitose in species with very short, densely branching rhizomes, to mat-forming plants in species are stoloniferous, with single leaf rosettes and culms occurring at intervals along a rhizome with very long internodes. Rhizomes may grow entirely horizontally, with branches occurring only in the horizontal plane, or they may have a combination of horizontal and ascending growths. In the cushion-plant genera, *Distichia, Oxychloe* and *Patosia*, the rhizomes are ascending, with no horizontal growth (Kirschner 2002a).

Some species, e.g. *Juncus stipulatus* Nees & Meyen and *J. cyperoides* Laharpe, may have a combination of rhizome growth patterns, with horizontal, stoloniform rhizomes sometimes giving rise to more densely branched rhizomes with shorter internodes, with an ascending growth pattern. It has been suggested that this may be an adaptation to unstable habitats, as these species often occur on sandy river banks (Balslev 1996).

Rhizome scales occur at the nodes, and are persistent or deciduous, and either membraneous or thick and sclereified in texture.

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Culms

The culms are usually erect, rarely procumbent (e.g. *J. bufonius*). They occur above ground and are short-lived, bearing a terminal inflorescence. They may either bear leaves along their length, or in a basal rosette.

The variation in the arrangement of leaves on the culms is of taxonomic importance, as it is usually constant within a species. However, certain species show some variation between individuals, e.g. *J. compressus* Jacq. and *J. squarrosus* L., as small plants may possess no cauline leaves, whereas larger individuals do (Buchenau 1906).

The culms are terete to laterally compressed in cross section. In *Juncus* sect. *Iridifolii* Snogerup & Kirschner the laterally compressed culms have acute edges, and are sometimes winged (Kirschner 2002a, b, c). The culm surface may be smooth or longitudinally striate or ridged, according to the presence or absence of subepidermal sclerenchymatous girders, and the size and shape of the strands (Balslev 1996).

Leaves

The most basal leaves on the culms often have fully developed sheaths but bear no or only rudimentary blades, and are referred to as cataphylls (Buchenau 1875, 1890, 1906). The length of the blade may vary, with the uppermost of these sometimes having more well-developed blades than the lower ones (Kirschner 2002a). The cataphylls may be very small, membranous and inconspicuous, to long, sclerified and pigmented structures which are very conspicuous in some taxa. In some species they are the only leaves present apart from the bracts and scales on the rhizomes and inflorescence (Balslev 1996).

Most species have foliar leaves which are inserted in a basal rosette, scattered along the stem, or both. They are usually spirally arranged, but rarely (*Distichia*) they are distichous. These consist of a leaf sheath and well-developed blade. In *Juncus* sect. *Juncotypus* Dumort. most species have no foliar leaves, but only cataphylls, although a few species in this section do have well-developed blades on some of the sheaths (Balslev 1996; Kirschner 2002c).

The sheath may be either closed (*Luzula*), or open, although the margins may sometimes overlap, completely sheathing the culm. Rarely, the sheath of *Juncus* is closed, but later split (Kirschner 2002a). The margins are sometimes membranous. The length of the sheaths in proportion to the blades varies, both between species and within an individual, according to the position on the plant. In *Luzula* the sheaths may be less than 1 cm on the basal rosette leaves, but up to 7 cm on the cauline leaves (Balslev 1996). In some species, e.g. of *Juncus* sect. *Stygiopsis* Kuntze, the uppermost leaf in the basal rosette may have an elongated sheath which surrounds the stem through part of its length, giving it the appearance of a single cauline leaf, although its point of insertion is within the basal rosette (Buchenau 1906).

The sheaths of many species terminate in two auricles at the junction between the sheath and the blade. These are often membranous, but vary in texture, being thicker in some species. They also vary in their size and shape. The variation is particularly noticeable in *Juncus* sect. *Steirochloa* Griseb., where it can be used to distinguish species (Balslev 1996). Some taxa do not have auricles, e.g. *Luzula* and some species of *Juncus*. Rarely, the auricles may be joined to form a ligule-like structure (Kirschner 2002a).

The leaf blades may be terete, with or without an adaxial groove, to dorsiventrally flattened in shape, or laterally flattened (*Juncus* sect. *Iridifolii*). They are linear to filiform, and the apex may be acute to acuminate, obtuse, or swollen (Kirschner 2002a). In *Luzula* subgen. *Marlenia* the apex extends to one or two hairs.

Leaf indumentum is largely absent in Juncaceae, but *Luzula* has simple multicellular hairs on the leaf margins; rarely, the abaxial leaf surface also bears trichomes (Kirschner 2002a). Some species of *Juncus* and *Luzula*, as well as *Patosia clandestina* (Phil.) Buchenau, have papillae on the leaf margins.

Inflorescences

Juncus and *Luzula* have a terminal inflorescence. Rarely, the lowest inflorescence bract is erect and stem-like, appearing to be a continuation of the stem. The inflorescence is pushed to the side and appears to be lateral, although it is not, and as a result it is often termed 'pseudolateral' (Balslev 1996; Kirschner 2002a, c).

The inflorescence is usually many-flowered in *Juncus* and *Luzula*; although it is rarely reduced to a few or a single flower in Juncus, no species are consistently single-flowered. In all other genera the inflorescence always bears a single flower, which is terminal in *Rostkovia* and *Marsippospermum*, and axillary in *Distichia*, *Oxychloe* and *Patosia*.

There is much variation in the arrangement of the inflorescence of *Juncus* and *Luzula*, particularly in *Luzula*, where the sectional classification is largely based upon inflorescence structure. It is either cymose or racemose, and panicle-like (the proximal branches do not extend beyond the distal branches) or anthelate (the proximal branches extend beyond the distal ones) (Buchenau 1865). It may be erect or nodding, and unbranched to frequently branched. The flowers may be borne singly, or congested into more or less dense clusters. The number of flowers in the clusters may also be used to distinguish species (Kirschner 2002a).

Variation also occurs in the appearance and arrangement of inflorescence bracts. The lowest inflorescence bract is often similar to foliar leaves in appearance, although it is usually smaller than the foliar leaves and the proportion of the bract formed by the sheath may also differ in comparison to the foliar leaves. The proportion of sheath also differs between some species, e.g. in *Juncus* sect. *Ozophyllum* the bracts tend to have reduced blades relative to the sheaths, whereas in sects. *Steirochloa* and *Juncotypus* the sheath is reduced. The lowest inflorescence bracts of some species of *Juncus* are erect and culm-like in appearance, with a very reduced sheath and cylindrical blade, and causing the terminal inflorescence to appear to be lateral. The total length of the lowest inflorescence bract compared to the inflorescence itself is also variable, and may be used to distinguish some species of *Juncus* and *Luzula*, as in some species it may be longer than the inflorescence, and in others shorter.

The inflorescence bracts show a tendency to become smaller and increasingly membranous at more distal nodes. There is variation between species in the texture of the bracts, with some species having largely membranous inflorescence bracts, and others with most bracts being leafy, while others show a gradual transition throughout the inflorescence. Their outline varies from linear to lanceolate, to semi-circular, and those of *Luzula* often have fimbriate margins (Balslev 1996).

In many species, small, membraneous bracts are present which are inserted on the pedicels and subtend the flowers. They do not support branches in their axils. They are termed 'prophylls' by Buchenau (1906) and 'bracteoles' by Balslev (1996) and Kirschner (2002a, b, c), and are referred to here as bracteoles. They may be absent, or one to four are present. Their presence or absence is of taxonomic importance in *Juncus*, as most authors have divided *Juncus* into two groups, usually at subgenus level, according to the presence or absence of bracteoles. Buchenau (1890, 1906) did so and called the groups 'prophyllati' and 'eprophyllati', but did not assign ranks. Kirschner (2002b, c) classifies those with bracteoles as subgenus *Agathryon* Raf., and those which lack them as subgenus *Juncus*. In *Juncus* they are directly associated with the form of the inflorescence, as those species with bracteoles have cymose inflorescences, and those which lack them have racemose inflorescences.

Flowers

The flowers of Juncaceae are usually small, from 2–8 mm long, although *Marsippospermum grandiflorum* (L.f.) Hook. has flowers to 40 mm long (Kirschner 2002a). They are actinomorphic, hypogynous, and usually hermaphrodite, but the genera *Distichia*, *Oxychloe* and *Patosia* are usually dioecious.

The perianth segments are arranged in two whorls of three. They may be more or less equal, or one of the whorls may be longer than the other. They are lanceolate, obtuse, acute or acuminate in shape, and vary in texture from membranous to non-membraneous. They often have membraneous margins, which may widen towards the top of the tepal to form two rounded flaps which are bent towards the centre of the flower (Balslev 1996).

The stamens are usually in two whorls of three, but in some species the inner whorl may be absent. The filaments are free, and filiform or flattened. The anthers are oblong to linear with lateral dehiscence. In some species the apical connective has a projection (Kirschner 2002a). The relative lengths of filament and anther show variation between taxa, and are usually constant within a species (Balslev 1996). The stamens may be shorter than the perianth, the same length, or exserted.

The superior ovary consists of three united carpels, and is 1-locular or 3-septate to 3locular. It is usually sessile, but in *Distichia* it is on a gynophore which lifts the capsule above the leaves on ripening. The single style branches into three stigmas which are papillose on their adaxial surface (Kirschner 2002a). Placentation is basal in *Luzula*, with a 1-locular ovary containing three ovules. In the other genera the placentation is parietal, on intruding septae or axile, and with many ovules per ovary. In *Juncus roemerianus* Scheele the placentae are swollen and spongy (Balslev 1996).

Fruit

The fruit of Juncaceae is usually a dry capsule which is globular to longer than wide, usually ranging from 1.5–5 mm long. The apex may be truncate, obtuse, acute or acuminate, and sometimes with a prominent beak. Dehiscence is usually loculicidal. In *Distichia, Oxychloe* and *Patosia* the mechanism of dehiscence is not well defined, but sometimes it is circumscissile (Balslev 1996). The capsules of some species, e.g. *Juncus densiflorus* Kunth, do not separate at the beak but dehisce by slits below the beak.

The lumen of the fruit is usually 1-locular, 3-septate or 3-locular, corresponding to the type of placentation in the ovary, but in *Juncus repens* Michx. and sometimes in *J. uruguensis* Griseb. the septae which are present in the ovary are lost, so the placentation of the fruit becomes free central (Balslev 1996).

Seeds

The seeds are ovoid, obovoid, ellipsoid, oblong or fusiform in shape (Balslev 1996). Their cross section may vary in shape, as they may be terete, or angular when many seeds are crowded in the capsule.

The embryo is small, and the largest part of the seed is occupied by a starchy endosperm. The embryo is more or less cylindrical or conical, and most of its volume consists of the cotyledon (Buchenau 1906).

The seed coat is derived from two integuments, but differs between taxa. In *Luzula* the inner seed coat is derived from the inner integument, and the outer integument forms a more or less shriveled outer seed coat at maturity, tightly surrounding the inner seed coat

(Buchenau 1890). In some species of *Juncus* the outer seed coat is also derived entirely from the outer integument, and forms a conspicuous, loose sheath surrounding the hard inner seed coat, which is derived from the inner integument. However, in most species of *Juncus* the derivation of the seed coat differs from that of *Luzula*. A hard seed coat forms from the fusion of the adjacent outer and inner integument cell walls. The outer seed coat forms forms from the outer cell layer of the outer integument (Buchenau 1890). This consists of thin-walled cells which, when moistened, swell to form a mucilaginous layer covering the seed, which may be an adaptation to help with seed dispersal (Salisbury 1974, Balslev 1996).

The outer seed coat of some species of *Juncus* and *Luzula*, and also of *Marsippospermum*, is sometimes extended to form apical or basal appendages. The inner seed coat is frequently textured, with variation in the pattern of the sculpturing, which has been studied by a number of authors including Balslev (1996), Brooks & Kuhn (1986), Buchenau (1868), Engelmann (1866, 1868) and Satake (1933). Buchenau described the sculpturing as costate, reticulate and transversely reticulate, while Balslev provides individual descriptions of the species studied, not attempting to divide the highly variable patterns into categories. The other authors looked at a smaller number of species, but found similar variation. The seeds appear to show variation in the regularity of the sculpturing and the degree to which they are sculptured, with some showing very little, and others with very distinct sculpturing. There may be longitudinal or transverse striations, or a combination, or a reticulated pattern which may be very distinct or faint.

Karyology

Diffuse centromeres

An unusual type of centromeric activity of the chromosomes of *Luzula elegans* Lowe was first noted by Malheiros & Castro (1947). They observed chromosomes in anaphase which formed U-shapes, with the ends towards the poles, which differs from the more usual v-shaped anaphase chromosomes with the ends away from the poles. They also noted that the chromosomes lacked obvious constrictions. These centromeres are referred to as diffuse or polycentric (Balslev 1996; Kirschner 2002a; Savile 1990) because the chromosomes lack a single, distinct centromere. Diffuse centromeres are known to occur

in *Luzula* and Cyperaceae, but the state for *Juncus* is unknown (Balslev 1996; Savile 1990).

Chromosome numbers

The chromosome numbers of Juncaceae show considerable polyploidy and aneuploidy. The presence of diffuse centromeres may allow a higher degree of chromosome fragmentation to occur than is usual in most organisms, as they may allow broken chromosomes to remain functional (Balslev 1996).

Chromosome numbers published for *Juncus* show that approximately two-thirds of the species are 2n = 40, and approximately one-third 2n = 80, but a small number of species have had numbers published which range from 2n = 18 to 2n = 130 (Balslev 1996). Although a number of different basic chromosome numbers have been suggested in the literature (Darlington & Wylie 1956; Harriman & Redmond 1976; Löve & Löve 1944; Snogerup 1963), based upon a review of the published chromosome numbers, Balslev (1996) suggests a basic chromosome number of x = 10 for *Juncus*.

The chromosome numbers for *Luzula* range from 2n = 6 in *Luzula elegans*, to 2n = 12 in the majority of *L*. subgen. *Luzula*, and multiples of 12 in the remaining species. Balslev (1996) considers that the low chromosome number of *L*. *elegans* is derived, and suggests a basic chromosome number of x = 6 for *Luzula*.

Oxychloe andina Phil. has 2n = 16 (Sasaki 1937). The chromosome numbers of other Juncaceae genera are not known.

Embryology

The anthers are tetrasporangiate, with a one-layered epidermis, endothecium and middle layer, and a glandular-secretory tapetum, which is uninucleate, and usually one-layered (Munro & Linder 1997), but in *Oxychloe* it has been recorded as two layered (Schnarf 1929; Johri *et al* 1992). Microsporogenesis is simultaneous, and the pollen is united in tetrads (Fritzsche 1832, 1833), which tend to be closely appressed so that they are somewhat rounded in outline. The arrangement of the grains is usually tetrahedral, but

other arrangements may also occur, such as rhomboidal or tetragonal (Buchenau 1890, 1906; Selling 1947).

The tetrads range in size between $30-70 \ \mu m$ (Balslev 1996; Table 2.3), with *Juncus* and *Luzula* tending to have the smallest tetrads. However, there is little further variation in the appearance of the pollen, and it is not considered to be taxonomically useful below family level (Balslev 1996).

Table 2.3 Pollen tetrad size of Juncaceae

Data taken from Balslev (1996).

Genus	Range of sizes (µm)
Juncus	36 - 50
Luzula	30 - 50
Marsippospermum	58 - 65
Oxychloe	46 - 55
Patosia	50 - 58
Rostkovia	30 - 70

2.2 Materials and methods

The morphological results are combined with the molecular results by mapping the characters onto the phylogenetic hypothesis obtained in Chapter 5 *Phylogeny*, in order to identify characters which support the phylogenetic hypothesis and to study character evolution within Juncaceae. In addition to this, cladistic analyses are carried out on the morphological data. A comparison of the groupings identified using the morphological analyses with those from the molecular analyses is used to assess the taxonomic and phylogenetic utility of the morphological data. The data collected must, therefore, serve several purposes. A cladistic study can only include discrete data, so wherever possible, the data is collected as categorical characters. However, measurements such as lengths are initially recorded as continuous data.

The choice of species to study was based upon current and previous classifications of Juncaceae (Buchenau 1906; Balslev 1996; Kirschner 2002a, b, c). The morphological part of this study is concerned with intergeneric relationships within Juncaceae (see Section 1.5), and the choice of species to study must represent the variation seen between the genera, subgenera and sections, in order to determine the relationships between genera, as well as the limits of the genera.

It was considered that at least three species within each section of *Juncus* and *Luzula* should be included wherever possible, and as many species as could be obtained for the remaining genera, which contain a small number of species. *Prionium* and *Thurnia* are used as outgroups, as they have previously been determined to be closely related to Juncaceae (Chase *et al* 1993, 1995, 2000; Givnish *et al* 1999; Muasya *et al* 1998; Munro & Linder 1998; Plunkett *et al* 1995; Simpson 1995). Herbarium specimens representing most genera and sections were obtained on loan from a number of herbaria. It proved difficult to locate a number of species. Several sections of *Juncus* and *Luzula* were poorly represented, for example *Juncus* sect. *Stygiopsis*, many species of which are of very small stature and occur at high altitudes or in subarctic regions. Where possible this was remedied by collecting trips to Scotland, Ireland, Guyana and Ecuador. Table A1 lists voucher specimens.

2.2 Materials and methods

The choice of morphological features to be studied was based in part by studying the literature. Characters which have been used to define taxa in classifications of Juncaceae were included, and descriptions of the morphology of Juncaceae were studied to identify other characters which showed variation. An initial study of specimens was also carried out to identify any further characters which might be useful.

Specimens were examined using non-destructive techniques where possible, using a hand lens or binocular microscope. However, for examination of the flowers and fruits it was necessary to remove material and rehydrate it by boiling for three to five minutes, to restore it to its approximate original dimensions and soften it to allow dissection.

2.3 Results

Based upon descriptions from the literature, reviewed in section 2.1.3 *Description of Juncaceae from the literature*, and upon a study of herbarium specimens, characters which showed variation between taxa were assessed for their potential use in the taxonomy of Juncaceae. The character list is given below, followed by a discussion of characters which were not included for analysis.

The data are given in Table A2, with characters numbered according to the list below. All measurements are in millimeters, and given as the average of at least three measurements per specimen examined wherever possible (see Table A1 for voucher specimens). 'Absence' or 'presence' characters are recorded as '0' or '1', respectively. As the dioecious species show differences in the forms of male and female flowers, inflorescence data is recorded separately for male and female plants.

It was not considered necessary to produce individual descriptions of the species, as detailed descriptions already exist in the literature; instead, where the morphology is found to differ from the literature, the characters involved are discussed (Section 2.4 *Discussion*). Some of these characters have been used previously in cladistic analyses or mapped onto phylogenies, and where this is the case references are given in Section 2.4 *Discussion*. Where the character states differ from those used previously, an explanation is given.

Character list

Habit

1. Habit

Habit is recorded as Non-rhizomatous '0'; Rhizomatous '1'; Cushion-forming '2'. Nonrhizomatous plants have a cluster of culms, but no rhizome can be identified. Rhizomatous plants have culms which develop from rhizomes which occur below ground or among the leaf-litter. Cushion-forming plants have a single type of erect, branching stem (Fig. 1.1).

Roots

2. Root diameter

Leaves

3. Leaf insertion distichous present/absent

4. Leaf sheaths closed or open

Closed leaf sheaths are scored as '0'; open sheaths as '1'.

5. Rosette of leaves present/absent

Leaves may be present in a rosette, with no length of culm visible between points of insertion of the leaves. This is always basal in Juncaceae (but in *Prionium* it is on an aerial stem). Cauline leaves may also be present, but these are inserted singly along an aerial stem. In some species there may be only a small number of leaves present; however, if there is more than one with a basal point of attachment a basal rosette is scored as present. If only a single basal leaf is present then this is considered indistinguishable from the cauline leaves and a rosette is scored as absent.

6. Cauline leaves present/absent

Cauline leaves are those which are inserted singly along the culm. A length of culm must be distingushable between the points of insertion of each leaf.

7. Leaves length

The total length of the leaves, including the sheaths, was recorded. If both basal and cauline leaves were present, both were measured.

8. Relative length of leaf blade to leaf sheath

The leaf blade length was divided by the sheath length. In some species (*Juncus* sect. *Graminifolii*) it was not always possible to identify the junction between the sheath and the blade, so data for this character are missing for some species.

9. Leaf tip

The shape of the leaf tip was recorded as acute/acuminate (0), obtuse (1), callose (2). Most Juncaceae have leaf tips which are acute to acuminate or obtuse, but some species of *Luzula* have thickened to slightly swollen leaf tips, described as callose.

10. Multicellular hairs on leaves present/absent

11. Papillae on leaves present/absent

Inflorescences

12. Plant height

13. Inflorescence length relative to plant height

This measurement is the inflorescence length divided by the plant height. The inflorescence length is measured from the point of insertion of the lowest inflorescence bract to the tip of the uppermost flower.

14. Pseudolateral inflorescence present/absent

The lowest inflorescence bract of some species is erect, continuing the line of the culm, and pushing the inflorescence to one side. It is also often culm-like in appearance, as it has a minute sheath, and the blade is unifacial and circular in cross-section. In these species (*Juncus* sect. *Juncotypus*) the inflorescence appears to be lateral, although it is actually terminal. It is usually termed 'pseudolateral'. In other species the bract is erect, but although it continues the line of the culm it is leafy in appearance. The leafy appearance may be due to a conspicuous sheath, or to a blade that is not circular in cross-section. 'Pseudolateral' in this sense is a combination of characters, so for the purposes of this study, 'pseudolateral' is used to refer to an inflorescence in which the lowest bract continues the line of the culm and pushes the inflorescence aside, regardless of whether it appears leafy or culm-like (Fig. 2.1 B, C). The relative proportions of the sheath and blade of the inflorescence bract are dealt with as a separate character.



Figure 2.1 Inflorescences. A *Juncus ecuadoriensis* racemose inflorescence. **B** *Juncus effusus* pseudolateral inflorescence. **C** *Rostkovia magellanica* fruit developed from pseudolateral, single-flowered inflorescence. **D** *Distichia muscoides* Fruit developed from a single flower. The leaves below the fruit have been removed. A, B, C taken from Balslev (1996); originally published in Harling, G. & Sparre, B. (eds.) (1979), Flora of Ecuador **11**: 1–45.

15. Inflorescence racemose or cymose

A racemose inflorescence is recorded as '0'; cymose as '1'.

16. Lowest inflorescence bract length relative to inflorescence

The relative length of the inflorescence bract to the inflorescence is calculated by dividing the bract length by the inflorescence length.

17. Lowest inflorescence bract relative proportion of blade to sheath

The relative proportions of the blade and sheath of the lowest inflorescence bract are calculated by dividing the blade length by the sheath length. In some species with flat leaves (*Juncus* sect. *Graminifolii*) it was not always possible to distinguish the sheath from the blade, so this character was not recorded for some species.

18. Number of flowers

The number of flowers per inflorescence was recorded as '0' = Many; '1' = Several (2 to 10 flowers); '2' = One flower. Individuals of the same species usually bear a variable number of flowers, and of those recorded as 'several', an occasional specimen would have a single flower per inflorescence, and more rarely, a specimen may be found with more than 10 flowers, but most inflorescences bear 2–10 flowers. Im contrase, specimens recorded as being single-flowered always bear a single flower.

19. Bracteoles present/absent

Bracteoles are small, membraneous bracts on the inflorescence which do not bear branches in their axils, and are inserted on the pedicels of each flower.

Flowers

20. Sex

Species are recorded as hermaphrodite (0) or dioecious (1).

21. Perianth length

An average value of the length of the perianth of one flower is obtained by measuring the outer segments. For species in which the inner perianth is longer, the inner perianth segments are measured instead.

22. Outer perianth length / inner perianth length

23. Outer perianth length / width

A measurement of the shape of the outer whorl of the perianth segments is obtained by dividing the length of each segment by its width.

24. Inner perianth length / width

A measurement of the shape of inner whorl of the perianth segments is obtained by dividing the length of each segment by its width.

25. Stamen length / perianth length

An average value of the anther length is obtained after taking measurements from at least three anthers from a single mature flower. A measurement of the degree to which the stamens are exserted from the flower is obtained by dividing by the perianth length.

26. Filaments flattened or filiform

Filaments may be filiform (0), with a narrow, more or less even diameter along the length. Flattened filaments (1) are conspicuously broadened laterally, and broader at the base than the apex.

27. Anther / filament length

The relative lengths of anthers to filaments are calculated by dividing the anther length by the filament length.

28. Anthers apical connective tip present/absent

29. Gynoecium length / perianth length

The degree to which the stigmas are exserted from the flower was calculated by dividing the total length from the base of the ovary to the tip of the stylodial branches by the perianth length.

30. Stigma length / style length

A measurement of the relative proportions of the length of the style and the length of the three branches forming the stigmas is obtained by dividing the length of the longest of the stigma branches by the style length.

31. Placentation type

Placentation is recorded as parietal (0), on intruding septae or axile (1), or basal (2). There is continuous variation between parietal, on intruding septae and axile placentation. With parietal placentation the ovules are attached to three longitudinal ridges on the outer wall of the ovary. With axile placentation, the ridges are extended to meet in the centre (but are not usually fused), dividing the ovary into three locules. There is variation in the degree to which the septae are extended, and in some species they do not meet in the centre. However, the small size and soft texture of the ovaries makes it difficult to take accurate measurements of the degree to which the septae intrude into the ovary, so this is recorded as a categorical rather than continuous character, and the two types are not distinguished.

Fruit

32. Capsule length

The measurement of the length of the capsule excludes any apical mucro.

33. Capsule exsertion

The degree to which the capsules are exserted from the flower was calculated by dividing the capsule length by the perianth length.

34. Capsule shape

A measurement of the capsule shape is obtained by dividing the length by the width.

35. Capsule apex shape

The capsule apex (excluding any apical mucro) is described as obtuse (0) or acute (1).

36. Capsule mucro length relative to capsule length

Many species have an apical mucro on the capsule, which is formed by the base of the style after the style has broken off. The mucro length is divided by the capsule length. In Table A2 the values given are $x \ 10^2$.

Seeds

37. Seed length

The length of the seed excludes any appendages, and also the outer seed coat when it forms a loose or spongy covering.

38. Seed shape

A measurement of the seed shape is obtained by dividing the length by the width.

39. Seed apical appendages present/absent

Apical and basal appendages of the seed are formed by extensions of the outer seed coat.

40. Seed basal appendages present/absent

Characters not included

A number of characters which showed potentially taxonomically informative variation were not used in the character list above. These are discussed below.

Rhizomes

Rhizome diameter

The diameter of the rhizomes could only be measured easily in specimens with long rhizome internodes. These formed a small proportion of the species which were examined. Many species do not possess rhizomes, and of those which do, in some cases the dense arrangement of the leaves, scales and culms obscured part or all of the rhizome. Sometimes only a small piece of rhizome was present, so to strip it of the leaves and scales would have caused excessive damage to the herbarium specimen.

Rhizome scales

As with the measurement of the rhizome diameter, in the majority of specimens it was difficult to measure the dimensions of the rhizome scales, especially when the rhizome internodes were very short. In addition, in some species with longer internodes the scales were in poor condition, often being split longitudinally, forming a fibrous layer covering the rhizome, so accurate measurements of the scales could not be made from the specimen.

Length between culm insertions on the rhizome

The lengths of rhizome between culm insertions show some variation between species, but there is also a very high degree of variation within species, even within a single specimen. Some specimens examined here showed a degree of variation far greater than the degree of variation between species, with some parts of the rhizome so short that the culms were directly adjacent to one another, and other parts with several centimetres between culms. A very large number of measurements would need to be taken to reflect differences between species rather than sampling error, and insufficient rhizome material was available to do this.

Culms

Culms diameter and cross-sectional shape

Some variation is known to occur in the cross-sectional shape of the culms, as the culms of some species are flattened. Measurement of the herbarium specimens showed that in many species the culms were flattened to some degree, and that this was not likely to be due to the pressing of the plants, as they were sometimes flattened in a plane perpendicular to the plane in which they were mounted. Measurement of the widest and narrowest diameters of the mid-point of the culm may show variation between taxa, but these measurements were difficult to take from herbarium specimens. The culms sometimes looked shrivelled, indicating that the measurements would not reflect those of living specimens, and frequently the culms were glued down, making it difficult to measure them accurately, or to remove a section for rehydration.

Leaves

Leaves width

The width of the leaves could be more accurately measured from the cross-sections taken for the anatomical work than directly from the herbarium specimens, so this measurement is included in that chapter.

Presence and dimensions of cataphylls

Among species examined here, cataphylls were found to be present in all of the species in which it was possible to distinguish the sheath from the blade. In other species, particularly in *Juncus* sect. *Graminifolii*, it was difficult to distinguish the sheath from the blade, and it was not possible to distinguish cataphylls from small foliar leaves. Because no variation was observed in the presence or absence of cataphylls it was not used. There was considerable variation in the size of the cataphylls, both within a single specimen and between species. However, as these are the most basal leaves on a plant, very frequently the outermost, or all, cataphylls were damaged in the herbarium specimens, or had died back while the shoot was still growing. It was considered too difficult to collect sufficient

data to carry out comparisons between the species, either on the number of cataphylls present or on their relative lengths.

Inflorescences

Inflorescence bracteoles dimensions

The bracteoles are very small and usually membranous, so they are delicate. On herbarium specimens they are often damaged, and an attempt to remove them to measure them may cause further damage. Accurate measurements of many species could not be obtained, and measurements of the size of the bracteoles were not included in the final list.

Flowers

Perianth tip shape

The perianth segment tips show continuous variation in shape, from acute to acuminate, sometimes with a mucro of varying length, and this is known to be taxonomically significant at species level (Kirschner 2002a, b, c).

The techniques used here (examination under a binocular microscope) obtained a precision of 0.1 mm in the measurements. However, the maximum variation in the tip shape may occur within approximately 10–20% of the total length, and as some perianth segments are as small as 2 mm long, this would require a precision of less than 0.1 mm. In addition, the perianth segments were difficult to handle, as they were often strongly keeled and could not be made to lie entirely flat without breaking them. Measurements of the variation in the shape of the tip were considered too difficult to obtain in this study.

Fruit

Dehiscence

Although some variation is seen in the capsule dehiscence mechanisms in Juncaceae, all the species included in this study that had capsules which had dehisced had done so with the same mechanism (loculicidal), so this character was not included in the character list for analysis. The capsules of *Oxychloe*, *Distichia* and *Patosia* have been described as having various mechanisms of dehiscence (Kirschner 2002a) but in the herbarium specimens observed here they proved difficult to study, as it was difficult to determine maturity. None were observed to have dehisced that could also be identified with confidence as fully mature, and not split due to mechanical damage during the preparation of the herbarium specimen.

Seeds

Seed coat sculpturing

The seed coat shows a high degree of variability in the degree of sculpturing and the pattern of the sculpturing; this variation is illustrated by Balslev (1996) and Brooks & Kuhn (1986). The variation appears to be continuous, but it would be difficult to take measurements which would reflect the variation seen, or to divide into distinct categories for recording. It was decided that although such variation may be of taxonomic use, it would not be used in this study, as the time involved in collecting the data relative to the number of characters obtained for analysis could not be justified.

Karyology

Chromosome numbers

The chromosome numbers show considerable variation, and they may be very informative about the evolutionary patterns within Juncaceae. They have not been included here because the published chromosome numbers cover only 40% of the species in this study. In addition, Balslev (1996) points out that many of these published numbers do not have voucher specimens, and as the identification of species is often difficult, the data may not be reliable. The time involved in obtaining the data in the lab would be too great for this study.

2.4 Discussion

A comparison of the characters described here is made with the descriptions in the literature, and also with their use in previous statistical analyses.

Habit

Characters relating to the habit, such as whether the plants are solitary, caespitose, or form loose or dense mats or cushions, are frequently used to distinguish species throughout the family (e.g. Balslev 1996; Kirschner 2002a, b, c), but have not been used previously in cladistic analyses. Armstrong (1997) did, however, use the categories 'Loosely tufted/rosette', 'Densely tufted' and 'Loose-densely tufted' in a phenetic analysis.

Previous descriptions of the habit of Juncaceae (e.g. Balslev 1996) have used a larger number of categories than are used here, distinguishing between rhizomatous plants on the basis of the frequency of branching and distance between culm insertion. These traits are considered here to be separate characters. Distance between culms is discussed in 'Characters not included'. The character states used for 'Habit' here represent differences in the points at which branches occur, as these differences determine whether the plants are solitary, caespitose, spreading or cushion-forming. Differences between the branching patterns are discussed in more detail in Section 5.4 *Character distribution and evolution*.

Roots

There is little variation in the roots of Juncaceae, and characters relating to the roots have not been used previously in cladistic or phenetic analyses, or to distinguish taxa. The measurements of the root diameter were included in order to investigate whether the variation is taxonomically significant.

Leaves

Juncus section *Juncotypus* is usually described as leafless (Balslev 1996; Kirschner 2002c). The leaf blades of these species are usually reduced to a rudimentary point on a large and well-developed sheath, which are referred to as cataphylls, but rarely the blade is

well-developed (Balslev 1996; Kirschner 2002c). Sheath and blade characters are recorded seperately here, and it was considered appropriate to treat the cataphylls of section *Juncotypus* as homologous to the sheaths of the leaves in other species. Therefore, a basal rosette of leaves is recorded as present in these taxa, and a separate character, the measurements of the relative sizes of the blade and sheath, indicate that these are cataphylls and not foliar leaves.

The presence of distichous leaves is used taxonomically to distinguish the genus *Distichia* within Juncaceae. Simpson (1995) scored plants on the basis of tristichous or distichous leaf insertion for cladistic analysis. Within Juncaceae, *Distichia, Oxychloe* and *Patosia* are scored as having distichous leaves, and the remaining genera as tristichous, following Dahlgren *et al* (1985). Munro & Linder (1998) added 'spiral' to the list of character states, 'to include taxa outside Juncales'. However, this is incorrect, as *Oxychloe* and *Patosia* do not have distichous leaves, and in addition, the phyllotaxis of Juncaceae is usually described as spiral rather than tristichous (Balslev 1996; Kirschner 2002a), so this distinction may not be correct. This character has been modified here to read 'distichous leaf insertion present/absent'

The presence or absence of multicellular hairs, and the presence or absence of closed leaf sheaths are taxonomically significant, as their presence distinguishes *Luzula* from the rest of Juncaceae. They were included in cladistic analyses by Simpson (1995) & Munro & Linder (1998), and the characters are used here without modification.

The presence or absence of papillae has not been used previously in cladistic or phenetic analyses, but they are present in some species of Juncaceae, particularly in *Luzula*, where they are used to distinguish certain species (Kirschner 2002a). Similarly, the shape of the leaf tip and the dimensions of the leaf have been used to distinguish species, and in some cases subgenera of *Luzula* (Buchenau 1906; Kirschner 2002a). They have not previously been used in cladistic analyses, but leaf tip shape and measurements of leaf width were used in phenetic analysis of closely related *Luzula* species by Armstrong (1997).

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Inflorescences

Of the inflorescence characters, only the number of flowers per inflorescence has been used in cladistic analyses previously, by Simpson (1995) and Munro & Linder (1998). They scored the number of flowers per inflorescence as solitary or more than one. An additional state has been added here: 'Several', to distinguish some species of *Juncus* which commonly have a very small number of flowers, varying from one to less than 10. Many of the other inflorescence characters recorded here have been used to distinguish taxa within Juncaceae (see Table 2.1), but they have not previously been included in cladistic analyses. These include the presence or absence of bracteoles subtending the flowers, and the relative lengths of the lowest inflorescence bract and inflorescence bract. Variation in the branching patterns and number of flowers were used in phenetic analysis by Armstrong (1997). Variation in the relative proportions of the lowest inflorescence bract has not been used taxonomically or in any analyses.

Flowers

A number of characters relating to the flowers have been used in cladistic analyses by Simpson (1995) and Munro & Linder (1998). Flower sex has been scored previously as bisexual or unisexual. The character states differ here only for the purposes of disinguishing male and female specimens while recording the data, as the flowers are dimorphic in some species. Usually, data from several specimens was combined to produce average values for a species, but data for male and female flowers of species with dimorphic flowers were not combined.

Perianth length has been used previously as a categorical character (Munro & Linder 1998; Simpson 1995), and scored as 0–7 mm long, or more than 7 mm long. It has been recorded here as a continuous character. A histogram of the character (Fig. A1, character 21) shows that the measurements do not indicate any points at which they could be split easily into categories, as the variation is continuous, but with the exception of the very large perianth segments of *Marsippospermum grandiflorum*. Instead the character is retained as continuous.

The presence or absence of a prominent apical connective projection on the anthers was used by Simpson (1995) and Munro & Linder (1998) for cladistic analyses. They scored it as present in *Marsippospermum, Oxychloe, Patosia* and *Rostkovia*, and absent in *Distichia, Juncus* and *Luzula*. The results here are slightly different. Both species of *Distichia* examined here possess apical connective projections, as does *Juncus trifidus* L... *Marsippospermum* is variable in this character (Kirschner 2002a), and the species investigated here does not have an apical connective projection, so it was scored as absent. An additional character, that of the relative lengths of the anthers and filaments, is known to be taxonomically significant (Kirschner 2002b, c), but has not been included in analyses. Other characters are rarely referred to in the literature, only occasionally being mentioned in species descriptions (Kirschner 2002a, b, c), but not generally treated as useful diagnostic characters. The degree to which the stamens are exserted, and the presence of filamentous or flattened filaments are included here to investigate whether they are taxonomically significant.

Variation in the gynoecium of Juncaceae is confined to the length of the style, the number of locules in the ovary and the placentation type, and all are of some taxonomic use (Balslev 1996; Buchenau 1906; Kirschner 2002a, b, c). Simpson (1995) and Munro & Linder (1998) scored for a multilocular or unilocular ovary. Placentation type was scored as various or basal by Simpson, and Munro & Linder changed this to apical, axile or basal. Apical placentation does not occur in Juncaceae, so it is not used here. The number of locules is not scored for at all, since it is usually linked to the placentation type; it is unilocular when the placentation is parietal, and trilocular when the placentation is basal, it is always unilocular. In this case it would be misleading to score for the number of locules, since the characters are linked; also, is not clear whether the unilocular ovaries of *Luzula*, with basal placentation, are homologous to the unilocular ovaries of *Juncus*, which are parietal or have intruding septae.

A further character which was used by Simpson (1995) is that of the fusion of the carpels, which is scored as solitary, fused in ovary region only, or fused with a single style. Munro & Linder (1998) also used this character, but recorded those with a solitary carpel as 'unknown'. Juncaceae was always scored as 'fused with a single style' by Simpson and Munro and Linder, and *Prionium*, used as an outgroup in this study, as 'fused in ovary

region only'. Juncaceae is not variable for these characters as it never has a solitary carpel, and always has a style, so they were not used here. Observations here also indicated the presence of a minute fused style in *Prionium*, somewhat similar in proportions to that of *Juncus capillaceus* Lam.. The character has been recorded here as a continuous one, as style length/stigma length.

Fruit

Simpson (1995) included data for fruit dehiscence in cladistic analysis, but it has been omitted here (see 'Characters not included'). Following the descriptions in Dahlgren *et al* (1985), Simpson scored most Juncaceae as having dehiscent fruit, and *Oxychloe* as polymorphic. However, the dehiscence or indehiscence of the fruit of *Distichia*, *Oxychloe* and *Patosia* is confusing and difficult to observe. It has been described as 'indehiscent(?)' by Buchenau (1906) for *Oxychloe andina*, and not described at all for *Distichia* or *Patosia*. Balslev (1996) describes the state as poorly defined and either indehiscent or dehiscent, and Kirschner (2002a) as dehiscent, and loculicidal, circumscissile or irregular. The fruit of the herbarium specimens observed here usually had not dehisced, but a very small number had split irregularly, and it was not possible to determine whether they had dehisced, or suffered mechanical damage.

Characters relating to the capsule shape and dimensions are used taxonomically, usually to distinguish closely related species (Balslev 1996; Buchenau 1906; Kirschner 2002a, b, c), and capsule valve length was included in phenetic analysis by Armstrong (1997). Otherwise they have not been included in analyses.

Seeds

The seeds of Juncaceae may have basal or apical appendages, and these are of differing forms. They are often referred to in the taxonomic literature and sometimes used to define subgenera or sections (Balslev 1996; Buchenau 1906; Kirschner 2002a, b, c). The presence or absence of an elaiosome has been used in cladistic analysis by Simpson (1995), who scored it as present in *Luzula* but otherwise absent from Juncaceae. Other seed appendages, which are sometimes present in the form of loose extensions of the outer seed coat, have not been used in analyses. Seed dimensions have not been used in cladistic

analyses. They are usually only referred to to distinguish closely related species (Kirschner 2002a, b, c), and they have been used in phenetic analyses of closely related species by Armstrong (1997). Seed dimensions are taxonomically significant, as they are larger in *Luzula* than in other Juncaceae, although this may be related to the number of seeds per carpel (one in *Luzula* and many in other Juncaceae).
2.5 Conclusions

The main aim of this chapter was to collect macromorphological data for cladistic analysis and for a discussion of character evolution within Juncaceae in Chapter 5 *Phylogeny*. 40 characters have been identified which are considered potentially taxonomically informative, and the data is presented in Table A2.

Many of these characters have been recognised previously as being potentially taxonomically informative within Juncaceae, and used in previous classifications (e.g. Buchenau 1906; Kirschner 2002a, b, c), but at the time of carrying out this investigation, they had not previously been used in cladistic or phenetic analyses; for example, the form and proportions of the lowest inflorescence bract have been noted by Balslev (1996) as variable, but a detailed investigation of this variation has not been carried out previously.

Chapter 3 Anatomy

3.1 Introduction

3.1.1 The use of anatomy in the taxonomy of Juncaceae

When characters are identified for use in the taxonomy of a group of organisms, emphasis tends to be put on characters which can be identified relatively quickly and easily, with the naked eye or with a hand-lens, and without the use of expensive or specialised equipment. However, there are situations where anatomy may be of use in addition to the macromorphology. The macromorphological organs of Juncaceae are considerably reduced, and a limited number of external morphological characters may be found which show taxonomically useful variation, whereas the anatomical variation is large.

Extensive use has been made of the anatomy of Juncaceae in taxonomic studies. Buchenau (1906) made detailed anatomical observations in his monograph of the family, and he gave much importance to some of these characters in his taxonomic treatment of the family. This approach has continued among all of the major taxonomic studies in Juncaceae since this date, with the range of anatomical characters used by Buchenau being built upon in more recent publications (Balslev 1996; Kirschner 2002a, b, c).

The anatomy is very variable within Juncaceae, between genera, and sometimes also within them (Buchenau 1906). However, at the generic level, anatomical characters are often referred to little, if at all, in most taxonomic publications, as there are sufficient external morphological characters showing variation between the taxa to distinguish them.

In addition, few of the genera are large enough for further subdivision to be useful, most genera containing only one to five species. Of the genera of Juncaceae, only *Juncus* and *Luzula* have been further subdivided, and of those only the subdivisions of *Juncus* rely on anatomical characters. Kirschner (2002a) divides *Luzula* into three subgenera and seven sections, but little reference is made to the anatomy. The anatomical descriptions of *Luzula* in the literature (Buchenau 1906; Cutler 1969) indicate that there is only limited variation between its species. The classification is based almost entirely upon

inflorescence characters, with reference also to seed appendages and the shape of the leaf tips. Although the classification of *Luzula* by Kirschner differs from previous classifications (Table 2.2), the range of morphological characters which have been identified as useful in the taxonomy of *Luzula* have remained similar throughout the various treatments of the genus (Grisebach 1844–1845; Buchenau 1890, 1906; Chrtek & Krisa 1974, 1980; Balslev 1996).

It is in the taxonomy of the largest genus, *Juncus*, that anatomical characters have been used extensively. At the subgeneric level there are relatively few characters showing taxonomically informative variation, whereas the anatomy provides many characters. Most subgeneric or sectional classifications of *Juncus* are based in part, or occasionally even entirely, upon anatomical characters.

In commenting on the anatomy of *Juncus*, Cutler (1969) observes that 'the most readily observed anatomical differences can be seen in the transverse section of the leaf blade', and this is reflected in all the classifications of *Juncus*. Buchenau (1875; 1890; 1906) published the most thorough works on the family at that time, and his study of the anatomy of the family provided him with many new characters of potential taxonomic use. His division of *Juncus* into eight subgenera was based on a combination of both macromorphological and anatomical characters (Table 2.1).

The anatomical characters Buchenau used are mostly of the leaf blade. They include the cross-sectional shape; the presence or absence, and quantity, of longitudinal canals present ('pluritubulose' or 'unitubulose') which may be with or without transverse septae; the presence and arrangement of air canals other than central cavities; and the presence or absence of stellate cells. Where septae are present, he recognises two types of septae: those which form complete partitions of the leaf ('perfectly septate'), and those which cross only a part of the cross-sectional area ('imperfectly septate'). Anatomical characters of the culms are referred to more rarely, and fewer culm characters are used in the classification than leaf characters. The presence of parenchyma throughout the culm is mentioned in the description of one subgenus, 'subgenus IV. *Junci thalassii*' (equivalent to sect. *Juncus*); and the cross-sectional shape is occasionally referred to, as it is usually terete, but it is laterally compressed in some species. Cutler (1969) found that according to the material examined for his work, Buchenau's '*Junci subulati*' (sect. *Forskalina* Kuntze)

may be distinguished anatomically from 'Junci genuini' (Section Juncotypus) by the presence of a sclerenchymatous cylinder in the culm of Juncus subulatus Forssk., which is absent in 'Junci genuini'. However, he also notes that a figure of the culm of Juncus subulatus shown in Buchenau (1906) shows no sclerenchymatous cylinder.

In later publications the subgeneric or sectional classifications of *Juncus* differ from those of Buchenau to varying degrees, but they all refer to similar anatomical characters, with some additional ones, and retain the emphasis on leaf anatomy. Balslev (1996) recognises six subgenera, based on a combination of macro-morphological characters and leaf and culm anatomy. The characters which Balslev refers to are similar to those referred to by Buchenau (1906), but with the addition of the arrangement of the vascular bundles. Balslev refers to the anatomy of the culms to differentiate *Juncus* subgenus *Thalassici* (equivalent to sect. *Juncus*) from other subgenera, using cross-sectional shape; the arrangement of vascular bundles; whether the pith is aerenchymatous or parenchymatous, and whether it is interrupted by lacunae or continuous. Reference is also made to the presence in some species of subepidermal sclerenchyma strands, evident as ridges on the outer surface of the stem.

Kirschner (2002a, b) has added further anatomical characters to the taxonomic treatment of *Juncus*. He divides *Juncus* into two subgenera, and these into 10 sections. This classification makes much use of the leaf anatomy, and a key to sections refers to leaf anatomy exclusively for several steps. The characters referred to include all those used previously, with the addition of the relative sizes of abaxial and adaxial epidermal cells. In the descriptions of the sections, and keys to species within each section, several other characters of the leaf and stem anatomy were used. Of the leaf anatomy, in addition to those characters already mentioned, the shape of the cells of the pith and the arrangement of stomata were used; and of the culm, the number of ridges on the outer surface of the culm. The pith in the stem is described as continuous (though sometimes degenerating in old stems) or interrupted with lacunae, and species may be identified according to whether the interruptions are reduced to septa-like plates with large lacunae, or with thicker portions of pith interrupted by small lacunae; and whether the stomata are sunken or superficial.

3.1.2 Aims

- 1. Anatomical descriptions in the literature are studied, and the organs which are likely to yield a large number of variable characters are identified for further study.
- 2. An anatomical study of the organs identified as potentially taxonomically useful is carried out, and the results presented as descriptions of Juncaceae and its genera, subgenera and sections.
- 3. Points where these results differ from the literature are discussed.
- 4. Characters which are of potential taxonomic use are identified, and a list of those characters generated for use in cladistic analyses, and character states determined.
- 5. Data is collected and recorded in a table for further analysis and for discussion in terms of character evolution in Chapter 5 *Phylogeny*.

3.1.3 Anatomical descriptions of Juncaceae in the literature

A detailed account of the anatomy of the roots, culms and leaves of Juncaceae has been provided by Cutler (1969), so it was not considered necessary to provide a review of the anatomy of Juncaceae. Instead, a discussion of the range of the anatomical variation which is described in the literature is given below.

Roots

General descriptions of the the root anatomy of the family are given by Buchenau (1906), Cutler (1969) and Balslev (1996). Cutler (1969) also gives separate descriptions for *Juncus* and *Luzula*, and certain differences between the root anatomy of *Juncus* and *Luzula* are also noted by Buchenau (1906) and Balslev (1996), but other genera are not described. In the case of Cutler (1969), this may be taken to mean that these taxa were adequately described by the general family description, as descriptions are generally given only when taxa show sufficient variation from the family description to require individual treatment. The most taxonomically significant variation appears to be between roots lacking air canals, as observed in *Luzula*, and those with air canals, as observed in *Juncus*. Variation in the thickness of cell walls is also seen, but it is noted (Balslev 1996) that, in certain cases, such variations may be linked to the size or age of the root, so they may be unreliable as taxonomic characters.

Rhizomes

The degree of variation between taxa in the rhizome is similar to that of the root, and can be similarly divided into two types based upon air spaces in the cortex. Other than this, the majority of the variation relates to cell wall thickening and numbers of cell layers, but these may be affected by age or environment, and may not be taxonomically significant.

Culms

The culms show a number of variable characters, many of which are potentially taxonomically significant. In particular, among other characters, the arrangement of vascular bundles and presence or absence and arrangement of sclerenchyma fibres in the ground tissue shows much variation. The number and arrangement of sclerenchyma girders, for example, has been used previously to distinguish between certain species of *Juncus* sect. *Juncotypus* (Buchenau 1906; Kirschner 2002c). The arrangement of air canals also shows some variation, as they may occur as a central canal or a ring of canals in the chlorenchyma, and the canals may have transverse septae, or not.

Leaves

There is not sufficient information in the literature to draw conclusions about the potential of the sheath to provide taxonomically informative characters. The sheaths are described by Cutler (1969) only for *Distichia* and *Oxychloe*, and sheaths are not described by other authors. However, the leaf blade anatomy shows a high degree of variation, particularly in the leaf outline and the presence or absence of an adaxial surface, the arrangement of the vascular bundles, the arrangement of sclerenchyma strands, and the arrangement of air canals, among other characters. Many of the characters appear to show a similar range of variation to that seen in the culm, especially in the arrangement of sclerenchyma strands and air canals in species of *Juncus*, as certain sections such as *Juncus* sect. *Juncus* show much anatomical similarity between the leaves and the culms. As is the case for these characters in the culms, the leaf characters are of taxonomic significance.

Of the leaf outline, much of the variation appears consistent between taxa, and may be used to distinguish sections of *Juncus* from one another (Balslev 1996; Buchenau 1906; Kirschner 2002b). However, there may be also be a degree of variation within species. Balslev (1996) notes that the thickness of the leaf may be related to environmental conditions, as forms of *Luzula racemosa* Desv. found at high altidude have thickened and adaxially grooved blades, whereas lower altitude specimens have thinner, flatter blades.

The epidermis shows variation in cell size and the degree of wall thickening, and although some of this may vary according to environmental conditions, much variation is also seen between species, such as the presence of bulliform cells on the adaxial surface of some species, and the loss or reduction of the adaxial surface in other species.

The variation in the vascular bundle sheaths may be taxonomically unreliable, as most of the variation described is between vascular bundles possessing a continuous inner sheath, and others with bundle caps only, and in the number of cell layers. This variation can be seen within a single species, as would be expected due to the different functions of vascular bundles within a leaf, so it does not appear likely to be taxonomically significant.

Conclusions

The anatomy of the roots and rhizomes of Juncaceae are not likely to yield useful taxonomic characters, and they have not previously been used to distinguish taxa, or included in cladistic and phenetic analyses. Although a small number of variable characters may be present in their anatomy, it was decided that the number of characters was not high enough to be worth the time involved in obtaining them for this study.

In contrast, the culms and leaves show a high degree of anatomical variation, and much of this appears to be taxonomically significant. Characters of both the culm and leaf anatomy have been used extensively in previous taxonomic works to distinguish subgenera, sections and species of *Juncus* (Balslev 1996; Buchenau 1906; Kirschner 2002b, c; Snogerup 1963). Some have been included in cladistic analyses by Munro & Linder (1998) and Simpson (1995). Two characters of the leaf anatomy were used: the presence of inverted vascular bundles in the leaves, which is unique to *Thurnia*, and the presence of air canals in the chlorenchyma, which is unique to *Prionium* (but see descriptions of *Juncus* and *Luzula* in Section 3.3 *Results*). One character of the culm anatomy was used: the presence or absence of scalariform perforation plates in the stem vessels. This character is only variable within Cyperaceae, and would not, therefore, be informative in this study. However, most of the characters have not been included in cladistic analyses previously.

Some similarity between the culms and the leaves can be seen, and many of the characters which appear to be of potential taxonomic use can also be found in the leaves. The leaves also show more anatomical variation than the culms, particularly in their cross-sectional shapes, bifaciality or unifaciality, and arrangements of air canals and sclerenchyma strands. It was decided that the largest number of potentially useful characters, and sufficiently large for this study, could be obtained from the leaves only.

Since the leaf sheaths have not previously been described it was decided that they, also, would be studied, to obtain new descriptions and to investigate their taxonomic utility.

They are known to differ from the blades in that they are always bifacial, whereas the leaves may be bifacial and unifacial, so it was thought possible that they may provide characters which are comparable between all species where the blades do not.

There is some further justification for the study of the sheaths of Juncaceae in parallel with a study of the blades. The unifaciality of the leaf blades is, in part, justified by the orientation of the vascular bundles in a complete ring, with the xylem poles facing inwards (Cutler 1969). Usually these leaves are terete, but they may be flattened, either laterally or dorsiventrally, in which case the 'ring' becomes two rows, with the xylem poles facing one another. This is also the case in Cyperaceae, where the flattened leaves of some species have two rows of vascular bundles, distinguishing them from most other species with flat leaves, which have a single row of vascular bundles. Cladium mariscus (L.) Pohl is one example, and its leaves have been described as 'pseudo-dorsiventral', as although the leaves appear to be dorsiventrally flattened, they possess two rows of vascular bundles, with the xylem poles facing one another. The entire external surface is interpreted as being homologous to the abaxial surface of a true dorsiventral leaf (Metcalfe 1971). If this interpretation is correct, the sheaths would be expected to contain a single ring of vascular bundles, as the sheath, being closed, clearly has an outer (morphologically abaxial) and inner (morphologically adaxial) epidermis, and is entirely bifacial. However, Guichard (1928) noted that the inverted bundles in the leaves of *Cladium mariscus* are also present in the sheath, and that the inverted bundles insert directly into the stem after turning through 180°. This implies that the interpretation of Metcalfe (1971) may not be correct, as the origin of the inverted bundles may not be due to the loss of the adaxial surface.

It is not known whether such a situation may also occur in Juncaceae. Cutler (1969) describes the sheath of *Distichia*, which contains a single row of vascular bundles, while the blade contains a single ring, as would be expected if the adaxial surface of the sheath becomes lost in the blade. Many other species of Juncaceae are also considered by Cutler to have unifacial leaf blades, including species of *Juncus*, which may have one or more rings of vascular bundles, or one or more rows, with the xylem poles facing inwards. The sheaths, however, are not described. The interpretation that these leaves are unifacial should be confirmed by a comparison with the vascular bundle orientation in the sheaths.

3.2 Materials and Methods

3.2.1 Selection of material and techniques

Wherever possible, the specimens studied were the same as those included in the macromorphology study, in order to maintain consistency and to enable the results to be combined with the macromorphological and molecular studies more easily. In some cases the flowering material used in Chapter 2 provided poor or insufficient leaf material, and it was necessary to use a different specimen. A list of specimens is given in Table A1.

The choice of anatomical characters to study was made by reference to both the literature and the plant material. A study of the literature (Section 3.1.3) was made to identify the organs which had the largest numbers of characters showing variation within Juncaceae. From the taxonomic literature (Kirschner 2002b, c; Balslev 1996; Buchenau 1906), a number of taxonomically informative anatomical characters were identified. In particular, in descriptions of subgenera and sections of Juncaceae much reference is made to the cross-sectional shape of the leaf blades, and also to the arrangement of vascular bundles, sclerenchyma fibres, and air canals in the leaves. Cutler (1969) studied the anatomy in detail, and the descriptions indicate potentially taxonomically useful variation in most organs. However, the greatest number of variable characters which have been noted as being unique to *Thurnia* (Cutler 1963) and *Prionium* (Cutler 1969) occur in the leaves.

Due to time constraints it was not possible to study the anatomy of many different organs. From the literature it was apparent that the leaf anatomy had the potential to provide the largest number of taxonomically informative characters, and it was decided that a study of the leaf anatomy alone would be sufficient.

In order to collect these data it was necessary to take transverse sections of leaf material. Many Juncaceae leaves are very small (approximately 0.5 mm diameter), and the mixture of hard sclerenchymatous and soft parenchymatous tissues resulted in the leaves of certain species being delicate and likely to break up if care is not taken in making the sections, particularly when taken from older herbarium specimens. Many of the leaves proved

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difficult to section by hand when initial attempts were made after rehydration, as the softer tissues tended to disintegrate, even after treatment with formalin acetic-acid alcohol (FAA - see below). In order to protect the structure of the material as much as possible it was considered necessary to embed it in paraffin wax. To maintain control over the thickness and quality of the sections taken, a microtome was used.

3.2.2 Methods

Leaf material was either removed from herbarium specimens or collected in the field and dried in silica gel. A small sample of material was boiled for approximately five minutes, until thoroughly rehydrated. This material was placed in sufficient FAA (formalin acetic acid alcohol: 90 ml 70% ethyl alcohol, 5 ml glacial acetic acid, 5 ml formalin) to cover the specimen, and left for two to four days. In order to store the material for future use it was transferred into 70% ethanol.

Dehydration, wax infiltration and embedding

The protocol followed was as published in Johansen, D. A. (1940), and set out below.

Dehydration of material stored in 70% ethanol was achieved by soaking the material in a series of dilutions of tertiary butyl alcohol (TBA) and ethanol with distilled water, to the following schedule:

Stock solution		Time in solution	
1	50% Ethanol/TBA		At least 2 hours
	(50ml distilled water, 40ml 95% Ethanol, 10ml TBA)		
2	70% Ethanol/TBA		Overnight
	(30ml distilled water, 50ml 95% Ethanol, 20ml TBA)		
3	85% Ethanol/TBA		1 hour
	(15ml distilled water, 50ml 95% Ethanol, 35ml TBA)		
4	97% Ethanol/TBA		1 hour
	(45ml 95% Ethanol, 55ml TBA)		
5	100% Ethanol/TBA		1 hour
	(25ml 100% Ethanol, 75ml TBA)		

Three further changes of the 100% Ethanol/TBA solution were carried out over a period of approximately 24 hours.

The material was then transferred to a mixture of equal parts paraffin oil and TBA. After at least one hour the material was placed in a jar 3/4 filled with solidified paraffin wax, the material being allowed to rest on top of the wax. This was then covered with a layer of the TBA/paraffin oil mixture. The jar was placed in an oven to allow the wax to melt slowly.

After 1 hour the wax and TBA/paraffin oil mixture was replaced with pure fresh melted wax. The wax was replaced twice during the following 6 to 12 hours.

The material was then placed in a mould containing molten paraffin wax (Paraplast Plus, melting temperature 56 °C), with a 'universal cassette' for mounting on the microtome, and the wax was allowed to set. The cassette and wax-embedded specimen were then removed from the mould and trimmed of excess wax.

Sections of 10–16 μ m thickness were cut from the block using a Leica SM 2000R sliding microtome.

Mounting and staining

Pre-washed slides were prepared with Haupt's adhesive 24 hours prior to preparing the sections to be mounted. A single drop of Haupt's adhesive was placed on each slide and spread across the area on which the sections were to be placed. The slide was placed on a slide warmer for a few seconds (until the adhesive was tacky to touch) and then stored in a slide box overnight, to protect the adhesive from dust.

The following day the sections were cut, several drops of formalin placed on the slide surface over the adhesive, and the sections placed on the slide so that the formalin allowed the wax to expand until flat. Excess formalin was drained off, and the slide was then heated briefly on a slide-warmer until the wax had softened sufficiently to remove any wrinkles, to ensure that the specimen lay completely flat on the slide. The slides were then stored for at least 24 hours before staining.

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The initial method used to stain the slides was with Fast Green, by passing them through the following series (from Johansen 1940):

Xylene	10 min. (or until all wax dissolved)
Xylene : 100% Ethanol 1:1	5 min.
100% Ethanol : Ether 1:1	5 min.
100% Ethanol	5 min.
95% Ethanol	5 min.
Fast Green (applied with dropper)	<15 s.
Clove oil : 100% Ethanol : Xylene 2:1:1	Dip 2–3 times to wash off excess stain
100% Ethanol : Xylene 1:1	5 min.
Xylene	15 min.
	Xylene Xylene : 100% Ethanol 1:1 100% Ethanol : Ether 1:1 100% Ethanol 95% Ethanol Fast Green (applied with dropper) Clove oil : 100% Ethanol : Xylene 2:1:1 100% Ethanol : Xylene 1:1 Xylene

This method was found to work well with most genera of Juncaceae, but the quality of the staining was variable, with some sections appearing very dark and some lighter, even on the same slide. The reasons for this were not identified. *Luzula* was found to stain very lightly, if at all, although longer times in the stain were tried (to 2 minutes).

An alternative staining method using Toluidine Blue was proven to be superior, as the stain was picked up well by all specimens. The slides were passed through the following series:

1	Xylene	10 min. (or until all wax dissolved)
2	Xylene : 100% Ethanol 1:1	5 min.
3	100% Ethanol : Ether 1:1	5 min.
4	100% Ethanol	5 min.
5	95% Ethanol	5 min.
6	75% Ethanol	5 min.
7	50% Ethanol	5 min.
8	30% Ethanol	5 min.
9	15% Ethanol	5 min.

10 Toluidine Blue

2 hours

11 Return through the series from 15% Ethanol to Xylene, using the same times.

One problem was identified using this method, which was that once the slides were placed in the more aqueous solutions (50% Ethanol and lower concentrations), some sections fell off the slides. Discussion with a colleague (Amornrat Prajaksood) identified that a possible reason for this may be insufficient time between placing the sections on the slides and staining them, as this appeared to happen only to those slides which were left for a short period (e.g. 24 hours) between the two stages, and those which had been prepared several days or weeks before staining did not fall off the slides.

DPX mountant was used to make permanent slides. A small quantity was added to the slide and a coverslip placed over it. The slides were then placed on a slide warmer for several hours or overnight until the DPX mountant was no longer tacky to touch.

3.3 Results

Leaf sheaths and leaf blades are described from transverse sections taken from the midpoint of the sheaths and blades. Longitudinal sections were also taken where it was considered necessary to examine structures which could not be seen in transverse sections, such as transverse septae. See Table A1 for a list of specimens examined.

Descriptions are given for the family and genera. They are also given for subgenera and sections of which at least one specimen was examined, although descriptions of some parts are omitted where they do not differ from the genus description.

3.3.1 Terminology

The terms used in the following descriptions may sometimes differ from the terms used in the literature. An explanation is given here for the choice of terms where this is the case.

Hypodermis

A hypodermis is considered to be a subepidermal layer which is distinct from the ground tissue. It may be derived from either the ground tissue or the protoderm (Esau 1965), so the term 'hypodermis' may refer to structures which are not necessarily homologous, as the origin can only be determined by developmental studies. In many species of Juncaceae distinct subepidermal layers are present, such as parenchymatous or sclerenchymatous cells. In *Rostkovia* cells described by Cutler (1969) as protective cells also form a distinct subepidermal layer in two longitudinal strips on the abaxial side of the leaf, corresponding to the presence of stomata in these regions. One of the aims of this chapter is to identify characters which can be used in cladistic analyses, so the terms used must indicate homologous structures, and the term 'hypodermis' is avoided here.

A hypodermis has been described as present in *Rostkovia* by Cutler (1969), consisting of sclerenchyma fibres, but absent throughout the rest of Juncaceae. The layers of sclerenchyma in *Rostkovia* are considered here to be homologous to the subepidermal girders present in many other Juncaceae species, as they appear to differ only in that they

occur under a larger proportion of the epidermis in *Rostkovia*, so they are referred to here as subepidermal girders.

Lobed and stellate cells

These are found among a number of species of Juncaceae, but they are most conspicuous in *Juncus* subgenus *Juncus*. They tend to be irregular in shape, but more or less isodiametric, with radiating lobes which are variable in number. The lengths of the lobes vary according to the species, but also according to the position within the leaf. Continuous variation can be seen from cells with very short, radiating lobes with very small intercellular air spaces between the lobes (e.g Fig. 3.19E), to cells with much longer lobes and proportionally larger intercellular air spaces (e.g. Fig. 3.19G). It was decided to refer to cells of which at least half of their diameter is formed by the lobes as 'stellate', and those with shorter lobes as 'lobed' cells. In the literature the term 'stellate' is used in a similar sense. The cells referred to here as 'lobed' are referred to as having 'short pegs', 'long pegs' or 'lobes' by Cutler (1969).

3.3.2 Descriptions of the leaf anatomy of Juncaceae

Leaf sheaths

Outline

In all genera except *Luzula*, the sheath is dorsiventrally flattened, and u- or v-shaped, or rolled to sheath the stem (Fig. 3.1A–C). The sheaths of *Juncus* sect. *Iridifolii* are a laterally compressed v-shape, with the two 'arms' sheathing the dorsiventrally flattened stem (Fig. 3.1E). The leaf sheath margins are free in all species except those of *Luzula*, and usually acuminate, with a reduction in the number of layers of ground tissue to a single layer near the tips. A continuation of the abaxial and adaxial epidermal layers often forms a membranous edge to the sheath, the epidermal layers being adjacent to one another with no ground tissue in between.

The leaf margins of *Luzula* are fused to form a cylinder (Fig. 3.1D). The region corresponding to the fused margins, i.e. the side furthest from the leaf blade, is very thin, sometimes membranous, and in mature leaves the sheath may split longitudinally in this region so the margins may sometimes appear to be free.

Leaf sheaths are always bifacial. The surface forming the inside of cylindrical sheaths is homologous to the adaxial surface in other genera, so the inner surface of cylindrical sheaths is subsequently referred to as the adaxial surface.

Epidermis

The leaf surface is usually smooth, but rarely it may have small papillae or ridges. The cuticle is usually thin, but in some species it is thickened.

The abaxial epidermal cells are square to circular or rectangular to oval in transverse section. They are usually isodiametric, but sometimes they are elongated tangentially up to four times as long as wide. The outer cell walls of the abaxial epidermis may be very thick to thin, and the anticlinal and inner walls may also be thickened (e.g. *Juncus maritimus* Lam.), but usually they are thin (Fig. 3.2A).



Figure 3.1 Leaf sheath outlines showing arrangement of vascular bundles and air canals, and epidermis. A *Oxychloe bisexualis* with single row of vascular bundles alternating with air canals. **B** *Marsippospermum* grandiflorum with large adaxial air canal. **C** *Juncus maritimus* with scattered vascular bundles and no air canals. **D** *Luzula campestris* fused sheath. **E** *Juncus xiphioides* v-shaped sheath with small unifacial section near the midrib.

The adaxial epidermal cells are similar in size to the abaxial epidermal cells or they are slightly larger, sometimes resembling the ground tissue in appearance. They are square to round or rectangular to oval in transverse section. They range from isodiametric to four times as long tangentially than radially, or to three times as long radially than tangentially. The cell walls of the adaxial epidermis are thin (Fig. 3.2B).

Stomata are often present on the abaxial surface, though they usually occur less frequently than on the leaf blades. In some species they may be very infrequent and several sections may need to be observed to find a single stoma, but in others several may be found in each section. In other species they may be entirely absent from the sheaths. The stomata of the sheaths are similar to those of the blades of the same species, being superficial or sunken from the leaf surface, and the guard cells often have small lips on the outer edge of the stoma, or on both the outer and inner edges.

Mesophyll

Most species have a small number of cell layers of ground tissue both abaxially and adaxially, either side of a row of air canals in the centre of the leaf. The ground tissue extends towards the centre at intervals to form longitudinal septae between the air canals. In some species of *Juncus* the ground tissue extends throughout the thickened sheath (Fig. 3.1C).

The cells of the ground tissue are usually isodiametric or only slightly elongated in shape, but in some sections of *Juncus*, e.g. sections *Juncus* and *Ozophyllum*, they are lobed or stellate, with intercellular air spaces (Fig. 3.2).

The ground tissue may be entirely chlorenchymatous, or chlorenchyma may be present on the abaxial side only, with parenchyma on the adaxial side (Fig. 3.2C). In some species, e.g. *Juncus subulatus*, the layers of chlorenchyma near the epidermis may become compressed. The leaf sheaths of many other species appear to contain no chlorenchyma, the ground tissue consisting entirely of parenchyma. However, in the specimens examined it was frequently difficult to identify chlorenchymatous cells, as cell contents were frequently absent. It is possible that small amounts of chlorenchyma may become degraded in the mature sheath.

3.3 Results



Figure 3.2 Leaf sheath epidermis and mesophyll. A *Marsippospermum grandiflorum* abaxial epidermis with thickened outer cell walls and superficial stoma. **B** *Juncus ecuadoriensis* adaxial epidermal cells (e) with thin walls, and ground tissue cells similar to the epidermal cells. **C** *Distichia acicularus* section of sheath showing 1-2 abaxial layers of chlorenchyma (c) and several adaxial layers of parenchyma (p). **D** *Juncus maritimus* stellate cells of abaxial side of sheath. **E** *Juncus subulitepalus* stellate cells and vascular tissue of transverse septa.

Vascular bundles

Vascular bundles are usually arranged in a single row, with large vascular bundles alternating with smaller ones. In some sections of *Juncus*, e.g. sections *Juncus* and *Ozophyllum*, they are often in several rows, or scattered throughout the section (Fig. 3.1), but the xylem poles are always orientated towards the adaxial side.

An outer vascular bundle sheath is sometimes present, consisting of a single layer of parenchyma cells which is frequently interrupted, but often it is not possible to distinguish a parenchymatous sheath from the surrounding tissue, in particular where vascular bundles are surrounded by parenchyma. However, in other species the outer sheath may be identified by the rounder shape or different size of the sheath cells than those of the surrounding tissue (Fig. 3.3A–D).

An inner vascular bundle sheath is usually present, consisting of several layers of sclerenchyma cells and usually forming a complete ring around the largest vascular bundles. In some species it may consist of more layers at the xylem and phloem poles than at the flanks, or a similar number of layers may be present all around the vascular bundle. In other species some vascular bundles may have a single cap of sclerenchyma on the xylem and phloem poles, or rarely on just the phloem pole (e.g. *Luzula gigantea* Desv.), or xylem pole (e.g. *L. racemosa* Desv.). Variation occurs within a single specimen, with smaller vascular bundles often having less sclerenchyma than the larger ones.

Sclerenchyma

Sclerenchyma is often only represented by the vascular bundle sheaths. In some species, e.g. of *Luzula* and *Juncus*, sclerenchyma strands may occur between or next to the vascular bundles, being similar in size and shape to smaller vascular bundles in the same specimen, but not associated with any vascular tissue (Fig. 3.3C). Extensions of the sclerenchymatous inner bundle sheath to form girders against the epidermis are rare, but one example was observed in a specimen of *Oxychloe andina*, and extensions occur to the adaxial or abaxial surface in some species of *Juncus* and *Luzula*. There are no girders next to the epidermis which are not associated with the bundle sheaths, as is the case with the leaf blades of many species.

3.3 Results



Figure 3.3 Leaf sheath vascular bundles and sclerenchyma. A *Luzula sylvatica* vascular bundle with complete inner sclerenchyma sheath, and outer parenchymatous sheath difficult to distinguish from surrounding ground tissue. B *Juncus maritimus* vascular bundle with an outer sheath (os) of a single layer of parenchyma. C *Luzula gigantea* large vascular bundle with sclerenchyma cap on phloem pole, small vascular bundle with complete sheath of sclerenchyma (vb), and sclerenchymatous strand (s) which is similar in appearance to small vascular bundle. D *Juncus trifidus* adaxial girder (g) formed by extension of the sclerenchymatous inner bundle sheath to the epidermis.

Air spaces

Most species have air canals in a single row, alternating with the vascular bundles (Fig 3.1). *Marsippospermum* also has an additional large air canal positioned adaxially to the vascular bundles (Fig. 3.1B).

Some species of *Juncus* have no large air canals, but the ground tissue of these species contains large intercellular air spaces, as they possess a pith of stellate or lobed cells (Fig. 3.2D).

Tannins, crystals and silica bodies

Crystals and silica bodies are absent. Tannins are rarely present, and were observed only in the sheaths of *Luzula multiflora* (Ehrh.) Lej.

Leaf blades

Outline

The leaf blades vary in the outline of the transverse section, from dorsiventrally flattened to oval or circular, with or without adaxial or abaxial grooves, or an intermediate shape (Fig. 3.4). When dorsiventrally flattened they may be entirely flat, or the leaf margins may curl towards the adaxial side, or they may be u- or v-shaped. *Juncus* sect. *Iridifolii* have ensiform leaves which are laterally flattened, the midrib forming one edge and the joined leaf margins forming the other edge.

Leaf blades are bifacial in *Luzula* and in many species of *Juncus*. Other species, e.g. some species of *Juncus*, show a reduction in the adaxial surface of the blade, and in some species, e.g. species of *Juncus* with cylindrical or ensiform leaves, they are entirely unifacial, with the adaxial surface being absent (further discussion of the unifaciality of the leaves is given in Section 3.4 *Discussion* and Chapter 4 *Auricles or ligules?*).

Epidermis

The leaf surface is usually smooth, but may be ridged or grooved, and rarely papillae may also be present on the leaf margins of some species, e.g. of *Juncus* and *Luzula*.

The epidermal cells are rectangular or square, to oval or circular in transverse section (Fig. 3.5). The cells of the abaxial epidermis range from isodiametric to two times as long tangentially as radially, or to two times as long radially as tangentially. They may become smaller at the margins, e.g. in some species of *Luzula*. The outer walls are usually thickened, but may be thin. The anticlinal and inner walls are usually thin, but sometimes they may be moderately thickened.

The cells of the adaxial epidermis are sometimes similar to the cells of the abaxial epidermis, but in many species they are larger, sometimes bulliform (Fig. 3.5C, D). They may be isodiametric, but are often longer radially than tangentially, to two times as long. The outer walls of the adaxial epidermis are either thinner than those of the abaxial epidermis, or of similar thickness, and the anticlinal and inner walls are thin. When the



Figure 3.4 Leaf blade outlines. A–C Bifacial leaf blades. A *Luzula sylvatica* dorsiventrally flattened leaf. B *Juncus capillaceus* oval leaf with adaxial groove. C *Juncus squarrosus* v-shaped leaf. D-F Unifacial leaf blades. D *Oxychloe bisexualis* terete leaf. E *Distichia acicularis* oval leaf with adaxial groove. F *Juncus ensifolius* ensiform leaf. Central air canal divided by longitudinal septae (ls).

adaxial cells are larger than the abaxial cells, they usually reduce in size towards the leaf margins, becoming similar to the abaxial cells.

Stomata are present on the abaxial epidermis only. Stomata may be sunken from the leaf surface in some species of *Juncus*, but are more usually superficial. The guard cells often have a lip on the outer surface, and sometimes also on the inner surface (Fig 3.5A, B).

Mesophyll

Chlorenchyma is present either throughout the ground tissue, or as several layers, either abaxially in bifacial leaves, or as outer layers of terete leaves, the remainder of the ground tissue consisting of parenchyma (Fig. 3.5). Palisade mesophyll is present in some species, and in others slightly elongated mesophyll cells may occur in the outer layers, becoming less elongated to isodiametric in the inner layers, or all the cells are isodiametric. The mesophyll often lacks conspicuous intercellular air spaces, but in some specimens spongy mesophyll is present in the inner portions of the leaf. There is no clear differentiation between palisade and spongy mesophyll in the leaf.

In some species of *Juncus* some mesophyll cells may be lobed or stellate in shape, with intercellular air spaces between the lobes (Fig. 3.6). In these species the cells tend to become more conspicuously lobed in the inner layers, and when transverse septae are present, the cells of the septae are usually more conspicuously lobed in shape than the rest of the ground tissue.

Parenchyma is sometimes present as large, very thin-walled cells which disintegrate with age, leaving air spaces in mature leaves. These are often evident as traces of cell walls remaining in the air spaces (Fig. 3.5E). In some species parenchyma which persists even in mature leaves is also present. When present in terete leaves, it may surround the vascular bundles as several cell layers between the chlorenchyma and a large central canal, or it may be present throughout the leaf. In bifacial leaves it may sometimes be present on the adaxial side of the leaf. In *Marsippospermum* a small area of parenchyma is also present next to the epidermis on the abaxial side.

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Figure 3.5 Leaf blade epidermis and mesophyll. A *Patosia clandestina* thickened outer walls of the abaxial epidermis with superficial stoma, and slightly elongated palisade mesophyll. **B** *Juncus ensifolius* abaxial epidermis with thin walls and sunken stoma. Epidermal cells are rounded and variable in size. **C** *Juncus bufonius* adaxial epidermis with bulliform cells, and spongy mesophyll. **D** *Juncus capillaceus* bulliform adaxial epidermal cells, and adaxial parenchyma which is similar in appearance to epidermal cells. **E** *Marsippospermum grandiflorum* outer mesophyll layers consisting of palisade mesophyll, and several inner layers of parenchyma (p), which are distinct from the innermost region of large, thin-walled cells which break down to form a cavity.

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Figure 3.6 Leaf blade mesophyll: stellate cells and septae. A *Juncus maritimus* stellate parenchyma cells in transverse section (stellate cell indicated by arrow). B *Juncus maritimus* stellate parenchyma cells in longitudinal section. C *Juncus biglumis* longitudinal section showing transverse septae crossing each canal at a different level. D *Juncus xiphioides* transverse septa in transverse section showing stellate chlorenchyma cells.

Vascular bundles

Vascular bundles are usually arranged in a single layer in bifacial leaves, or a single ring in unifacial leaves, often with larger bundles alternating with smaller bundles. Sometimes they may be in more than one layer, or scattered throughout the leaf, with the smaller bundles closest to the abaxial surface (Fig. 3.4). In bifacial leaves they are orientated with the xylem poles towards the adaxial surface, and in unifacial leaves the xylem poles are orientated towards the centre of the leaf.

The vascular bundles usually have two sheaths: an outer sheath consisting of a single layer, sometimes interrupted, of large, thin-walled parenchymatous cells, and an inner sheath of sclerenchymatous cells (Fig. 3.7A). When the vascular bundles are surrounded by parenchyma the outer sheath may be difficult to distinguish from the surrounding tissue. However, in other species the outer sheath may be identified by the rounder shape or different size of the sheath cells than those of the surrounding tissue. In species where the vascular bundle is surrounded by chlorenchyma, if a parenchyma sheath is present it is clearly distinguishable from the surrounding tissue, but it may sometimes be absent. In some species of *Luzula* the outer sheath may extend to the adaxial or abaxial epidermis.

The sclerenchymatous inner sheath may be a single layer to several layers of cells forming a complete sheath, or it may be present only as caps on the xylem and phloem poles of the vascular bundle. Variation often occurs within a specimen, with larger vascular bundles having complete sheaths, and smaller ones having caps. In some taxa (e.g. *Oxychloe* and some species of *Juncus*), a sclerenchymatous sheath is present only at the xylem or phloem poles, and a parenchyma sheath is present only at the flanks between the poles. Some smaller vascular bundles of the same species may have a single, complete or interrupted, parenchyma sheath and no sclerenchyma sheath, or a cap of sclerenchyma on the xylem pole only, and parenchyma on the phloem pole and on the flanks. In many species the inner sheath may extend to the abaxial, or rarely adaxial, epidermis to form a sclerenchymatous girder.

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Figure 3.7 Leaf blade vascular bundles and sclerenchyma. **A**. *Juncus bufonius* vascular bundle with inner sheath of sclerenchyma forming caps on the xylem and phloem poles, and an interrupted outer sheath of a single layer of parenchyma cells. **B**. *Oxychloe bisexualis* vascular bundle with girder formed by extension of the sclerenchymatous inner vascular bundle sheath to the epidermis. **C** *Juncus maritimus* sclerenchyma girder at margin of unifacial leaf. **D** *Juncus capillaceus* sclerenchyma girder at margin of bifacial leaf.

Sclerenchyma

Sclerenchyma is present in the leaves as vascular bundle sheaths. In some species the sclerenchyma of the bundle sheaths may extend to the abaxial or adaxial epidermis to form a girder (Fig. 3.7B). In other species girders are present next to the epidermis, but not associated with vascular bundles (Fig. 3.7C, D). Often such girders are present only at the margins of bifacial leaves, but in *Juncus* sect. *Juncus* they occur in a ring just inside the epidermis, sometimes resulting in a ridged or grooved appearance of the surface the leaf.

Air spaces

Air canals are frequently present in a series alternating with the vascular bundles (Fig. 3.4A). In terete leaves they may also occur as large central canal (Fig. 3.4D, E), or as several central canals divided by plates of parenchyma or chlorenchyma cells (Fig. 3.4F). The canals are often continuous, but septae may sometimes be present dividing the air canals transversally. When several central canals are present, the septae often divide individual canals at different levels in the leaf, so a single septa does not cross the entire width of the leaf (Fig. 3.6C).

Air canals are formed by the breakdown of a pith of large, thin-walled cells, which may be evident as remnants of cell walls in the air space but with no identifiable cell structure (Fig. 3.4D, E), or the canal may be entirely open (Fig. 3.4F). In sections taken from very young leaves pith may still be present in this region.

Some species do not have air canals, but the spongy mesophyll tends to form very large air spaces between the vascular bundles. Some variation is seen between specimens with spongy mesophyll and no clearly defined air canals but with very large intercellular air spaces between the vascular bundles, and other specimens with no spongy mesophyll, but well-defined air canals. Both these types, and intermediate stages, may be seen in different specimens of the same species. This is discussed in more detail for *Luzula* and *Rostkovia*, but similar variation was also observed in *Juncus*.

Tannins, crystals and silica bodies

Tannin, crystals and silica bodies are absent.

Anatomical description of leaves of Distichia

Leaf sheaths Fig. 3.8

Epidermis

The abaxial epidermal cells are isodiametric or very slightly elongated radially (up to 1.5 times as long as wide). They have thickened outer walls, moderately thickened anticlinal walls and thin inner walls. The adaxial epidermal cells are larger than the abaxial epidermal cells, and elongated up to three times as long tangentially as radially, and all walls are thin.

Stomata were observed on the abaxial surface of *D. acicularis* Baslev & Laegaard, but were infrequent, with two stomata occurring among a total of four sections examined. They were not observed at all on the sheaths of *D. muscoides*.

Mesophyll

In *D. acicularis* one cell layer of chlorenchyma is present on the abaxial side of the sheath, increasing to 2-3 layers next to the phloem pole of the vascular bundles. The cells are isodiametric or irregular in shape, to slightly elongated radially. Chlorenchyma was not observed in the transverse sections taken of the sheaths of *D. muscoides*.

Longitudinal sections indicate that the number of layers of chlorenchyma increases towards the leaf blade, with a small number being present in the sheaths near to the leaf blade, but no chlorenchyma is present in the middle section of the sheath of *D. muscoides*. The sheaths of *D. acicularis* examined were longer, and a greater proportion of the length contained chlorenchyma than in *D. acicularis*, some being present in the middle section, so the number of layers of chlorenchyma observed is dependent on the point at which the section is taken.

The remainder of the ground tissue is parenchymatous. In the sections examined parenchyma was present both abaxially and adaxially in *D. muscoides*, but only adaxially in *D. acicularis*, although the longitudinal sections indicate that some abaxial parenchyma

is present nearer the base of the sheath. The cells are very large and thin-walled, and were often fragmented in *D. muscoides*.

Vascular bundles

Vascular bundles are arranged in a single row, alternating with air canals in the centre of the sheath.

The vascular bundle sheaths consist of an outer sheath of a single layer of parenchyma, which is sometimes difficult to distinguish from the surrounding tissue, but often it is distinguishable by the smaller size of the sheath cells than in the surrounding tissue. Sclerenchyma forms a complete inner sheath, consisting of 2–3 cell layers.

Sclerenchyma

In *D. acicularis* sclerenchyma is represented only by the vascular bundle sheaths. In *D. muscoides* the vascular bundles nearer the margins show a reduction in the proportion of vascular tissue in relation to the sclerenchyma. At the margins 1-2 strands of sclerenchyma are present. They are circular in outline and with outer parenchyma sheaths similar to those of the vascular bundles, but with no associated vascular tissue. The strands of sclerenchyma appear to be a continuation of the row of vascular bundles.

Air spaces

A single row of air canals occurs between the vascular bundles.



Figure 3.8 *Distichia* leaf sheaths. A *D. acicularis*. B *D. acicularis* abaxial epidermis with stoma. C *D. muscoides* small vascular bundle and adaxial eidermis, near margin. D *D. muscoides* sclerenchyma strand. E *D. acicularis* large vascular bundle and abaxial chlorenchyma.

Leaf blades Fig. 3.9

Outline

Leaves are unifacial and round, or slightly laterally flattened to oval in shape, with or without an adaxial groove.

Epidermis

The epidermal cells are isodiametric, with very thick outer, moderately thickened anticlinal and thin inner cell walls. Stomata are superficial with outer lips on the guard cells.

Mesophyll

Chlorenchyma is present as 2–5 layers of palisade cells, elongated to 2–4 times as long radially as tangentially in the outer layers, but tending to become isodiametric in the inner cell layers. Very large, thin-walled, non-chlorenchymatous cells occur in the central region, but they disintegrate with age to form an air canal.

Vascular bundles

Vascular bundles are arranged in a single ring at the edge of the chlorenchyma, with the xylem pole projecting into the air canal.

An incomplete outer sheath of a single layer of parenchyma surrounds the vascular bundles, although in *D. muscoides* two layers may be present at the flanks of some of the larger vascular bundles. An inner sheath of sclerenchyma is present either as a complete sheath of 3–7 cell layers, but with a smaller number of layers on the flanks between the poles than at the poles, or it is present only as caps on the xylem and phloem poles.

Sclerenchyma

Sclerenchyma is represented only by the vascular bundle sheaths.

Air spaces

A single, large central air canal is present in mature leaves.

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Figure 3.9 *Distichia* leaf blades. A *D. acicularis*. B *D. muscoides*. C *D. acicularis* vascular bundle with xylem pole projecting into central air canal, and mesophyll showing a tendency for the inner cell layers to become less elongated than the outer layers. D *D. muscoides* vascular bundle.
Anatomical description of leaves of Juncus

Of the genera of Juncaceae, *Juncus* shows the greatest anatomical variation, and most of the organs show the full range of variation which is described for Juncaceae.

Leaf sheaths

Mesophyll

The sheaths of most species have a small proportion of ground tissue compared to the area of the sheath. It is often present as 1–4 cell layers on the abaxial and adaxial sides, extending towards the the centre at intervals to form longitudinal septae beween the air canals. In some taxa, e.g. *Juncus* sect. *Juncus* which does not have air canals, more cell layers are present, the number varying according to the size of the sheath.

The cells of the ground tissue are usually isodiametric or only slightly elongated in shape, but they may be slightly lobed with small intercellular air spaces, or more rarely stellate with larger intercellular air spaces. Where lobed or stellate cells occur, the outer cell layers are usually unlobed or have shorter lobes than those of the inner layers.

In some species chlorenchyma is clearly present, often also with parenchyma present. The chlorenchyma may occur on both sides of the sheath, or just the abaxial side. The cells are usually more or less isodiametric, although in some species they may be elongated either radially or tangentially, or rarely lobed to stellate in shape. When chlorenchyma is present only on the abaxial side, parenchyma is present on the adaxial side, with cells which are often elongated tangentially, but sometimes isodiametric, or lobed to stellate in shape. The cells of the parenchyma are often larger than those of the chlorenchyma, but in some species they may be smaller.

Some species also have plates of cells forming transverse septae across the air canals, which are usually formed of a continuation of the layers of chlorenchyma surrounding small vascular bundles, but the cells may be more lobed or stellate in shape than the rest of the chlorenchyma. The septae usually occur at different levels in each canal to those of other canals, so the sheath is rarely divided across the entire width at a single point.

Vascular bundles

Vascular bundles are usually arranged in a single row, alternating with air canals in the centre of the sheath. In several sections of *Juncus* subgen. *Juncus*, and in *J.* subgen. *Agathryon* sect. *Forskalina*, additional, smaller vascular bundles occur abaxially of the air canals. In some species where this arrangement is seen, some small vascular bundles may occur directly abaxially of the largest vascular bundles. In other species several rows of vascular bundles may be identified, particularly in species with leaves which grow very large, e.g. *J. subulatus*.

In *Juncus* sect. *Juncus* and in *J. squarrosus*, vascular bundles are scattered throughout the section, and do not alternate with air canals in a recognisable pattern. The central row contains the largest vascular bundles, and the size of the bundles decreases in the rows that occur abaxially and adaxially. In species which have transverse septae, small vascular bundles also occur throughout the septae.

Vascular bundles may have a parenchymatous outer sheath which can be distinguished by the different size or rounder shape of the sheath cells to those of the ground tissue. In other species a parenchyma sheath is difficult to distinguish from the surrounding ground tissue, and in some species a parenchyma sheath cannot be distinguished at all.

An inner bundle sheath consists of several (2-10) cell layers of sclerenchyma, forming a complete sheath on the larger vascular bundles. There may be a similar number of cell layers in all areas of the sheath, or there may be fewer layers at the flanks between the xylem and phloem poles than there are at the poles. Smaller vascular bundles may also have a complete sheath, or they may have caps of sclerenchyma on the xylem and phloem poles, or just the xylem pole. Very rarely a sclerenchyma cap may be present only on the phloem pole (*Juncus squarrosus*).

Sclerenchyma

In some species sclerenchyma is only represented by the bundle sheaths. In *Juncus trifidus* the sclerenchyma of the bundle sheaths is extended to the adaxial epidermis near the sheath margins to form girders. The width of the extension increases towards the epidermis to form a triangle, with a flat edge against the epidermis and a 'corner' formed by the

vascular bundle. In most species no extensions of the bundle sheaths occur to the epidermis. However, in many species the vascular bundles occupy a large proportion of the width of the leaf sheath, and the bundle sheaths may then occur next to the epidermis, although they may consist of a similar number of cell layers all around the sheath, with no additional layers forming a girder.

In other species circular strands of sclerenchyma occur among the vascular bundles, similar in appearance to small vascular bundles and often forming a continuation of the row of vascular bundles, but not associated with any vascular tissue. Usually they occur near the margins of the leaf, but in *J. squarrosus* they occur on the abaxial and adaxial sides of the sheath. In both cases they occur where the smallest vascular bundles might be expected to be found.

Air spaces

Longitudinal air canals are present in most species as a single row alternating with the vascular bundles, often occupying a large proportion of the area of the sheath. They may be continuous, or interrupted by transverse septae, which usually occur at different levels in each longitudinal canal. Rarely (*Juncus* subgen. *Juncus*) no air canals are present, but irregular and poorly-defined air spaces may be formed by the deterioration of ground tissue in the central regions of the sheath.

Leaf blades

Epidermis

The abaxial epidermal cells are usually isodiametric, but in some species they may be slightly elongated up to approximately 1.5 times as long tangentially or two times as long radially. The outer cell walls are usually thickened, though they may be thin. Anticlinal and inner cell walls may show some thickening, but are usually thin. The adaxial epidermal cells, when present, are often larger than the abaxial epidermal cells, sometimes bulliform, or they may be similar in size. They are isodiametric or slightly elongated radially. The outer adaxial cell walls may show similar thickening to the outer abaxial cell walls, or they are thinner, and the anticlinal and inner cell walls are thin.

Stomata are usually only present on the abaxial surface, but rarely they are also present on the adaxial surface. They are usually superficial, but sometimes sunken from the leaf surface.

Mesophyll

In flat-leaved species chlorenchyma is usually present throughout the ground tissue, but in some species it is present only on the abaxial side of the leaf, and parenchyma is present on the adaxial side. In terete-leaved species it is usually present in a ring around the outside of the leaf, sometimes with a central air canal directly inside the chlorenchyma layers, and sometimes with parenchyma inside the layers of chlorenchyma.

The chlorenchyma may consist of palisade mesophyll, or the cells may be only slightly elongated to isodiametric in shape. When the cells are elongated, they tend to become less elongated or isodiametric in the innermost layers. In specimens with isodiametric mesophyll cells they may have a spongy arrangement, especially in the inner portions of the leaf, or they may be closely arranged with no conspicuous air spaces. There is no clear differentiation between spongy and palisade mesophyll. In some species the mesophyll cells are sometimes lobed, with small intercellular air spaces, or less frequently stellate, with large intercellular air spaces. In species with septate air canals, the septae usually consist of lobed or stellate cells.

When present, parenchyma cells tend to be larger than the chlorenchyma cells, and they are usually isodiametric, but they may be lobed to stellate in shape.

Vascular bundles

The arrangement of vascular bundles shows the full range of variation which is described for Juncaceae.

Vascular bundles often have an outer sheath consisting of a single, continuous or interrupted layer of parenchyma cells, but it is sometimes difficult to distinguish from the surrounding tissue in species where the vascular bundles are surrounded by parenchyma.

An inner sheath usually consists of 1–4 cell layers of sclerenchyma, less frequently to eight layers. It may be complete, but with more cell layers on the xylem and phloem poles than on the flanks, or it may be present only as caps on the xylem and phloem poles. When it is present as caps, the outer parenchymatous sheath is sometimes present only at the flanks between the poles. Smaller vascular bundles tend to have less sclerenchyma associated with them than the larger ones. When the larger bundles have complete sclerenchyma sheaths, the smaller ones often only have caps of sclerenchyma. Rarely, the smaller vascular bundles have no sclerenchyma associated with them.

Air spaces

Some species do not have air canals, but the spongy mesophyll tends to form very large air spaces between the vascular bundles. Some variation may also be seen between specimens with spongy mesophyll and poorly defined air canals, and specimens of the same species with palisade mesophyll and well-defined air canals. In *Juncus* sect. *Juncus* no air canals are present, but intercellular air spaces are present between stellate cells.

Tannins

Cells containing tannins were not observed.

Anatomical descriptions of subgenera of Juncus

Most of the variation described for *Juncus* is represented in each of its two subgenera. Detailed descriptions of the subgenera are unnecessary, as they would differ very little from the description of the genus. Points where they do differ from the genus description are given.

Juncus subgenus Agathryon

Leaf blades

Air spaces

Air spaces may be represented only by the intercellular air spaces in spongy mesophyll in some specimens, but air canals are present in others. Air canals are always continuous.

Anatomical description of Juncus section Forskalina

Leaf sheaths Fig. 3.10A-C

Epidermis

The abaxial epidermal cells are isodiametric. The outer walls are very thick, and anticlinal and inner walls are thin. The adaxial epidermal cells are approximately 2–3 times as long tangentially as radially, and all walls are thin.

Stomata are frequent on the abaxial surface, and are sunken from the leaf surface. The guard cells have indistinct or absent lips, but the walls of the surrounding epidermal cells project over the stomata.

Mesophyll

Several (2–4) cell layers of chlorenchyma are present on the abaxial side of the leaf sheath. They are usually isodiametric or slightly elongated tangentially. On the adaxial side are 3–4 cell-layers of parenchyma, which also extend towards the abaxial side as longitudinal septae, 5–7 cell layers wide, between the central air canals.

Some stellate cells occur in the central region. In the transverse sections examined stellate cells were only observed on the adaxial side of the central air canal, near the junction between the leaf sheath and leaf blade, and the remainder of the central region consisted of an air canal formed by the breakdown of pith.

Vascular bundles

Vascular bundles are usually arranged in one row, but very small vascular bundles may occur close to the largest bundles, usually laterally and slightly abaxially of them, very rarely directly abaxially of them. The largest vascular bundles occur in the parenchyma which forms bridges between the abaxial and adaxial sides, and alternate with air canals. The smaller ones usually occur on the abaxial side of the air canals.

An outer vascular bundle sheath consists of a single layer of parenchyma, which may sometimes be difficult to distinguish from the surrounding ground tissue, and appears to be absent on the smallest vascular bundles near the sheath margins. An inner sheath of several layers of sclerenchyma is present, which may be thicker (4–7 cell layers) on the xylem and phloem poles of large vascular bundles than on the flanks, or it may consist only of caps on the xylem and phloem poles on small vascular bundles.

Sclerenchyma

Some sclerenchyma fibres are present near the margins of the sheath, forming strands of irregular outline. They appear to be a continuation of the row of vascular bundles. No distinguishable outer sheath is present, but in this respect they are similar to the smallest vascular bundles near the sheath margins.

Air spaces

A series of large air canals alternate with the largest vascular bundles.

Leaf blades Fig. 3.10D-G

Outline

Leaf blades are terete and unifacial, with irregularly ridged or grooved outlines.

Epidermis

The epidermal cells are isodiametric to slightly elongated tangentially, and have very thick outer walls, while anticlinal and inner walls are thin. Stomata are sunken from the leaf surface. The guard cells have indistinct or absent lips, but the walls of the surrounding epidermal cells form papillae which project over the stomata.

Mesophyll

The outer cell layers consist of chlorenchyma, but were frequently compressed in some areas in the specimen examined, resulting in the ridged appearance of the leaf outline. When not compressed, three cell layers could be distinguished of palisade mesophyll, the

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cells being 3–5 times as long as wide. 4–6 cell-layers of parenchyma occur inside the layers of chlorenchyma, surrounding a large central air canal.

Vascular bundles

Vascular bundles are usually arranged in one ring, but some very small vascular bundles may occur close to the largest bundles, usually laterally and slightly abaxially of them. The larger vascular bundles occur in the parenchyma, but the phloem pole is directly adjacent to the outer layer of chlorenchyma. The phloem pole of smaller vascular bundles is adjacent to or embedded in the chlorenchyma layers, while the xylem pole is in the parenchyma.

Vascular bundle sheaths have an outer, single cell-layer of parenchyma which is difficult to distinguish from the surrounding ground tissue, except where it is adjacent to chlorenchyma. An inner sheath of several layers of sclerenchyma is present, which may be thicker (3–4 cell layers) on the xylem and phloem poles of large vascular bundles than on the flanks, or it may only consist of caps on the xylem and phloem poles on smaller vascular bundles.

Sclerenchyma

Sclerenchyma is present only as sheaths around the vascular bundles.

Air spaces

A single, large air canal is present in the centre of the leaf.



Figure 3.10 *Juncus subulatus*. A–C Leaf sheaths. B Sclerenchyma strands near the margin. C Abaxial epidermis and vascular bundles. D–G Leaf blades. E Abaxial epidermis and chlorenchyma. F Stellate cells near junction between leaf sheath and leaf blade. G Vascular bundle in parenchyma layer, with two small lateral bundles, and abaxial chlorenchyma.

Anatomical description of leaves of Juncus section Steirochloa

Leaf sheaths Fig. 3.11

Epidermis

Epidermal cells are usually similar in size and shape to the cells of the ground tissue on each side or sometimes slightly smaller, being slightly elongated tangentially on the adaxial side, and isodiametric on the abaxial side. The epidermal cells of both surfaces may become smaller at the margins. The outer cell walls of the abaxial epidermis are thickened, anticlinal walls may be moderately thickened or thin, and inner walls are thin.

Stomata are present on the abaxial surface, but very infrequent. They were observed in a very small proportion of the sections taken of *J. capillaceus* and *J. trifidus*, and were not observed in the sections taken of *J. squarrosus*. They are superficial with a small outer lip on the guard cells.

Mesophyll

Mesophyll consists of 1–2 cell layers of isodiametric cells on the abaxial and adaxial sides in *J. capillaceus* and *J. trifidus*, but numerous layers, varying in number according to the size of the leaf, are present in *J. squarrosus*. They are usually isodiametric, but they may be slightly irregular in outline with small intercellular air spaces.

Vascular bundles

Vascular bundles are present in a single layer in *J. capillaceus* and *J. trifidus*, alternating with large air canals in the centre of the leaf. In *J. squarrosus* they are present in several rows, with the largest bundles near the adaxial surface.

The vascular bundles have an outer sheath consisting of a single layer of parenchyma cells, sometimes interrupted. An inner sheath of sclerenchyma forms a complete sheath on larger vascular bundles, consisting of a similar number of cell layers (3–8) all around the vascular bundles, or with fewer layers at the flanks between the xylem and phloem poles. The smaller vascular bundles have less sclerenchyma, sometimes still forming a complete sheath, though there may be fewer cell layers at the flanks between the xylem and phloem

poles than the poles. Sometimes the sclerenchyma is only present as caps on the xylem and phloem poles of smaller vascular bundles, or just the phloem pole (*J. squarrosus*) or xylem pole (*Juncus trifidus*).

Sclerenchyma

In addition to the inner vascular bundle sheaths, sclerenchyma is present in some species as strands which are usually circular in outline, but variable in size, up to 11 cells in diameter. In *Juncus squarrosus* they occur among the smallest vascular bundles on the adaxial and abaxial sides of the leaf sheath, and in *J. capillaceus* and *J. trifidus* they occur at either end of the row of vascular bundles, near the margins. Sclerenchyma strands are absent in *J. gerardii* Loisl..

In *Juncus trifidus* the sheaths of the vascular bundles near the margins are extended to the adaxial epidermis to form girders, and girders which are not associated with vascular bundles are also present near the margins.

Air spaces

Air canals are usually arranged in a single row alternating with the vascular bundles. In *J. squarrosus* a small number of air canals are present in a single row in the centre of the leaf, or slightly closer to the abaxial side than the adaxial side.

3.3 Results



Figure 3.11 Juncus sect. Steirochloa leaf sheaths. A J. trifidus. B J. squarrosus. C J. capillaceus abaxial epidermis and vascular bundle. D J. squarrosus vascular bundle. E J. squarrosus vascular bundle with sclerenchyma cap on phloem pole. F J. trifidus adaxial girder (g) formed by extension of vascular bundle sheath. G J. trifidus girders (g) not associated with vascular tissue. H J. squarrosus sclerenchyma strand.

Leaf blades Fig. 3.12

Outline

Leaves are oval with a concave adaxial surface, or a thickened v-shape, with margins which are either rounded or acute. Leaves are bifacial, but *Juncus squarrosus* shows reduction in the adaxial surface to a small proportion of the total leaf surface, whereas the adaxial surface of *J. capillaceus* and *J. trifidus* extends for most or all of the width of the leaf.

Epidermis

The abaxial epidermal cells are isodiametric to slightly elongated tangentially. The adaxial epidermal cells are isodiametric, and either slightly larger than the abaxial epidermal cells or bulliform. They may show a slight reduction in size next to sclerenchyma girders, e.g. in *J. capillaceus*. All outer cell walls are moderately thickened, anticlinal cell walls are thin or slightly thickened, and inner cell walls are thin. Stomata are superficial and present on the abaxial surface only.

Mesophyll

The ground tissue consists of isodiametric or slightly irregularly shaped cells, which have few or no intercellular air spaces in some specimens, but in other specimens spongy mesophyll is present.

In *J. capillaceus* and *J. squarrosus* parenchyma is present in several (1-5) cell layers on the adaxial side. The parenchyma cells are usually very similar in appearance to those of the epidermis, but with all walls thin.

Vascular bundles

Vascular bundles are usually arranged in a single row, but in several rows in *J. squarrosus*. A layer of thickened cells occur between the xylem and phloem tissue in *J. capillaceus* and *J. trifidus*, but not in *J. squarrosus*.

An outer sheath consists of a single layer of parenchyma, and it may be complete or interrupted. An inner sheath of sclerenchyma is present, usually with more cell layers (3-7) on the xylem and phloem poles than on the flanks. The inner sheath of smaller vascular bundles may have caps on the xylem and phloem poles only, or sometimes (*J. squarrosus*) on the phloem pole only.

Sclerenchyma

In *J. capillaceus* and *J. gerardii* the inner sheath of the largest vascular bundles extends from the phloem pole to the abaxial epidermis to form a girder, but in *J. trifidus* and *J. squarrosus* it does not. Most species also have a girder next to the epidermis of the leaf margins which is not associated with a vascular bundle, but *Juncus trifidus* does not.

Air spaces

Air spaces are present in spongy mesophyll, which often tends to form very poorly-defined air canals between the vascular bundles. In specimens where the mesophyll is not spongy, well-defined air canals occur between vascular bundles.

3.3 Results



Figure 3.12 Juncus sect. Steirochloa leaf blades. A J. capillaceus. B J. trifidus. Arrowheads indicate points where anticlinal divisions of the epidermis have occurred. C J. squarrosus reduced adaxial surface. Arrows indicate margins of adaxial surface. D J. trifidus leaf margin. E J. squarrosus vascular bundle. F J. trifidus vascular bundle and abaxial girder. H J. squarrosus vascular bundle sclerenchymatous phloem cap.

Anatomical description of leaves of Juncus section Tenageia

Leaf sheaths Fig. 3.13A, B

Epidermis

Epidermal cells are similar in size and shape to the cells of the ground tissue on each side, being isodiametric on the abaxial side, and isodiametric, irregularly shaped or slightly elongated tangentially on the adaxial side. The outer cell walls of the abaxial epidermis are thickened, and all other cell walls are thin. No stomata were observed.

Mesophyll

The ground tissue consists of 1-3 cell layers on the abaxial and adaxial surfaces, with air spaces in between. The cells on the abaxial side are isodiametric, while the cells on the adaxial side are slightly smaller, and isodiametric or slightly elongated tangentially.

Vascular bundles

The vascular bundles alternate with air canals in the centre of the leaf. An outer parenchymatous sheath is difficult to distinguish from the surrounding tissue. A sclerenchymatous sheath consists of a complete ring around larger vascular bundles, with more cell layers (3–4) on the xylem and phloem poles than on the flanks between the poles. Smaller vascular bundles may have sclerenchyma caps on the xylem poles only, or on both xylem and phloem poles.

Leaf blades Fig. 3.13C-F

Outline

Leaves are bifacial, and oval with a slightly concave adaxial surface.

Epidermis

The adaxial epidermal cells are isodiametric, reducing slightly in size towards the leaf margins. The adaxial epidermal cells are very large, appearing similar to bulliform cells. The outer cell walls of both epidermal surfaces are moderately thickened, and the anticlinal and inner cell walls are thin. Stomata are present on the abaxial surface only, and are superficial.

Mesophyll

The ground tissue consists of spongy mesophyll of isodiametric cells, with larger air spaces towards the middle portions of the leaf.

Vascular bundles

Three large vascular bundles are present, alternating with four small vascular bundles. The large vascular bundles have an interrupted outer sheath of a single layer of parenchyma cells, and an inner sheath consisting of 1–4 cell layers of sclerenchyma forming caps on the xylem and phloem poles. The smaller vascular bundles usually have a complete sheath consisting of 1–2 layers of parenchyma cells, and no sclerenchyma.

Sclerenchyma

In addition to the vascular bundle sheaths, small sclerenchyma girders which are not associated with vascular bundles are present next to the epidermis at the leaf margins.

Air spaces

No air canals are present, and air spaces are present only as the large intercellular spaces occurring in the spongy mesophyll.

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3.3 Results



Figure 3.13 *Juncus bufonius*. A Leaf sheath. B Leaf sheath vascular bundle. C Leaf blade. D Stoma and marginal sclerenchyma strand. E Vascular bundle. F Small vascular bundle with parenchymatous sheath but no sclerenchyma.

Anatomical description of leaves of Juncus subgenus Juncus

Leaf sheaths

Air spaces

Air canals are present in most species, and may be either continuous or interrupted by transverse septae. In section *Juncus* air spaces are represented only by intercellular air spaces between stellate cells.

Leaf blades

Air spaces

Air spaces may be represented only by intercellular spaces in spongy mesophyll or between lobed or stellate cells, or by air canals. Air canals may be either continuous or interrupted by transverse septae.

Anatomical description of leaves of Juncus section Graminifolii

Leaf sheaths Fig. 3.14A–E

Leaf sheaths were observed for J. cyperoides only.

Epidermis

The epidermal cells are usually isodiametric, sometimes irregular in shape, and the adaxial cells are larger than the abaxial cells. The adaxial cells tend to be reduced to the size of the abaxial cells at the margins. All cell walls are thin. Stomata are superficial.

Mesophyll

The mesophyll consists of a single cell layer of chlorenchyma on both abaxial and adaxial sides next to the epidermis, and 1-2 cell layers surrounding the vascular bundles, which alternate with large air canals. The chlorenchyma cells may disintegrate with age, leaving areas with no ground tissue so the epidermal cell layers are directly adjacent to the air canals, but the chlorenchyma tends to persist next to the phloem poles. Near the margins the ground tissue consists of 1-2 cell layers of parenchyma.

Vascular bundles

Vascular bundles are present in a single row, and occasionally two of the longitudinal bundles are linked by a small transverse bundle, blocking the air canal transversally at that point.

An outer sheath consists of a single layer of parenchyma cells, usually forming a complete sheath, and distinguishable from the mesophyll by the rounder shape of the sheath cells. An inner sheath consists of several layers of sclerenchyma cells, which is sometimes thicker (4–10 cell layers on large vascular bundles) at the xylem pole than at the phloem pole (2–4 cell layers on large vascular bundles), or it may be present as caps on the xylem and phloem poles only, or just the xylem pole.

Air spaces

Air canals alternate with vascular bundles. Occasionally individual air canals may be blocked by a transverse vascular bundle.

Leaf blades Fig. 3.14F–H

Outline

Leaves are dorsiventrally flattened and bifacial, and v-shaped, or flat with the margins curved upwards adaxially. The margins vary in outline between species, from acute to rounded. There may be little or no dorsiventral differentiation in some species, while others show some dorsiventral differentiation.

Epidermis

Epidermal cells are usually isodiametric in shape, though they may be irregularly shaped or slightly elongated radially. All cell walls are thin, or the outer cell walls may be moderately thickened. Stomata are superficial, and have a small outer lip on the guard cells. In *Juncus caespiticius* E.Mey. and *J. cyperoides* the adaxial cells are larger than the abaxial cells, up to about three times the width and four times the length of the abaxial cells in *J. caespiticius*. The adaxial cells are reduced to the size of the abaxial cells at the margins. Stomata are present on the abaxial surface only.

Mesophyll

The mesophyll cells may have a dense arrangement with few or no intercellular air spaces throughout the leaf in some specimens, and in others spongy mesophyll is present in the inner layers. The cells are usually isodiametric or irregularly shaped, although in some species slightly elongated cells may occur in the outer layers, but becoming isodiametric in the inner layers.

Vascular bundles

Vascular bundles are arranged in a single row. Vascular bundles have an outer sheath of parenchyma cells, which may be complete or interrupted, sometimes present only at the

flanks between the xylem and phloem poles on bundles where the sclerenchymatous inner sheath forms caps on the poles. The parenchymatous outer sheath may be less frequently interrupted on smaller vascular bundles than on larger ones. An inner sclerenchymatous sheath consists of 2–8 cell layers, and is complete, or present as caps at the xylem and phloem poles only.

Sclerenchyma

Sclerenchyma is represented only by the inner bundle sheaths.

Air spaces

In leaves with a mesophyll which is not spongy, well-defined air canals alternate with vascular bundles. In leaves with spongy mesophyll, the mesophyll tends to form poorly defined air canals in the centre of the leaf, or the entire leaf may be filled with spongy mesophyll which has very large air spaces in the areas between vascular bundles. Some variation may occur between specimens of a single species.



Figure 3.14 *Juncus* sect. *Graminifolii*. A–E Leaf sheaths. B *J. cyperoides* sheath margin. C *J. cyperoides* abaxial chlorenchyma and stoma, and small vascular bundle with adaxial sclerenchyma cap, and parenchyma sheath adjacent to adaxial epidermis. D *J. cyperoides* vascular bundle. E *J. cyperoides* air canal blocked by layer of chlorenchyma associated with a transverse vascular bundle. F–H Leaf blades. F *J. cyperoides* leaf tip with no parenchyma or sclerenchyma. G *J. caespiticius* bulliform adaxial cells and spongy mesophyll. H *J. cyperoides* adaxial cells (a) slightly larger than abaxial cells.

Anatomical description of Juncus section Iridifolii

Leaf sheaths Fig. 3.15

Outline

The leaf sheaths are a laterally compressed v-shape. The 'arms' of the v-shape are bifacial, but the adaxial surfaces become fused as they approach the mid-rib, where the sheath becomes unifacial.

Epidermis

The abaxial epidermal cells are rounded and very variable in size, with large cells interspersed with smaller ones, giving an uneven texture to the leaf surface. Some cells may be further enlarged or elongated to form small papillae, particularly near the midrib. The cells of the adaxial surface are larger than those of the abaxial surface, or similar in size. All epidermal cells are thin-walled.

Stomata are present on the abaxial surface, and are sunken from the leaf surface, with small outer and inner lips on the guard cells.

Mesophyll

Several cell layers (3–5) of chlorenchyma occur abaxially in the bifacial parts of the sheath, and outermost in the unifacial part, but air canals occupy the majority of the sheath. The outer cell layers may be slightly elongated radially, being isodiametric to 1.5 times as long as wide, and some cells may be very slightly and irregularly lobed, leaving small intercellular air spaces. Inner cell layers tend to be isodiametric or slightly and irregularly lobed, although the cell layer next to the vascular bundles may have cells which are elongated tangentially, to approximately five times as long as wide in *J. xiphioides* E.Mey.. 1–2 cell layers of parenchyma occur on the adaxial side. Bridges of a small number of parenchymatous cell layers also extend from one side to the other, dividing the air canals longitudinally.

Plates of tissue are associated with transverse vascular bundles, and these form septae across the central air canals. These consist of cells which are slightly larger than those of the rest of the chlorenchyma and with longer lobes, sometimes stellate in shape, resulting in larger intercellular air spaces.

Vascular bundles

Vascular bundles are arranged in a single row in the bifacial parts, and two rows in the unifacial part of the sheath. The large vascular bundles alternate with the air canals, and smaller bundles are present on the abaxial side of the air canals. Small vascular bundles are also present in the transverse septae.

A parenchymatous outer sheath is easily distinguishable from the surrounding chlorenchyma, consisting of a single cell layer which may be complete or interrupted. An inner sheath consists of small amounts (2–3 cell layers) of sclerenchyma forming a complete sheath on larger vascular bundles, or caps on the phloem and xylem poles, or sometimes just on the xylem poles on small vascular bundles. When it forms a complete sheath it is always thicker on the phloem and xylem poles than on the flanks between the poles.

Air spaces

The central regions of the sheath are hollow, containing several discontinuous longitudinal air canals, each canal being divided by transverse septae which may occur at different levels in the leaf to those of other air canals.



Figure 3.15 *Juncus* sect. *Iridifolii*. Leaf sheaths. A *J. xiphioides*. **B** *J. xiphioides* vascular bundle with sclerenchymatous sheath forming adaxial girder. **C** *J. xiphioides* transverse septa showing lobed cells. **D** *J. xiphioides* papillose epidermis and midrib. **D** *J. xiphioides* longitudinal septa.

Leaf blades Fig. 3.16

Outline

Leaves are ensiform, being laterally compressed, with the fused margins forming one edge, and the midrib forming the other edge. They are unifacial, having lost the adaxial surface.

Epidermis

The epidermal cells are round and very variable in size, with large cells interspersed with smaller ones, giving a slightly uneven texture to the leaf surface, but they remain within a similar range of sizes around the circumference of the leaf. Some cells may be further enlarged or elongated to form small papillae, particularly on the edges.

All cell walls of the epidermis are thin. Stomata are slightly sunken from the leaf surface, and small outer lips are present on the guard cells.

Mesophyll

Chlorenchyma occupies the outer part of the leaf in approximately 4–7 cell layers. The cells are elongated up to two times as long radially as tangentially in the outer layers, and tending to become isodiametric in inner layers, although elongated cells tend to radiate away from the larger vascular bundles. The cells are sometimes slightly lobed, with very small intercellular air spaces.

The longitudinal septae dividing the air canals from one another consist of parenchymatous cells which are isodiametric in transverse section, and elongated axially. Transverse septae are present dividing the air canals, consisting of chlorenchymatous cells which are irregularly lobed, with larger intercellular air spaces than in the rest of the mesophyll.

Vascular bundles

Vascular bundles usually occur in a single ring, with the phloem poles adjacent to or embedded in the mesophyll, and the xylem poles projecting into the air canals. Small vascular bundles also occur in both the longitudinal and transverse septae. Vascular bundles have a conspicuous outer sheath of a single layer of large parenchyma cells, usually complete. An inner sheath of sclerenchyma is present, often consisting of 1-3 cell layers forming either a complete sheath or caps on the xylem and phloem poles of the larger vascular bundles, and sometimes absent from the smaller vascular bundles.

Sclerenchyma

Sclerenchyma is present only in the vascular bundle sheaths.

Air spaces

Air spaces occur as several longitudinal canals, occupying the majority of the volume of the leaf. Transverse septae are present, dividing each longitudinal canal, often with each longitudinal canal being divided at different levels to the other canals.



Figure 3.16 Juncus sect. Iridifolii leaf blades. A J. ensifolius. B J. ensifolius edge formed from fused margins. C J. ensifolius epidermis and stoma. D J. ensifolius vascular bundle and mesophyll. E J. ensifolius longitudinal section showing transverse septa, and air spaces in mesophyll which are not visible in transverse section.

Anatomical description of leaves of Juncus section Juncus

Leaf sheaths Fig. 3.17

Epidermis

The abaxial epidermal cells are isodiametric, with the outer walls very thick, and the inner and anticlinal walls thickened to a lesser degree. The adaxial epidermal cells are elongated to approximately 1.5–2 times as long tangentially as radially, and all walls are thin. Stomata are not present on the leaf sheath.

Mesophyll

On the abaxial side, the outermost cell layers of the ground tissue may show moderate cell wall thickening, with isodiametric cells. The cells in the next 1–2 layers are larger, more or less isodiametric in shape, but the inner of these may show a tendency to become slightly lobed in shape. Next to these layers are 4–7 layers of lobed to stellate cells with large intercellular air spaces. Next to the larger vascular bundles more layers of these cells are present, the stellate cells becoming more elongated in shape and tending to radiate outwards from the vascular bundles.

In the centre of the sheath the ground tissue consists of larger, very thin-walled cells, which are fragmented. On the adaxial side are 5–6 cell layers of unlobed parenchyma cells, which are elongated approximately 1.5–3 times as long tangentially as radially.

Vascular bundles

Vascular bundles are in several layers, with the smallest bundles on the abaxial and adaxial sides, and the largest in the centre of the section. Cells with thick walls are present in 1-2 layers between the xylem and phloem.

The vascular bundles have an outer sheath of a single layer of parenchyma cells, which may have moderately thickened cell walls, and an inner sheath consisting of a complete

3.3 Results



Figure 3.17 *Juncus maritimus* leaf sheaths. A Leaf sheath. B Adaxial epidermis, parenchyma and sclerenchymata strand. C Lobed/stellate cells become elongated towards the vascular bundles (right). D Vascular bundle.

sheath of sclerenchyma, of 6–9 cell layers at the poles of the large vascular bundles, and slightly thinner (3–5 cell layers) at the flanks between the xylem and phloem poles.

Sclerenchyma

In addition to vascular bundle sheaths, sclerenchyma is sometimes present as circular strands arranged in one row adaxially, between the lobed and unlobed layers of parenchyma cells. The largest are up to 11 cells in diameter, and they become smaller towards the margins. They are surrounded by a single sheath of parenchyma cells. Strands which look similar to these also occur on the abaxial side, but less frequently, and they are easily confused with similar-looking vascular bundles which contain very small amounts of vascular tissue surrounded by many layers of sclerenchyma.

Air spaces

Parenchyma cells between the largest vascular bundles show a tendency to deteriorate with age, forming some small air canals, or no air canals are present.

Leaf blades Fig. 3.18

Outline

Leaf blades are round in cross-section and unifacial.

Epidermis

Epidermal cells are rounded and isodiametric in cross-section, with the outer cell walls very thick, the anticlinal walls moderately thickened, and the inner walls thin. Stomata are superficial, with small and indistinct inner and outer lips on the guard cells.

Mesophyll

Palisade mesophyll is present as 4–7 layers of cells which are elongated to approximately four times as long as wide. The inner layers may be lobed with small intercellular air spaces. Within these layers are approximately 6–8 cell layers of small stellate cells, while the remainder of the ground tissue in the centre of the leaf consists of rounded parenchyma

cells with small intercellular air spaces, which are up to three times the diameter of those in the outer layers. These cells are sometimes fragmented.

Vascular bundles

Vascular bundles are scattered throughout the sections, with the smallest near the edge of the leaf and the largest near the centre. Cells with thick walls are present in 1-2 layers between the xylem and phloem.

The vascular bundles are surrounded by a continuous or interrupted parenchyma sheath in a single cell layer, which may be distinguishable from the surrounding parenchyma cells by the smaller size and more regular, rounded shape. An inner sheath is present as several layers of sclerenchyma, which is thicker at the xylem and phloem poles (4–7 cell layers in large vascular bundles) than at the flanks between the poles, or it is present only as caps at the xylem and phloem poles.

Sclerenchyma

Strands of sclerenchyma are present as girders next to the epidermis. They are triangular in cross-section, 6–12 cells wide next to the epidermis, extending through most of the depth of the palisade mesophyll, and each girder is separated by 4–7 mesophyll cells next to the epidermis.

Strands of sclerenchyma are also present in the central region of the leaf blade. These are circular to oval in outline, and surrounded by a sheath consisting of a single layer of parenchyma.

Air spaces

Air spaces are present only as intercellular spaces between the stellate or lobed cells.



Figure 3.18 *Juncus maritimus* leaf blade. A Leaf blade. B Sclerenchyma girders. C Stoma. D Small vascular bundles with abaxial sclerenchyma caps, and stellate/lobed cells (a). E Longitudinal section showing chlorenchyma in outer layers (right), vascular bundle with parenchymatous sheath, and lobed parenchyma in inner layers. F Vascular bundle.

Anatomical description of Juncus section Ozophyllum

Leaf sheaths Fig. 3.19

Epidermis

Very small ridges are present throughout the abaxial surface of *Juncus subulitepalus* Balslev, although they may be slightly more pronounced in the areas near large vascular bundles. Other species have smooth surfaces.

The abaxial epidermal cells are isodiametric or very slightly elongated radially. The outer walls are thickened, and the anticlinal and inner walls are thin. The adaxial epidermal cells are similar in appearance to, and indistinct from, the adaxial ground tissue, being isodiametric or slightly elongated tangentially, and larger than the abaxial epidermal cells. All adaxial epidermal cell walls are thin. Superficial stomata are present on the abaxial surface.

Mesophyll

Chlorenchyma is present only on the abaxial side. The cells are isodiametric or irregularly shaped, sometimes slightly elongated radially to approximately 1.5 times as long as wide in the outer layers. In the inner layers they are sometimes irregularly lobed with small intercellular air spaces. Parenchyma is present on the adaxial surface, with isodiametric and unlobed, or irregularly lobed or sometimes stellate cells, which are larger than the chlorenchyma cells.

The number of cell layers of the ground tissue varies according to species, the size of the leaf sheath, and the position relative to vascular bundles, as the number of layers on both abaxial and adaxial sides increases towards the larger vascular bundles in the inner layers, eventually extending towards the centre of the leaf from both sides to form longitudinal septae dividing the central air canals.

Transverse septae also occur across the air canals, often with the septae in each canal occurring at different levels to other canals. The cells of the septae are smaller than the rest of the chlorenchyma cells, and irregularly lobed to stellate.
Vascular bundles

Vascular bundles are arranged in one to several rows. The number of rows varies according to the size of the sheath, rarely with a single row present in small sheaths. The largest vascular bundles alternate with the air canals in a row. Further rows of vascular bundles occur abaxially, decreasing in size towards the abaxial surface. In the sheaths of large species such as *Juncus subulitepalus* there may be two rows of air canals, with the second row of vascular bundles alternating with the second row of air canals. Small vascular bundles also occur in the transverse septae.

An outer vascular bundle sheath of parenchyma is usually difficult to distinguish from the surrounding ground tissue, but is sometimes distinguishable by the smaller size and rounder shape of the sheath cells. An inner sheath of sclerenchyma usually forms a complete sheath of 2–6 cell layers on large vascular bundles, which is often thicker at the xylem and phloem poles than at the flanks, or it may only form caps on the xylem and phloem poles on smaller vascular bundles.



Figure 3.19 Juncus sect. Ozophyllum Leaf sheaths. A J. ecuadoriensis. B J. bulbosus. Vascular bundle (vb) abaxial of air canal. C J. subulitepalus vascular bundles in more than one layer. D J. subulitepalus abaxial epidermis with ridged surface, and slightly lobed chlorenchyma cells. E J. articulatus abaxial epidermis and lobed cells with small intercellular air spaces (space indicated by arrow). F J. articulatus vascular bundle. G J. subulitepalus transverse septa showing stellate cells and vascular tissue.

Leaf blades Fig. 3.20

Outline

Leaves are unifacial (but see section 3.4.1 *Reduction of the adaxial epidermis* regarding *J*. *bulbosus* L.), and round or oval in outline with or without abaxial or adaxial grooves.

Epidermis

The epidermal cells are isodiametric or slightly elongated to approximately 1.5 times as long radially as tangentially. The outer cell walls are thickened, the anticlinal walls may be moderately thickened or thin, and the inner walls are thin. Stomata are superficial with an outer lip on the guard cells.

Mesophyll

The outer 2–6 cell layers of the ground tissue consist of chlorenchyma. The cells are isodiametric or elongated, up to three times as long radially as tangentially in *Juncus articulatus* L., and less so in the other species examined. When elongated, the inner cell layers are often less elongated, or isodiametric or irregularly shaped. The cells of the outer layers may sometimes have very small lobes with small intercellular air spaces, and those of the inner layers are more frequently lobed, with slightly larger intercellular air spaces than those of the outer layers. The cells of the transverse septae tend to be more lobed than those of the remaining chlorenchyma, to stellate in shape, with larger intercellular air spaces.

Inside the chlorenchyma layers are several layers of parenchyma. In species with a single central air canal the parenchyma forms a ring 2-7 cell layers deep surrounding the air canal. In *J. bulbosus*, which has several longitudinal canals, the parenchyma is present in the centre of the leaf and also forms the longitudinal septae dividing the canals. The parenchyma cells are larger, but similar in shape to the cells of the inner layers of chlorenchyma, being isodiametric, irregularly shaped, or slightly lobed.

Vascular bundles

The vascular bundles are usually arranged in a single row, but in large-leaved species such as *Juncus subulitepalus* a second row may also be present, as small vascular bundles may occur abaxially of the largest vascular bundles of the first row. Small vascular bundles also occur in the transverse septae.

Sclerenchyma

Sclerenchyma is present only as vascular bundle sheaths.

Air spaces

A single central air canal is present in most species, which is always divided at intervals by transverse septae. In other species, several longitudinal air canals are present, each of which is divided by transverse septae, each canal usually being divided at different levels to other canals in the leaf.

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Figure 3.20 Juncus sect. Ozophyllum Leaf blades. A J. articulatus. B J. bulbosus. C J. articulatus mesophyll consisting of outer layers of chlorenchyma and inner layers of parenchyma. D J. subulitepalus vascular bundles in 2 layers.

Anatomical description of leaves of Juncus section Stygiopsis

Leaf sheaths Fig. 3.21A–C

Epidermis

The abaxial surface has small papillae, which become more pronounced near the margins.

The abaxial epidermal cells are isodiametric or slightly irregularly shaped, with very thick outer walls, and all other walls thin. The cell walls of the abaxial epidermis become smaller and the walls have more thickening at the margins, with the anticlinal and inner walls thickened as well as the outer walls. The adaxial epidermal cells resemble the ground tissue cells, being irregular in shape from isodiametric to slightly elongated to up to two times as long tangentially as radially, and with thin walls.

Stomata are present on the abaxial surface, and are superficial with outer lips on the guard cells.

Mesophyll

The ground tissue cells are irregularly shaped but usually more or less isodiametric, sometimes slightly lobed with small intercellular air spaces. They are present in 1-2 layers on the abaxial side and one layer on the adaxial side, with large air canals in between, bridged by a row of vascular bundles, each of which is surrounded by a further 1-2 layers of ground tissue.

Vascular bundles

The largest vascular bundles are arranged in a single row alternating with the air canals in the centre of the leaf. Smaller vascular bundles are also arranged abaxially to the largest air canals, with the xylem pole projecting into the air canal.

An outer vascular bundle sheath of parenchyma is difficult to distinguish from the surrounding ground tissue, but may sometimes be distinguished by a more regular shape than the surrounding tissue. An inner sheath of sclerenchyma is present as a complete

sheath, sometimes with more cell layers (3-5) at the xylem and phloem poles than at the flanks (1-2 cell layers).

Leaf blades Fig. 3.21D, E

Outline

Leaves are unifacial in the middle region of the blade (but see 3.4.1 *Reduction of the adaxial epidermis*). They are round or oval with an adaxial groove, and an indistinct abaxial groove.

Epidermis

The epidermal cells are isodiametric, with thickened outer walls, slightly thickened anticlinal walls, and thin inner walls. Stomata are superficial and an outer lip is present on the guard cells.

Mesophyll

Chlorenchyma forms 2–6 cell layers in a ring in the outer part of the leaf, but it is reduced to 2–3 cell layers at the adaxial side. The cells are usually isodiametric in shape, but those of the outermost layer may be up to two times as long as wide radially. Thin-walled cells form a bridge across the central air canals, but they show a tendency to deteriorate, leaving a bridge of broken cell walls with unclear cell structure.

Vascular bundles

Vascular bundles are arranged in a single ring around the leaf, in the inner cell layers of the chlorenchyma, with the xylem poles adjacent to the central air canals. Smaller vascular bundles occur on the adaxial side of the leaf than those on the abaxial side.

A complete outer sheath of a single cell layer of parenchyma is present. An inner sheath of sclerenchyma is present as a complete sheath on the larger vascular bundles, or as caps on the xylem and phloem poles of the smaller vascular bundles. It is usually thin (1–2 cell

layers) but more cell layers (up to five) may be present on the phloem pole of large vascular bundles.

Sclerenchyma

Sclerenchyma is present only in the vascular bundle sheaths.

Air spaces

Two large air canals are present in the centre of the leaf, divided by a bridge of parenchyma which crosses between the largest abaxial vascular bundle (midrib) and the adaxial side of the leaf.

3.3 Results



Figure 3.21 *Juncus triglumis*. A–C Leaf sheaths. B Abaxial epidermis near margin, with ridged surface. C Vascular bundle and adaxial epidermis. D–E Leaf blades. E Vascular bundle.

Anatomical description of leaves of Luzula

Leaf sheaths

Outline

Leaf sheaths always have fused margins to form a cylinder, with the adaxial surface forming the inside of the cylinder. The sheath is narrower at the adaxial side of the cylinder than at the abaxial side, sometimes membranous.

Epidermis

The epidermal cells range in shape from isodiametric to slightly elongated tangentially, rarely up to four times as long as wide on the adaxial surface. All epidermal cell walls are usually thin, but the outer walls may sometimes be slightly thickened. Stomata are sometimes absent from the leaf sheaths, but they are present on the abaxial surface in other species, and are superficial.

Mesophyll

The ground tissue always consists of isodiametric or irregularly shaped cells. Often they are parenchymatous, but young sheaths may contain chlorenchyma. A small number of cell layers is usually present, usually with 1–4 cell layers on the adaxial and abaxial surfaces. Large air spaces are present in between, bridged by vascular bundles, and with 1–3 cell layers of mesophyll surrounding each vascular bundle.

Vascular bundles

Vascular bundles are always arranged in a single ring. The bundles tend to become smaller and more widely spaced on the adaxial side of the sheath.

Vascular bundles always have an inner sheath of sclerenchyma. Larger vascular bundles usually have a complete sclerenchyma sheath, consisting of a similar number of cell layers all around the vascular bundle, or with more layers on the xylem and phloem poles than on the flanks. Smaller vascular bundles may have a sclerenchyma cap on both the xylem and phloem poles, or only on the xylem pole (e.g. *Luzula racemosa*), or phloem pole (e.g. *Luzula gigantea*).

Usually a parenchyma sheath cannot be distinguished from the surrounding tissue, but rarely it can be detected as a single, frequently interrupted layer of cells, or as 2–4 cells on the flanks between the xylem and phloem poles, particularly on vascular bundles where the inner sheath forms bundle caps.

Sclerenchyma

In most species sclerenchyma is present only as vascular bundle sheaths. In a few species strands of sclerenchyma may occur between the vascular bundles, similar in size and shape to the smaller vascular bundles, but consisting entirely of sclerenchyma identical to that of the vascular bundle sheaths, and not associated with any vascular tissue.

Air spaces

Air spaces are present as air canals alternating with vascular bundles, and often constitute a large proportion of the leaf sheath.

Leaf blades

Outline

The leaves are always dorsiventrally flattened and bifacial. They may be v-shaped or flat, sometimes with the margins rolled towards the adaxial surface, but some of the variation in the specimens examined may have occurred due to lab procedures.

Leaf margins are variable in shape, from acuminate to rounded to square or irregularly shaped, or thickened.

Epidermis

The abaxial epidermal cells are usually isodiametric or slightly elongated, up to two times as long tangentially as radially. The adaxial epidermal cells are usually isodiametric to slightly elongated, up to two times as long radially as tangentially. They are often larger than the abaxial cells, sometimes appearing similar to bulliform cells. All epidermal cell walls are often thin, but the outer walls are sometimes thickened. The cells at the margins may show more thickening of the outer walls than other cells.

Stomata are superficial, and are present on the abaxial epidermis only. In some species (e.g. *Luzula luzuloides* (Lam.) Dandy & E.Willm.) stomata may appear to occur on the adaxial surface near the margins, but Cutler (1969) interprets this to be due to an extension of the abaxial epidermis around the margins onto the morphologically adaxial surface, an interpretation which is accepted here (see Section 3.4.2 *Stomata*).

Mesophyll

The mesophyll always consists of isodiametric or slightly irregularly shaped cells. Other than differences between the abaxial and adaxial epidermis, little dorsiventral differentiation is evident.

In some specimens the mesophyll cells are closely packed with very small or no intercellular air spaces. In these specimens well-defined air canals occur in the centre of the leaf between vascular bundles. In others, the ground tissue in the central portion of the leaf consists of spongy mesophyll, being loosely packed cells with conspicuous intercellular air spaces. The spongy mesophyll usually tends to form very poorly defined air canals in the centre of the leaf.

Variation between leaves containing spongy mesophyll and leaves that do not may occur within a species, as specimens of *Luzula racemosa* examined showed both states. Both plants examined were collected at the same site and close together, but it is possible that the conditions varied over short distances, e.g. in terms of soil moisture content, as the ground was marshy but uneven. It is known that environmental conditions can affect the proportions of spongy mesophyll in leaves of certain other plant families (Esau 1965), and that xeromorphic leaves have a more strongly developed palisade tissue than mesomorphic leaves (Shields 1950). It is possible that a continuous variation between leaves with a ground tissue consisting of spongy mesophyll, and of leaves with no spongy mesophyll but with well defined central air canals, may occur in *Luzula* due to environmental conditions.

Vascular bundles

Vascular bundles always occur in a single layer, sometimes with large vascular bundles alternating with smaller ones.

An outer vascular bundle sheath consists of a single layer of parenchyma but it is sometimes poorly developed, or absent. It may be evident as 2–3 parenchyma cells on the flanks between the xylem and phloem poles of larger vascular bundles, particularly when the inner bundle sheath is thicker at the poles than at the flanks, but in other species it may be distinguishable as a single cell layer, frequently interrupted, around the vascular bundles. Where it is interrupted around the larger vascular bundles it may be less frequently interrupted or continuous around smaller bundles.

An inner vascular bundle sheath is usually present as several cell layers of sclerenchyma. It may surround the vascular bundles completely, though often consisting of more cell layers at the xylem and phloem poles than at the flanks between the poles, or it may consist only of caps on the xylem and phloem poles. It sometimes extends from the larger vascular bundles to the abaxial or adaxial epidermis to form girders.

Sclerenchyma

Many species have sclerenchymatous girders next to the epidermis formed by the inner vascular bundle sheaths. In addition to these, small amounts of sclerenchyma are often also present as girders next to the epidermis at the margins of the leaves; these are not associated with vascular bundles.

Air spaces

Continuous variation appears to occur between leaves with spongy mesophyll, with larger intercellular air spaces in the centre of the leaf which tend towards forming poorly defined air canals, and leaves with palisade mesophyll and well-defined air canals, as discussed in 'mesophyll'.

Anatomical description of leaves of Luzula section Alpinae

Leaf sheaths Fig. 3.22A, B

Epidermis

Epidermal cells of both surfaces are similar to the ground tissue cells, being isodiametric or slightly elongated tangentially, and with no cell wall thickening. Stomata are absent.

Mesophyll

Cells are isodiametric or irregularly shaped, and present in 1-2 layers on the abaxial and adaxial sides, and one layer or none surrounding the vascular bundles which are interspersed between large air spaces in the middle of the sheath.

Vascular bundles

The larger vascular bundles are surrounded by a complete sheath of 3–6 cell layers of sclerenchyma. Smaller vascular bundles may have sclerenchyma caps on both xylem and phloem poles, but the phloem cap may sometimes be very small, rarely absent. A parenchyma sheath may be present but is difficult to distinguish from the surrounding parenchyma tissue.

Sclerenchyma

Sclerenchyma is represented only by the vascular bundle sheaths.

Leaf blades Fig. 3.22C-F

Outline

Leaf margins are rounded to acute in outline.

Epidermis

The abaxial epidermal cells are isodiametric to slightly elongated tangentially. The adaxial epidermal cells are large, appearing similar to bulliform cells, but reducing in size towards the margins. The adaxial epidermal cells are isodiametric to slightly elongated radially, up to two times as long as wide. The epidermal cells at the margins are similar in size to the cells of the abaxial epidermis, or they may be smaller than the cells of the abaxial epidermis.

The outer cell walls of the abaxial epidermis are moderately thickened, and the outer walls of the adaxial epidermis are thin to slightly thickened. The anticlinal and inner walls are thin. The epidermal cells at the margins may have a similar degree of wall thickening to that of the abaxial epidermis, or they may be slightly thicker.

Mesophyll

The mesophyll consists of isodiametric cells. Spongy mesophyll may be present throughout most of the leaf, or closely packed cells may surround large, well-defined air spaces between the vascular bundles.

Vascular bundles

Vascular bundles often have an interrupted outer parenchyma sheath, although in *Luzula racemosa* it may be evident only as 2–3 cells at the flanks between the xylem and phloem poles. An inner sclerenchymatous sheath may be continuous on the largest vascular bundles, but thicker (3–6 cell layers) at the xylem and phloem poles than at the flanks, or it may be present as caps on the xylem and phloem poles only. The smallest vascular bundles, near the margins of the leaf, may have no sclerenchyma associated with them.

Sclerenchyma

Sclerenchyma may be present at leaf margins, as 5-12 fibres at the margins. It is sometimes absent in *Luzula racemosa*, but sometimes present in small quantities.

3.3 Results



Figure 3.22 *Luzula* sect. *Alpinae*. **A–B** leaf sheaths. **A** *L*. *racemosa*. **B** *L*. *racemosa* large vascular bundle with complete sclerenchyma sheath, and small bundle with sclerenchyma cap on xylem pole. **C–F** leaf blades. **C** *L*. *spicata*. **D** *L*. *spicata* leaf tip with sclerenchyma girder. **E** *L*. *racemosa* spongy mesophyll with poorly defined air canals. **F** *L*. *racemosa* mesopyll with few intercellular air spaces and well-defined air canals.

Anatomical description of leaves of Luzula section Anthelaea

Leaf sheaths Fig. 3.23

Epidermis

Cells of the abaxial epidermis are isodiametric to slightly elongated tangentially, up to two times as long as wide. Cells of the adaxial epidermis are slightly larger than those of the abaxial epidermis, and elongated up to three times as long tangentially as radially. All cell walls of the epidermis are thin or slightly thickened. No stomata were observed in *L. sylvatica*, but stomata were frequent in *L. luzuloides*.

Mesophyll

The mesophyll consists of isodiametric or irregularly shaped cells.

Vascular bundles

The larger vascular bundles have a complete sheath of sclerenchyma which is thicker at the xylem and phloem poles (2–6 cell layers) than at the flanks between the poles. Smaller vascular bundles may only have caps of sclerenchyma on the xylem and phloem poles. A parenchyma sheath is not distinguishable from the surrounding tissue.

Sclerenchyma

In addition to the vascular bundle sheaths, small strands of sclerenchyma which may be circular or irregular in outline may be present near the membranous part of the sheath. The sclerenchyma of the strands appears distinct from that of the bundle sheaths, as it has much more wall thickening. However, a proportion of the cells of the sheaths of the vascular bundles closest to the strands may also have similarly thickened cells.

3.3 Results



Figure 3.23 *Luzula* sect. *Anthelaea* leaf sheaths. A *L. sylvatica* sheath split in the membranous region. B *L. sylvatica* vascular bundle. C *L. luzuloides* small vascular bundle near adaxial side of sheath showing very thick-walled sclerenchyma cells. D *L. luzuloides* sclerenchyma strand.

Leaf blades Fig. 3.24

Outline

Leaf margins are rounded to square or slightly irregular in outline, often slightly thicker than adjacent parts of the leaf.

Epidermis

All epidermal cells are isodiametric to slightly elongated tangentially, up to 1.5 times as long as wide, but the adaxial cells are larger than the abaxial cells, sometimes appearing similar to bulliform cells. The cells of the adaxial epidermis often decrease in size opposite the largest vascular bundles. The cells of the abaxial epidermis may show a slight increase in size opposite the largest vascular bundles, or they may remain similar in size to the other abaxial epidermal cells. Epidermal cells at the margins are similar in size to those of the abaxial surface, or sometimes slightly smaller.

The outer cell walls of the epidermis on both surfaces are thickened, and all other walls are thin. Epidermal cells at the leaf margins may show increased wall thickening compared to those of the abaxial and adaxial epidermal cells.

Mesophyll

Spongy mesophyll fills the ground tissue, intergrading into poorly defined air canals between the vascular bundles (*L. luzuloides*), or the mesophyll does not have conspicuous intercellular air spaces, but surrounds well-defined air canals between the vascular bundles (*L. sylvatica*). Rarely the mesophyll extends through air canals to form a transverse septum which is associated with small transverse vascular bundles. The mesophyll cells are usually isodiametric or slightly irregularly shaped, but in the transverse septae they are lobed to stellate in shape, with small intercellular air spaces.

Vascular bundles

Small transverse vascular bundles may occasionally occur between the larger longitudinal bundles.

A parenchyma sheath is usually distinguishable from the surrounding tissue either completely surrounding the vascular bundles, or interrupted, and sometimes extending to each epidermis from the medium sized vascular bundles to form girders of parenchyma. An inner sclerenchymatous sheath consists of 2–5 cell layers, and may be complete on larger vascular bundles, or present as caps on the xylem and phloem poles of smaller vascular bundles. The sclerenchyma sheath may extend to the the abaxial and adaxial epidermis from the largest vascular bundles.

Sclerenchyma

In addition to the vascular bundle sheaths, a small quantity (1-2 cell layers) of sclerenchyma is present as girders next to the epidermis at the leaf margins, and sometimes girders are also formed from extensions of the vascular bundle sheaths to the abaxial and adaxial epidermis.



Figure 3.24 Luzula sect. Anthelaea leaf blades. A L. sylvatica. B L. luzuloides. C L. sylvatica leaf tip. D L. sylvatica bulliform adaxial cells reduce in size by large vascular bundles. E L. sylvatica transverse vascular bundle associated with lobed mesophyll cells. F L. luzuloides vascular bundle with adaxial sclerenchymatous girder formed from bundle sheath. G L. luzuloides vascular bundle with extensions of the parenchymatous outer sheath to adaxial and abaxial epidermis.

Anatomical description of leaves of Luzula section Diprophyllatae

Leaf sheaths Fig. 3.25A, B

Epidermis

Both abaxial and adaxial epidermal cells are similar, and similar to the cells of the ground tissue, being more or less isodiametric and with no wall thickening. No stomata are present.

Mesophyll

The mesophyll consists of 1–2 layers of parenchyma cells on the adaxial and abaxial surfaces, between which air canals are divided by vascular bundles. At the thinner, adaxial side of the sheath which corresponds to the fused margins, a small section is entirely parenchymatous, the parenchyma cells appearing similar to the abaxial and adaxial epidermis.

Vascular bundles

Vascular bundles are almost circular in outline. A parenchyma sheath is difficult to distinguish from surrounding tissue. A sclerenchyma sheath forms a complete ring around the vascular bundles, often with fewer layers and less wall thickening on the xylem pole. Often, on the largest vascular bundles it is absent from the xylem pole but surrounds the phloem pole and the flanks between the poles. The smallest vascular bundles often have reduced vascular tissue surrounded by several layers of sclerenchyma.

Sclerenchyma

Sclerenchyma is often present as circular strands 4–10 cells in diameter, interspersed among the ring of vascular bundles, and appearing to differ from them the smallest bundles only in the total absence of vascular tissue.

Leaf blades Fig. 3.25C-E

Outline

Leaf margins are acute, but a widening at a point just inside the tip of the margins corresponds to the presence of a strand of sclerenchyma.

Epidermis

The abaxial epidermal cells are elongated up to approximately two times as long tangentially as radially, and the adaxial cells to approximately two times as long radially as tangentially. The adaxial cells are very large to bulliform. All epidermal cells show a reduction in size and increased wall thickening at the margins.

All epidermal cells have thickened outer walls, and thin tangential and inner walls. Epidermal cells at the leaf margins show a reduction in size and increase in cell wall thickening at the leaf margins compared to those of the abaxial and adaxial epidermal cells. Stomata are present on the abaxial surface only.

Mesophyll

Spongy mesophyll fills the ground tissue, intergrading into poorly defined air canals between vascular bundles. The mesophyll cells are isodiametric or irregularly shaped.

Vascular bundles

Small transverse vascular bundles occur infrequently between the larger, longitudinal vascular bundles.

Vascular bundles have an outer sheath of a single, interrupted layer of parenchyma. They have a single, complete sheath of cells with moderately thickened cell walls, with more cell layers (5–7) at the xylem and phloem poles than at the flanks.

Sclerenchyma

Girders of sclerenchyma are present at the leaf margins.

3.3 Results



Figure 3.25 *Luzula gigantea*. A–B leaf sheaths. A Sheath showing vascular bundles and sclerenchyma strands, and region of parenchyma on adaxial side. B Large vascular bundle with very little wall thickening of adaxial side of bundle sheath, and sclerenchyma strand. C–E leaf blades. D Leaf tip. E Abaxial and adaxial epidermis, and vascular bundle.

Anatomical description of leaves of Luzula section Luzula

Leaf sheaths Fig. 3.26A–D

Epidermis

Abaxial cells are isodiametric or slightly irregularly shaped. Adaxial cells are 3–4 times as long tangentially as radially. All epidermal cell walls are thin. Stomata are rarely present, but were observed infrequently in both species examined.

Mesophyll

Cells are isodiametric or irregularly shaped. Some layers were seen to have become compressed next to the adaxial epidermis in *Luzula campestris* (L.) DC.. Large intercellular air spaces or small air canals are present between vascular bundles.

Vascular bundles

Vascular bundles are very closely spaced, with a minimum of two ground tissue cell layers between the sheaths with very small air canals between them.

An outer parenchymatous bundle sheath is difficult to distinguish from the surrounding tissue. A sclerenchymatous sheath of 2–7 cell layers surrounds larger vascular bundles completely, the number of cell layers sometimes varying with no clear pattern around each vascular bundle, but often with more cell layers present at the adaxial side, or they may have distinct lateral extensions on the adaxial side.

The smaller vascular bundles do not have a complete sheath, but sclerenchyma caps occur on the adaxial side, sometimes also with the sclerenchyma extending laterally into the ground tissue. The smaller vascular bundles usually alternate with the larger bundles, but a series of small bundles occurs in the thinnest, adaxial side of the sheath. The sclerenchyma caps which occur on the small bundles in the thinnest part of the sheath may be proportionally larger than those on the small bundles in the rest of the sheath, and extend further into the ground tissue laterally.

Leaf blades Fig. 3.26E–G

Outline

Leaf margins are rounded.

Epidermis

The epidermal cells of both surfaces are usually isodiametric, though they may be slightly irregularly shaped. The outer cell walls are slightly thickened or thin, and the anticlinal and inner cell walls are thin. The adaxial cells are larger than the abaxial cells, sometimes appearing similar to bulliform cells. Epidermal cells at the leaf margins show a reduction in size and increased wall thickening compared to the abaxial and adaxial epidermal cells. Stomata are present on the abaxial surface.

Mesophyll

Spongy mesophyll fills the ground tissue, often intergrading into air canals between vascular bundles. The mesophyll cells are isodiametric or irregularly shaped.

Vascular bundles

Vascular bundle sheaths sometimes have 2–4 parenchyma cells at the flanks between the xylem and phloem poles, while sclerenchyma is usually present as a complete inner sheath on the larger bundles. Small bundles may have xylem and phloem caps only, or lack a sclerenchyma sheath and have only a parenchyma sheath. The sclerenchyma sheath of large vascular bundles is frequently adjacent to the adaxial epidermis. The sclerenchyma sheath of the largest vascular bundle may be adjacent to the abaxial surface, but there is usually at least one cell layer of ground tissue between the sclerenchyma sheath and abaxial epidermis for other vascular bundles.

Sclerenchyma

In addition to the vascular bundle sheaths, sclerenchyma girders are present at the leaf margins.

3.3 Results



Figure 3.26 *Luzula* sect. *Luzula*. A–D leaf sheaths. A *L. campestris*. B *L. campestris* vascular bundle with lateral extensions of the sclerenchyma sheath. C *L. multiflora* small vascular bundle in thinnest part of sheath with laterally extended sclerenchyma cap on adaxial side. D *L. campestris* series of small vascular bundles in thinnest part of sheath with laterally extended sclerenchyma caps on the adaxial side. E–G leaf blades. E *L. campestris*. F *L. multiflora* leaf tip. G *L. multiflora* vascular bundle.

Anatomical description of leaves of *Luzula* section *Thyrsanochlamydeae*

Leaf sheaths Not observed.

Leaf blades Fig. 3.27

Outline

Leaf margins are acute.

Epidermis

The abaxial epidermal cells are isodiametric, with moderately thickened outer walls and thin anticlinal and inner walls. The adaxial epidermal cells are bulliform, and isodiametric to slightly longer radially than tangentially, with moderately thickened outer walls and thin anticlinal and inner walls. The epidermal cells at the leaf margins are similar to the abaxial epidermal cells.

Mesophyll

The chlorenchyma cells are isodiametric or irregularly shaped. They are arranged into a very loose spongy mesophyll, often with large air spaces next to the stomata and between vascular bundles.

Vascular bundles

Vascular bundles have an interrupted outer sheath of a single layer of parenchyma. A sclerenchyma sheath 1–3 cell layers deep surrounds the vascular bundles, or is present as caps on the xylem and phloem poles.

Sclerenchyma

In addition to the bundle sheaths, very small girders consisting of 1–4 fibres are present at the margins.

3.3 Results



Figure 3.27 *Luzula arcuata* leaf blades. **B** Leaf tip with small sclerenchyma girder. **C** Epidermis, mesophyll and vascular bundle.

Anatomical description of leaves of Luzula subgenus Pterodes

Leaf sheaths Not observed.

Leaf blades Fig. 3.28

Outline

The margins are thickened and irregularly, due to the presence of subepidermal girders.

Epidermis

The abaxial epidermal cells are slightly elongated tangentially, and with thickened outer walls, and thin anticlinal and inner walls. The adaxial cells are isodiametric or slightly elongated radially, and very large or bulliform, with all walls moderately thickened. The epidermal cells of both surfaces become slightly smaller at the margins and next to large vascular bundles, where the sclerenchyma sheath is adjacent to the epidermis.

Mesophyll

The mesophyll cells are isodiametric or irregularly shaped, and present in very few (usually 1–2) cell layers adaxially, abaxially and surrounding the vascular bundles, with very loose spongy mesophyll intergrading with large air canals in the centre of the leaf.

Vascular bundles

Vascular bundles have no distinguishable outer sheath except on the phloem and xylem poles, where a single cell layer of parenchyma may occur between the inner sheath and the epidermis. A sclerenchymatous sheath surrounds the vascular bundles completely (3–5 cell layers, but 1–2 cell layers thinner at the flanks between the xylem and phloem poles).

Sclerenchyma

In addition to the bundle sheaths, sclerenchyma is present as girders at the leaf margins, usually as a layer 2–5 cells deep, and sometimes partially encircling the end of the leaf.

3.3 Results



Figure 3.28 *Luzula johnstonii* leaf blades. A Leaf outline. **B**, **C** Irregularly shaped, callose leaf tips. **D** Vascular bundle.

Anatomical description of leaves of Marsippospermum

Leaf sheaths Fig. 3.29

Epidermis

Abaxial epidermal cells are isodiametric to approximately 1.5 times as long tangentially than radially. They have very thick outer walls, moderately thickened anticlinal walls, and slightly thickened or thin inner cell walls. The adaxial epidermal cells are isodiametric or up to two times longer tangentially than radially, and thin-walled. Stomata are present on the abaxial surface but infrequent, and are superficial with small inner and outer lips on the guard cells.

Mesophyll

The ground tissue consists entirely of parenchyma, with cells which are more or less isodiametric, and larger on the adaxial side than on the abaxial side.

Vascular bundles

Vascular bundles are present in a single row. A parenchymatous outer sheath is not distinguishable from the surrounding parenchyma tissue. A sclerenchymatous sheath is usually complete, but much thicker (6–10 cell layers) on the xylem and phloem poles than at the flanks, or it may only form bundle caps on the smaller vascular bundles.

Air spaces

A single row of air canals alternates with the vascular bundles. In addition, a large canal is present, bounded by several layers of parenchyma on the adaxial side, and on the abaxial side, several layers of parenchyma containing the row of vascular bundles alternating with the small air canals.

3.3 Results



Figure 3.29 *Marsippospermum grandiflorum* leaf sheaths. **B** Epidermis with thickened outer walls and stoma, and parenchyma. **C** Vascular bundle. **D** Small vascular bundle with reduced vascular tissue (left), and sclerenchyma strand near margin of sheath.

Sclerenchyma

Small strands of sclerenchyma which are circular in outline and up to five cells in diameter are present near the margins, appearing to be a continuation of the row of vascular bundles, with 1-2 strands present at the ends of the row of vascular bundles.

Leaf blades Fig. 3.30

Outline

Leaf blades are terete, with a large adaxial groove and a smaller abaxial groove.

Epidermis

The cuticle is very thick. Cuticular ridges are present on the adaxial and abaxial grooves, but the cuticle is smooth elsewhere.

Epidermal cells are more or less isodiametric with very thick outer walls, and slightly thickened anticlinal and inner walls. The adaxial epidermis is extremely reduced and present only in the centre of the adaxial groove. The adaxial cells are larger than the abaxial cells, but reduced in size towards the edges, and they have similar cell wall thickening to the abaxial cells. Stomata are superficial and present in all areas except on the abaxial and adaxial grooves.

Mesophyll

Chlorenchyma is present as a ring of 5–9 cell layers around the outside of the leaf, but it is interrupted on the abaxial side, opposite the mid-rib, and on the adaxial side, opposite the adaxial epidermis, where parenchyma extends to the epidermis. The chlorenchyma cells are elongated up to five times as long radially as tangentially, but shorter in the inner layers. Sometimes they are slightly lobed with very small intercellular air spaces. Inside the chlorenchyma are several cell layers of parenchyma, with cells which are larger than the chlorenchyma and isodiametric or irregular in shape.

Vascular bundles

The vascular bundles are arranged in a single ring in the parenchyma, immediately adjacent to the chlorenchyma. They are associated with a large amount of sclerenchyma which forms caps on the xylem and phloem poles, with more cell layers (7–9) on the phloem pole than on the xylem pole (5–7 layers). On the largest vascular bundles the caps are joined at the flanks of each vascular bundle by moderately thickened cells. An outer sheath of parenchyma is evident only where the phloem pole is adjacent to the chlorenchyma; elsewhere it is not distinguishable from the ground tissue.

Air spaces

A large central canal is present, and a ring of small air canals alternates with the vascular bundles.

Sclerenchyma

Sclerenchyma is represented only by the vascular bundle sheaths.

3.3 Results



Figure 3.30 *Marsippospermum grandiflorum* leaf blades. **B** Abaxial epidermis with cuticular ridges, and abaxial parenchyma. **C** Epidermis with stoma and smooth cuticle, and chlorenchyma. **D** Adaxial epidermis with cuticular ridges, and parenchyma. **E** Vascular bundle. Parenchyma sheath is only distinguishable next to chlorenchyma (left). **F** Small vascular bundle next to adaxial groove with extended sclerenchyma sheath.
Anatomical description of leaves of Oxychloe

Leaf sheaths Fig. 3.31A–D

Epidermis

The abaxial epidermal cells are isodiametric or slightly elongated radially up to 1.5 times as long as wide, and with thick outer walls and thin anticlinal and inner walls. The adaxial epidermal cells are rectangular or rounded, up to twice as long tangentially as radially, and all walls are thin. Stomata are absent.

Mesophyll

2-3 layers of parenchyma cells are present on both the adaxial and abaxial sides of the sheath. Between these is a series of air canals, divided by narrow bridges consisting of 2-4 further cell layers extending from the abaxial and adaxial sides of the sheath.

Most ground tissue cells are isodiametric or irregular in shape, sometimes slightly elongated radially. In *O. bisexualis* Kuntze the cell layers forming the bridge between the abaxial side and the phloem poles of the vascular bundles consist of irregularly shaped to slightly lobed cells with large intercellular air spaces.

Vascular bundles

Vascular bundles occur in a single row in the centre of the leaf, alternating with air canals. An outer, parenchymatous sheath is sometimes distinguishable from the surrounding parenchyma tissue by the smaller size of the sheath cells, but often it is not possible to distinguish it. An inner, sclerenchymatous sheath is present on all vascular bundles, either as caps on the xylem and phloem poles only, or as a complete sheath, but always consisting of more cell layers (2–4) on the xylem and phloem poles than on the flanks.

One specimen of *O. andina* was observed which had an extension of the sclerenchymatous sheath of the largest vascular bundle to form a girder next to the epidermis.

Sclerenchyma

In addition to vascular bundle sheaths, sclerenchyma is sometimes present as 1-2 circular strands, variable in size but up to eight cells in diameter, near the margins and appearing to be a continuation of the row of vascular bundles.

Air canals

One specimen of *O. andina* was observed with a transverse septum across one of the air canals between vascular bundles. It consisted of stellate cells associated with a small, transverse vascular bundle.

Leaf blades Fig. 3.31E–H

Leaf blade outline

The leaves are unifacial and terete, entirely round or slightly flattened laterally with a concave adaxial side. There are often slight ridges corresponding to the presence of subepidermal sclerenchyma girders.

Epidermis

The epidermal cells are rounded and isodiametric, with very thick outer walls, moderately thickened to thin anticlinal walls, and thin inner walls. Stomata are superficial.

Mesophyll

Chlorenchyma is present as 5-10 layers in a ring in the outer region of the leaf. The cells are either isodiametric or elongated up to two times as long as wide in the outer layers, and isodiametric in the inner layers. Inside the ring of chlorenchyma are very large, thin-walled parenchyma cells which break down to form a single large central canal, so there is no parenchyma present in older leaves.

Vascular bundles

Vascular bundles are arranged in a single ring in the chlorenchyma. A parenchyma sheath is most evident on small vascular bundles, which may sometimes lack a sclerenchymatous sheath. On large vascular bundles a small number of parenchyma cells (usually 2–4) are found on each flank, between the xylem and phloem poles, while sclerenchyma forms caps on the xylem and phloem poles. The xylem cap usually consists of 3–5 layers of sclerenchyma on larger vascular bundles, and the phloem cap often extends to the epidermis, with up to 20 layers in some specimens, to form a girder.

Air spaces

Air canals are present only as a single large central canal.



Figure 3.31 *Oxychloe*. **A–D** Leaf sheaths. **A** *O. bisexualis*. Arrow indicates transverse septum. **B** *O. andina* epidermis, mesophyll and vascular bundle with extension of the sclerenchyma sheath to the epidermis. **C** *O. andina* stellate cells associated with small transverse vascular bundle. **D** *O. bisexualis* vascular bundle with sclerenchyma caps. **E–H** Leaf blades. **E** *O. bisexualis*. **F** *O. andina*. **G** *O. bisexualis* Epidermis, and sclerenchymatous vascular bundle sheath extended to epidermis to form a girder. **H** *O. bisexualis* Small vascular bundle with complete parenchyma sheath and no sclerenchyma.

Anatomical description of leaves of Patosia

Leaf sheaths Leaf sheaths were not observed.

Leaf blades Fig. 3.32

Leaf blade outline

The leaves are unifacial, and oval, often with a concave adaxial surface.

Epidermis

The epidermal cells are isodiametric, with very thick outer walls and slightly thickened or thin anticlinal and inner walls. Stomata are superficial.

Mesophyll

Chlorenchyma is present as 5-8 layers in a ring in the outer region of the leaf. The cells are elongated 1.5-2.5 times as long as wide in the outer layers, and isodiametric in the inner layers. Inside the ring of chlorenchyma are large, thin-walled cells which break down to form a single, large central canal.

Vascular bundles

Vascular bundles are arranged in a single ring in the chlorenchyma, with the xylem pole adjacent to or projecting into the outer layers of parenchyma. The vascular bundle sheaths are predominantly sclerenchymatous. A small number of parenchyma cells (usually 2–3) are found on each flank, while sclerenchyma forms caps on the xylem and phloem poles; very rarely on just the xylem pole. The xylem cap usually consists of 3–6 layers of sclerenchyma, and the phloem cap may be similar or slightly larger. Sometimes the xylem cap of the largest vascular bundle extends to the epidermis to form a girder.

Air spaces

Air canals are present only as a central canal, following breakdown of parenchyma pith.

3.3 Results



Figure 3.32 *Patosia clandestina* leaf blades. A Leaf blade with slight adaxial groove. **B** Leaf blade with large adaxial groove. **C** Epidermis with superficial stoma, and slightly elongated palisade mesophyll. **D** Vascular bundle with sclerenchyma caps on xylem and phloem poles, and parenchyma sheath on the flanks between the poles. **E** Vascular bundle with sclerenchyma cap on the xylem pole, and parenchyma sheath on phloem pole. **F** Large vascular bundle with extension of the sclerenchyma to the epidermis to form a girder.

Anatomical description of leaves of Rostkovia

Leaf sheaths Fig. 3.33

Epidermis

The abaxial epidermal cells are isodiametric, but may become slightly elongated radially near the margins. They have very thickened outer walls, moderately thickened anticlinal and thin inner walls. The adaxial epidermal cells are slightly longer (to 1.5 times) radially than tangentially. The outer walls are slightly thickened or thin, and the other walls thin. Stomata are absent.

Mesophyll

The mesophyll consists entirely of parenchyma cells, with 1-3 layers on the adaxial side, and 2-4 layers on the abaxial side. Between these layers, the series of air canals is divided only by the vascular bundles.

Vascular bundles

Vascular bundles are present in a single layer, alternating with the air canals. An outer sheath is difficult to distinguish from the parenchymatous ground tissue, and only visible when adjacent to the air canals. A sclerenchymatous sheath forms a complete ring of 3–6 cell layers.

Sclerenchyma

Small strands (approximately 4–5 cells) of sclerenchyma are present as 1–3 individual strands near each margin. In addition to these is a girder of sclerenchyma at the margins, in the form of 1–2 cell layers of sclerenchyma fibers between the epidermal layers.

3.3 Results



Figure 3.33 *Rostkovia magellanica* leaf sheaths. **B** Vascular bundle. **C** Sclerenchyma strands near sheath margin. **D** Sclerenchyma girder at sheath margin.

Leaf blades Fig. 3.34

Outline

The leaf blades are oval, with a small adaxial and larger abaxial groove.

Epidermis

The epidermal cells are isodiametric, with very thick outer, and moderately thickened anticlinal and inner walls, and a thickened cuticle. The adaxial cells are slightly larger than the abaxial cells. Superficial stomata occur only in two broad bands on the abaxial side of the leaf, either side of the mid-rib.

Mesophyll

The mesophyll is mostly chlorenchymatous, the cells usually isodiametric, but the outer layers may be elongated up to two times as long as wide. The chlorenchyma cells are sometimes very slightly lobed, resulting in very small intercellular air spaces. Palisade mesophyll occurs in the outer parts of the leaf and throughout the margins, but the mesophyll tends to become spongy in some specimens near the stomatal bands, and between the vascular bundles in the centre of the leaf. In other specimens the mesophyll may be entirely palisade, with well-defined air canals occurring between the vascular bundles.

A layer of cells occurring beneath the stomatal bands is clearly distinguished from the surrounding chlorenchyma. They have moderately thickened walls and irregularly lobed shapes with intercellular air spaces. These are referred to by Cutler (1969) as protective cells.

On the adaxial side of the leaf a single, discontinuous layer of parenchyma cells occurs, adjacent to the epidermis and interrupted by sclerenchymatous cells. The parenchyma cells are radially elongated, and larger than the chlorenchyma cells.

Vascular bundles

Five vascular bundles are present in a single row. A parenchymatous outer sheath may be distinguishable, particularly at the flanks between the xylem and phloem poles. A complete sheath of sclerenchyma surrounds the largest vascular bundle in the centre, and extends from the phloem pole to the epidermis to form a girder. The smaller vascular bundles usually only have sclerenchyma caps at the xylem and phloem poles, or just at the phloem pole.

Sclerenchyma

Sclerenchyma is present as vascular bundle sheaths, and also as 1–2 cell layers next to the epidermis, in all regions except by the stomatal bands. These layers are discontinuous near to the adaxial groove.

Air spaces

Four air canals usually alternate with the vascular bundles. The edges of the canals in some specimens are clearly defined, being surrounded by palisade mesophyll, but in other specimens they may be poorly defined, and they may also extend towards the stomatal bands, intergrading with areas of loose spongy tissue.

3.3 Results



Figure 3.34 *Rostkovia magellanica* leaf blades. A leaf blade of specimen from wet habitat with palisade and spongy mesophyll. **B** Leaf blade from specimen from wetter habitat than A, with proportionally more spongy mesophyll (sm) abaxially. **C** Stomatal band with subepidermal 'protective' cells. **D** Leaf blade tip with subepidermal sclerenchyma and palisade mesophyll. **E** Adaxial epidermis with subepidermal sclerenchyma (sc), interrupted by parenchyma cells (p). **F** Midrib. **G** Small vascular bundle with sclerenchyma caps on xylem and phloem poles. **H** Small vascular bundle with sclerenchyma cap on phloem pole only.

3.3.3 Character scoring for analyses

In order to include the results in cladistic analyses all those characters were chosen which showed variation within Juncaceae and which were potentially taxonomically informative. They were scored as bistate characters, with the exception of the leaf blade dimensions, which are recorded in mm. Absence or presence characters were recorded as '0' or '1', respectively. The data are given in Table A3.

Character list

Leaf blade characters

41. Leaf blade bifacial (0); bifacial but with a significantly reduced adaxial surface (1); unifacial (2).

Where no clear differentiation occurs in the adaxial and abaxial leaf surfaces, adaxial and abaxial surfaces were identified by the arrangement and orientation of vascular bundles. Xylem poles are orientated towards the adaxial surface and phloem towards the abaxial surface. If the vascular bundles form a complete ring with the xylem poles orientated towards the leaf, the leaf is recorded as unifacial.

Leaves with a reduced adaxial epidermis (e.g. *Juncus bulbosus*) may appear to be unifacial in the middle part of the leaf blade, but sections taken lower down the blade show that they are bifacial, but with a reduced adaxial epidermis. Although the results in Section 3.3.2 *Descriptions of the leaf anatomy of Juncaceae* refer only to the middle part of the leaf blade, it was considered misleading to score these leaves as unifacial. These are scored as having a reduced adaxial epidermis, even when the description in Section 3.3.2 describes the specimens as unifacial.

42. Leaf blade width

The width is measured between apparent margins to obtain the widest dimension of the leaf, regardless of where the 'true' anatomical margins may occur.

43. Leaf blade shape

A measurement of the leaf blade is obtained by dividing the width at the widest point by the width at the narrowest point.

44. Leaf margin outline

Leaf margins are described as rounded (0) or acute (1).

Many leaves have distinct margins, but in species with a reduced adaxial epidermis these margins may not be associated with the junction between the abaxial and adaxial epidermis, as shown by Guédès (1967), but both the abaxial and adaxial sides of the apparent margins are bounded by the abaxial epidermis. Therefore the margins are taken to be the lateral points of the leaf blade, marking the boundary between the adaxial and adaxial abaxial sides of the leaf, but not the boundary between the true abaxial and adaxial epidermis.

45. Relative adaxial cells size

Adaxial cells which are similar in size to abaxial cells are recorded as '0'. Adaxial cells which are at least 50% larger than abaxial cells are recorded as '1'.

46. Sunken stomata present/absent

47. Parenchyma present/absent

- 48. Stellate or lobed chlorenchyma present/absent.
- 49. Stellate, non-chlorenchymatous pith present/absent.

50. Leaf blade central cavity present/absent

The central cavity is formed by the breakdown of large, thin-walled cells. This process is related to the age of the leaf. In younger specimens the cells are still present, while in others intermediate stages are seen, with some of the cells still present while others have disappeared (e.g. Fig. 3.31E, F). These specimens are scored as '1' regardless of whether the cells have or have not disintegrated to form the cavity.

51. Longitudinal septae present/absent if leaf is hollow.

52. Transverse septae present/absent.

Transverse septae may occur in longitudinal canals in the leaf, within a central cavity or in air canals between vascular bundles. Many authors distinguish between 'complete' and 'incomplete' transverse septae, referring to septae which cross the entire width of the leaf as 'complete', and those which do not cross the entire width of the leaf as 'incomplete'. This is related to the number of longitudinal air canals. The septae usually form a complete division of each air canal, so if a single air canal is present, the septae are 'complete', and if more than one are present, the septae are often 'incomplete', as the divisions across each canal often occur at a different point in the leaf. As the number of air canals is recorded elsewhere (character 51) it is not necessary to distinguish between complete and incomplete transverse septae.

53. Longitudinal air canals between vascular bundles present/absent

These differ from the central canal in their arrangement and position in the leaf, being interspersed between the vascular bundles, usually alternating with them. In some specimens (e.g. *Marsippospermum*) these may be present in addition to a central canal (character 50), so this may be considered to be a separate character.

54. Vascular bundles arranged in more than one layer or row present/absent

- 55. Abaxial girders formed by the inner bundle sheath extending to epidermis present/absent
- 56. Adaxial girders formed by the inner bundle sheath extending to epidermis present/absent

57. Sclerenchymatous girders next to the abaxial epidermis but not associated with vascular bundle sheaths present/absent

In some leaves girders are present next to the epidermis at the leaf margins. However, in species with a reduced adaxial epidermis (e.g. *J. squarrosus*) it has been shown that the apparent leaf margins do not correspond with the junction between the abaxial and adaxial

epidermis (Guédès 1967), so the 'marginal' girders are adjacent to the abaxial epidermis only. In addition, in certain species of *Luzula* Cutler (1969) interprets the epidermis to extend around the margins on to the morphologically adaxial surface (see Section 3.4.2 *Stomata*). This implies that the marginal strands of *Luzula* could also be interpreted as abaxial. It was decided that scoring marginal girders as a separate character to nonmarginal girders would be misleading, so marginal girders are included in this character.

58. Sclerenchymatous strands in ground tissue present/absent

Sclerenchymatous strands are usually circular in outline, and they have sheaths which are similar in appearance to those of the small vascular bundles in the same specimen.

Leaf sheath characters

59. Stomata very infrequent or absent (0); present (1)

Some species have stomata on the sheaths, but they are present so infrequently (e.g. a single stoma is present in one section among several examined) that it is sometimes difficult to determine whether stomata are absent or infrequent, unless large numbers of sections are examined. Other species have at least one stoma per section, and often several. Stomata are recorded as 'very infrequent or absent' if they occur at a frequency of fewer than one stoma per section of $10-24 \mu m$ diameter, and 'present' if they occur at a frequency of at least one stoma per section.

60. Stellate or lobed cells present/absent

61. Vascular bundles in more than one row or scattered present/absent

In most species a single row of vascular bundles alternate with air canals. In others they are scattered or in several rows. Where air canals are present in these species, the largest bundles are arranged in a row alternating with the air canals, and the other rows are arranged abaxially to this row, decreasing in size in each additional row. The first additional row is usually arranged with each bundle placed half way between the positions of the bundles of the first row, and thus approximately half way along the air canals, so that the xylem poles are adjacent to the air canal (Figs. 3.19A, C).

In other species most vascular bundles occur in a single row and alternate with air canals, but some additional, smaller vascular bundles are arranged abaxially to the air canals. This may be interpreted as either two rows or as a single row, as the smaller vascular bundles rarely, if ever, occur directly abaxially of any other vascular bundles (e.g. Fig. 3.19B). However, this arrangement is similar to that of the first two rows in species which more clearly have several rows, so they are recorded has having more than one row.

62. Air canals present/absent

63. Transverse septae present/absent

- 64. Abaxial girders formed by the inner bundle sheath extending to epidermis present/absent
- 65. Adaxial girders formed by the inner bundle sheath extending to epidermis present/absent

66. Sclerenchymatous strands in ground tissue present/absent

Sclerenchymatous strands are usually circular in outline, and they have sheaths which are similar in appearance to those of the small vascular bundles in the same specimen.

Characters not included

A number of characters which showed variation between taxa were considered unsuitable to be included in the analyses. These characters, and the reasons for excluding them, are discussed in the following section.

Chlorenchyma in the leaf sheaths

In the sheaths of some species (e.g. *Juncus bulbosus*, Fig. 3.19B) chlorenchyma is only present abaxially, and parenchyma is present adaxially. In other species (e.g. *J. maritimus*, Fig. 3.17) the chlorenchyma appears to be lacking in the sheath, and in some (e.g. *J. cyperoides*, Fig. 3.14B) chlorenchyma is present both abaxially and adaxially. This is of interest because although the sheaths are always bifacial, the leaf blades of many species

are unifacial and terete, and do not show dorsiventral differentiation, so these characters cannot be compared in the leaf blades. However, it is difficult to compare the arrangement of chlorenchyma among the sheaths of the specimens studied here. The age and condition of the herbarium specimens used, and also the methods used to produce the slides, made accurate identification of photosynthetic tissue difficult in some specimens, as the cell contents were frequently lost, and in a small number of older specimens the cell walls themselves had suffered some degradation.

It is also noted (3.3 *Results: Distichia* leaf sheaths) that variation in the arrangement of chlorenchyma also occurs longitudinally in the sheath, so the point at which a transverse section is taken significantly affects the results. With the very small (approx. 1 cm in length) sheaths found in some species, including species of *Distichia*, it was difficult to ensure that the exact middle portion was sectioned, and an error of even 1 mm could make a significant difference in the observations made.

Variation in form and number of cell layers in the vascular bundle sheaths

There is some variation between taxa in the number of layers of sclerenchyma forming inner bundle sheaths, but considerable variation also occurs within a species, and even within a specimen. Large vascular bundles are likely to have more layers of sclerenchyma than small ones within a section, and different sections may also differ from one another. In addition, although there is variation between species regarding the form taken by the inner bundle sheaths, with some species usually having caps on the xylem and phloem poles, and others with complete sheaths, a significant amount of variation may also occur within a single section. It is possible that the variation may be due in part to the age of the leaf or to environmental conditions, and any variation of taxonomic significance would be difficult to isolate from variation due to other factors.

Palisade and spongy mesophyll

There is much variation in the cell shape and arrangement in the chlorenchyma, and some may be taxonomically significant, as some taxa only possess palisade mesophyll, while others may have isodiametric cells, which may be arranged into spongy mesophyll or arranged closely with inconspicuous intercellular air spaces. However, many species show states which are intermediate, illustrating a continuous variation in the shape and arrangement of the mesophyll, some of which may be caused by environmental influence. The outermost layers of chlorenchyma may consist of cells which are consipicuously elongated into palisade mesophyll, but they often become isodiametric in the inner layers. The occurrence of spongy mesophyll is equally variable, with some specimens of a species sometimes containing spongy mesophyll, and others of the same species containing none.

3.4 Discussion

Although many of the species described here have already been described in detail in the literature (Cutler 1969), new observations have been made. Aside from the differentiation between species with closed leaf sheaths and those with open sheaths, the leaf sheaths of Juncaceae have received little attention in the literature. With the exception of Distichia and Oxychloe, their anatomy has not been described previously, so the descriptions of leaf sheaths given here are new. There is less variation in the anatomy of the leaf sheaths than the leaf blades, and much of the variation that occurs in the sheaths also occurs in the blades. However, a small number of characters are of interest. This derives in part from the fact that the leaf sheaths are always bifacial, whereas the leaf blades of many species have lost their adaxial surface and become unifacial. Even when the leaf blades remain bifacial they may show a degree of anatomical difference from the sheaths, for example the number of layers of vascular bundles may be reduced from several in the sheaths to a single layer in the blades, and the presence and arrangement of sclerenchyma strands also varies. Transverse septae have been found to be more easily observed in the sheaths of some species than in the blades, and have been observed in taxa in which they have not previously been recorded.

In addition to these, the descriptions of the leaf blades of several taxa are also new, as the anatomy of the leaves of *Distichia acicularis*, *Juncus capillaceus*, *J. caespiticius*, *J. cyperoides*, *J. ecuadoriensis* Balslev, *J. subulitepalus*, *Luzula gigantea*, *L. johnstonii* Buchenau, *L. racemosa* and *L. spicata* (L.) DC. have not been described previously (*J. capillaceus* is figured by Buchenau (1906), but with limited detail). For those species which have been described previously, a comparison of the descriptions given here with those in the literature shows a broad agreement, but additional observations are discussed in the following sections. A discussion of the implications for the taxonomy of Juncaceae is given in Chapter 5 *Phylogeny*.

3.4.1 Reduction of the adaxial epidermis

It is known that some species of *Juncus* have bifacial leaf blades, and others are entirely unifacial, due to a suppression of the development of the adaxial epidermis (Adamson

1925; Buchenau 1890; Guédès 1967). The identification of unifacial leaves is made possible by the arrangement and orientation of the vascular bundles. In unifacial leaves the vascular bundles are arranged in a ring (or several rings) with the xylem poles orientated towards the centre of the leaf. This can be explained if the adaxial epidermis has become lost, the entire surface then becoming equivalent to the abaxial surface of a bifacial leaf. The points equivalent to the margins of a bifacial leaf are fused, and the vascular bundles, which would normally occur in an arc in a bifacial leaf, form a complete ring.

However, this interpretation makes the assumption that the vascular bundles of bifacial leaves are orientated with the xylem poles towards the adaxial surface, but inverted vascular bundles have been observed in the sheaths of some Cyperaceae (Guichard 1928), so this assumption may not always be true. However, the observations made here show it to be correct for Juncaceae. The sheaths of all species studied here have the xylem poles orientated towards the adaxial side, and as the leaf sheaths are known to be bifacial (the adaxial and abaxial surfaces are continuous with the adaxial and abaxial sides of the point of insertion on the stem) it can be concluded that no inverted bundles are present. The progression from the bifacial sheath to a unifacial blade is described in more detail in Section 4.3.2 Descriptions of the junctions between leaf sheaths and blades.

For some species, however, such as *Juncus squarrosus*, interpretations of the relative proportions of adaxial surface to abaxial surface differ between authors. Although all authors agree that it is bifacial, Buchenau (1890, 1906) interprets the junction between the abaxial and adaxial epidermis of *J. squarrosus* as being indicated by the position of marginal sclerenchyma strands, whereas Adamson (1925) and Guédès (1967) consider the junction to be marked by a change in the mesophyll cells, with parenchyma occurring under the adaxial epidermis, and chlorenchyma occurring under the abaxial epidermis (Fig. 3.12C). According to the interpretation of Adamson and Guédès, the apparent margin, containing strands of sclerenchyma, does not mark the true junction between the adaxial epidermis. The apparent margins are entirely surrounded by abaxial epidermis, whereas the adaxial epidermis is present only in a small region in the groove on the adaxial side of the leaf, and thus may show an intermediate state between bifacial and unifacial leaves, as the adaxial surface is present but reduced in area. Guédès supports this interpretation by showing that the xylem poles of the vascular bundles are orientated

towards the parenchymatous region, and away from the apparent margins. This interpretation is followed here.

In all the specimens observed here, when parenchyma is present in bifacial leaves it is adjacent to the epidermis on the adaxial side only, and within unifacial leaves it is central, and never next to the epidermis; these observations are supported by the orientations of the vascular bundles. The loss of the adaxial surface causes the portions of the leaf which would normally be adaxial to occur in the centre of the leaf (Adamson 1925; Buchenau 1890), so the central parenchyma in unifacial leaves is likely to be homologous to the adaxial parenchyma in bifacial leaves. However, some of the species observed here have no parenchyma present in the leaf at all, e.g. *J. trifidus* (Fig. 3.12B). It is concluded here that the absence of adaxial parenchyma can only be used to indicate the loss of the adaxial surface if parenchyma *is* present centrally; in contrast, the presence of adaxial parenchyma is present at all then the orientation of the vascular bundles must be used to identify the presence or absence of an adaxial surface.

J. bulbosus is described in Section 3.3.2 Descriptions of the leaf anatomy of Juncaceae as unifacial (Fig. 3.20B), but Adamson (1925) and Cutler (1969) describe it as bifacial, with a reduced adaxial epidermis. The sections of the leaf blades examined here have parenchyma in the central portion of the leaf, but chlorenchyma forms a complete ring next to the epidermis, containing a ring of vascular bundles with the xylem poles orientated towards the centre of the leaf, indicating a unifacial leaf. In contrast, the bifacial leaf sheath has parenchyma on the adaxial side and chlorenchyma on the abaxial side (Fig. 3.19B). The sections examined here differ from those described by Adamson and Cutler, which did have a small quantity of parenchyma on the adaxial side of the leaf blade, indicating a bifacial leaf.

It is possible that different results may be found at different points along the leaf blade. In the figures of *J. squarrosus* given by Guédès (1967) a series of sections are shown throughout the leaf blade. The further along the leaf blade the sections were taken, the smaller the proportion of the leaf surface consisting of adaxial epidermis; eventually, near to the tip of the blade, the leaf becomes entirely unifacial. The series of sections presented in Chapter 4 *Auricles or ligules*? support this. Sections of the leaf blade of *J. bulbosus* are bifacial in the region close to the sheath (Fig. 4.17D), but the adaxial surface is progressively reduced along the length of the blade, which becomes unifacial (Fig. 3.20B). The differences between these observations and those of Cutler (1969) may be explained if the sections examined by Cutler were taken relatively further down the blade than those examined here. Alternatively, it is possible that variation may occur between specimens in the point at which the blade becomes unifacial.

The leaf blade of *Juncus bulbosus* should be described as bifacial with a reduced adaxial epidermis, as stated by Adamson and Cutler; although the upper portion of the blade is unifacial, the lower portion of the blade is bifacial. A similar situation is noted with *Juncus triglumis*. The leaf appears to be unifacial in the sections taken of the middle region of the leaf blade (Fig. 3.21D), but sections of the region showing the transition between the leaf sheath and leaf blade (Fig. 4.20C) indicate that the lower region of the leaf blade is bifacial.

3.4.2 Stomata

Stomata are found to be frequent on the leaf sheaths of a number of taxa of Juncaceae, although it is generally considered that stomata are absent on the leaf sheaths (Cutler, 1969; although Cutler does mention the presence of stomata on the sheaths of *Distichia*). When present, they occur on the abaxial epidermis only, and are similar in appearance to those on the blades.

A problem arises in *Luzula* which relates to the identification of the adaxial epidermis. The stomata of Juncaceae are usually abaxial, but on the leaf blades of a few species, e.g. *L. luzuloides*, they appear to be present adaxially, but only near to the margins. Cutler (1969) states that the stomata are always abaxial, but that in some species of *Luzula* the abaxial epidermis 'extends around the margins onto the morphologically adaxial surface', thus explaining the apparently adaxial stomata.

There is no parenchyma present in *Luzula* to indicate the position of the adaxial epidermis, as sometimes (but not always) occurs in *Juncus*, and the leaves are always dorsiventrally flattened. The xylem poles of vascular bundles are always orientated towards the adaxial side, but the regions in which stomata have been observed adaxially here do not extend

very far along the adaxial side, and they do not extend beyond any vascular bundles, so the orientation of the vascular bundles is not informative. However, the cells on the adaxial surface show considerable variation in size. They are usually much larger than those of the abaxial epidermis, but near the margins they are smaller, and identical in appearance to those of the abaxial epidermis. Stomata only occur very close to the margins, in the region where the epidermal cells appear similar to those on the abaxial epidermis. They are absent from the areas where the adaxial epidermis appears distinct from the abaxial epidermis, and they are absent from the adaxial surface of the sheaths. This arrangement is similar to that in some species of *Juncus* where there is a tendency for the adaxial surface to become reduced. For example, in *Juncus squarrosus* the apparent margins, which contain sclerenchyma strands, are not the 'true' margins; the abaxial surface extends around the apparent margins and the adaxial surface is only present in the central part of the leaf (Adamson 1925; Cutler 1969; Guédès 1967; see also Section 3.4.1 *Reduction of the adaxial epidermis*). In conclusion, the interpretation by Cutler (1969) that stomata are abaxial only is accepted here.

3.4.3 Adaxial parenchyma

The figure of *Juncus trifidus* in Buchenau (1906) shows one layer of nonchlorenchymatous cells next to the adaxial epidermis, but not extending along the full length of the adaxial side. Cutler (1969) records no such layer, and the specimens studied here lack a subepidermal layer. However, in Fig. 3.12B two areas are indicated on the adaxial epidermis where an epidermal cell appears to have divided anticlinally. This may indicate the origin of the subepidermal layer observed by Buchenau.

The observations of *Rostkovia magellanica* here also differ from those in the literature, as a subepidermal parenchyma layer was observed on the adaxial side (Fig. 3.34E), but this was not noted by Cutler. Although the majority of the adaxial subepidermal layers are sclerenchymatous, there are gaps where there is no sclerenchymatous subepidermal layer, and a layer of parenchyma cells occurs in these areas.

3.4.4 Lobed and stellate cells

The only previous detailed descriptions of the mesophyll are given by Cutler (1969). Further detail may be added to his descriptions here, particularly regarding the presence and form taken by lobed or stellate chlorenchyma and parenchyma cells. Lobed cells are described by Cutler as present in a number of taxa, in the chlorenchyma of *Distichia*, *Marsippospermum*, *Oxychloe* and *Juncus* sections *Iridifolii* and *Ozophyllum*. The cells of the chlorenchyma of *Luzula* are described as 'sometimes lobed', but he does not give any further detail and does not mention lobed cells in the individual descriptions. Stellate chlorenchymatous cells are described as occurring in the septae of *Juncus* sections *Iridifolii* and *Ozophyllum*, and stellate non-chlorenchymatous pith in sect. *Forskalina*.

There are some differences between Cutler's (1969) descriptions and the results presented here. Lobed cells were not observed in *Distichia*, and they were not observed in the leaf blades of *Oxychloe*, as described by Cutler. However, stellate cells were found in the sheaths of *Oxychloe andina* (Fig. 3.31C) and *O. bisexualis*, associated with transverse vascular bundles and forming transverse septae (see following section '*Septae*'). Similarly, lobed chlorenchyma cells were not observed in *Luzula*, but stellate cells were observed in the leaf sheaths and blades of *L. sylvatica*, associated with transverse vascular bundles and forming transverse (Fig. 3.24E).

The observations of lobed or stellate cells in *Marsippospermum*, and *Juncus* sects. *Forskalina*, *Iridifolii* and *Ozophyllum* presented here are similar to those of Cutler, but lobed cells are found to be present in additional sections of *Juncus*. Lobed cells were found in the leaf sheath of *J.* sect. *Stygiopsis*, although they were infrequent. This does not contradict the observations in the literature, as only the leaf blades have been described previously.

The ground tissue of *Juncus* sect. *Juncus* has not previously been described as containing lobed or stellate cells, but the leaves examined here were found to contain both lobed and stellate cells. The outer layers of the leaf blade are chlorenchymatous, the inner layers of which are only slightly lobed, with very small intercellular air spaces. Adjacent to this are several cell layers of parenchymatous stellate cells. In longitudinal section they have four lobes and appear cross-shaped, with large intercellular air spaces (Figs. 3.18E), but in

transverse section they are more or less radially symmetrical and unlobed, and are less conspicuous (Figs. 3.18D). In the leaf sheath the ground tissue is more conspicuously stellate in shape; the cells are irregular with a variable number of lobes, but with very large intercellular air spaces, and they occur throughout a large proportion of the section (Fig 3.17C).

3.4.5 Septae

In *Juncus* sects. *Forskalina*, *Iridifolii* and *Ozophyllum* transverse septae occur across the longitudinal air canals at intervals. The cells of the septae are lobed to stellate in shape, as described by Cutler (1969), and they are associated with small transverse vascular bundles. Transverse septae are also known to occur in *Juncus* sect. *Stygiopsis* (Cutler 1969; Kirschner 2002b).

In the results presented here, transverse septae have been found to occur in a number of other taxa. They were found to be present in the sheaths of *Oxychloe*, and both the sheaths and blades of *Luzula sylvatica*. They are anatomically similar to the transverse septae occurring in *Juncus* sections *Iridifolii* and *Ozophyllum*, consisting of a plate of stellate chlorenchyma cells surrounding small transverse vascular bundles, thus forming a transverse septa across one of the longitudinal air canals.

Within *Juncus*, septae were observed in the sheaths of section *Graminifolii*. They were infrequent, as only a single example was observed, in *J. cyperoides*. They were not observed in the leaf blades, which tend to have air canals which are reduced in area in comparison with the sheath. The relatively high proportion of mesophyll relative to air canals would mask a tendency to form transverse septae across the air canals.

The occurence of transverse septae in these taxa may be of taxonomic significance, particularly within *Juncus*. The observation of transverse septae in sect. *Graminifolii* shows that they occur in every section of subgenus *Juncus*, with the exception of section *Juncus*, which does not contain air canals in either the sheaths or the blades. In contrast, transverse septae have not been observed in any sections of subgenus *Agathryon*.

3.4.6 Vascular bundle arrangement

The arrangement of vascular bundles in the sheaths shows a small degree of variation, but it also sometimes differs from that in the leaves, often together with a change in the arrangement of air canals. In *Juncus* sects. *Forskalina* and *Ozophyllum* the sheaths have a row of vascualar bundles alternating with a row of air canals. In the sheaths of large species, e.g. *Juncus subulitepalus*, the vascular bundles are clearly arranged in more than one row, with the additional rows occurring abaxially of the row of air canals (Fig. 3.19C). In the sheaths of smaller species or individuals, additional rows of vascular bundles may be less frequent. However, their arrangement in relation to the arrangement of the air canals is similar to that in sheaths where two or more rows are disinct and easily identifiable, with large vascular bundles alternating with the air canals, and smaller ones occurring abaxially of the air canals.

This is in contrast to the blades of the same species, which have a single central air canal, and lack air canals between vascular bundles. Furthermore, vascular bundles usually occur in a single ring: the occurrence of bundles directly abaxially of other bundles is infrequent, and in this study it was only observed in very large leaves, e.g. those of *J. subulitepalus* (Fig. 3.20D).

The arrangement of vascular bundles of *Juncus squarrosus* into several layers is unusual within subgenus *Agathryon*, with the exception of *J. subulatus*. It differs from previous observations by Buchenau (1906) and Cutler (1969), where *J. squarrosus* is described or figured as having a single row of bundles. However, Adamson (1925) mentions that vascular bundles sometimes occur in more than one row. Guédès (1967) figures a series of sections of the leaf blade of *J. squarrosus*, and while some of the figures indicate a poorly defined row of vascular bundles, others indicate a more scattered arrangement, with some bundles occurring in more than one layer.

The leaves of *J. squarrosus* differ from the leaves of other species with multiple layers of bundles in that they are bifacial, although the adaxial surface is reduced. The leaf blades of other species observed to have vascular bundles in several layers are unifacial. They also differ in that the bundles appear scattered throughout the section, without a clear

alternation with air canals. In this respect they bear some similarity to the leaves of *Juncus* sect. *Juncus*, which also has vascular bundles scattered throughout the section, but they differ anatomically in other ways, such as the unifaciality of the leaf blades and presence of lobed cells in sect. *Juncus*.

3.4.7 Subepidermal sclerenchyma girders

Buchenau (1906) illustrated *Juncus bufonius* with sclerenchymatous girders extending from the sheaths of one or several vascular bundles to the abaxial epidermis. Cutler found *J. bufonius* to have no such girders. These results support the observations of Cutler, as there are no abaxial girders in the specimen examined. There are also differences regarding *Juncus trifidus*, as Buchenau figures it with adaxial girders, but I have not found this to be the case. Cutler does not indicate whether his specimens possessed girders or not.

In some species of *Luzula* the vascular bundle sheaths extend towards the adaxial or abaxial epidermis to form a girder. Usually these are sclerenchymatous, but in some specimens the parenchymatous outer bundle sheath forms an extension to the epidermis. These have not been noted previously, but they are sometimes very distinctive in appearance (e.g. Fig. 3.24G), forming a parenchymatous bridge across the width of the leaf, up to five cells wide.

In *Juncus maritimus*, a series of sclerenchymatous girders are present which are not formed from extensions of the vascular bundle sheaths. Cutler describes specimens in which some of the girders connected to the vascular bundle sheaths, but this was not the case in any of the specimens examined here.

3.4.8 Sclerenchymatous strands

Strands of sclerenchyma occur in the ground tissue of a number of species throughout Juncaceae. Although they lack any vascular tissue, they look similar to small vascular bundles, as they sometimes possess a parenchymatous bundle sheath similar to the vascular bundles in the same section, and their position in the section is usually consistent with the position in which small vascular bundles would be expected to occur, such as near the margins at the ends of a row of vascular bundles, or alternating with large vascular bundles.

Cutler (1969) notes their presence in the centre of the leaf blades of *Juncus* sect. *Juncus*, and suggests that they may be vestigial vascular bundles. Such strands are observed here in a number of other taxa in addition to sect. *Juncus*. However, with the exception of sect. *Juncus*, in all cases they were present only in the sheaths. Their distribution may be of taxonomic significance, as they are frequent among species of subgenus *Juncus*, but they are absent from subgenus *Agathryon*, with the exception of *J. subulatus*. They were also observed in *Distichia* and some species of *Luzula*.

3.5 Conclusions

The study of the anatomy of Juncaceae has resulted in new descriptions of the anatomy of the leaves of the genera, subgenera and sections. New observations have been made, particularly of the leaf sheaths, which had not previously been described for most of the taxa examined here. The study of the leaf sheaths has resulted in the identification of several characters which may be taxonomically informative. A smaller degree of variation is present in the sheaths than in the blades, as the sheaths are always bifacial, but the blades may be bifacial or unifacial, differ in their cross-sectional shapes and in the arrangement of air canals. But because of this comparisons between some characters become easier. For example, in the sheaths, within a row of vascular bundles air canals alternate with the vascular bundles, so that additional rows of vascular bundles may be identified easily as they appear abaxially of the air canals. This is not always the case in the blades, where the air canals may become lost.

A dataset of 26 characters has been obtained, and the data is presented in Table A3. Most of these characters have not previously been used in statistical analyses, and the distribution of many of these characters have not previously been examined in relation to taxonomic classifications of Juncaceae.

Chapter 4 Auricles or ligules?

4.1 Introduction

Morphological cladistic studies of Juncales have made use of the presence or absence of ligules, recording them as present in some genera and absent in other genera within both Cyperaceae and Juncaceae (Munro & Linder 1998; Simpson 1995). The presence or absence of ligules within Cyperaceae has been well documented in most genera, and it has been considered to be a useful diagnostic character within the family (Camelbeke & Goetghebeur 1999). In Juncaceae there are often structures present at the junction between the leaf sheath and blade, and sometimes these bear a resemblance to the ligules of other families, such as those of Cyperaceae and Poaceae (Figs. 4.1, 4.2). They have been referred to variously in the taxonomic literature as auricles (Balslev 1996; Kirschner 2002a), stipule-like lobes (Dahlgren et al 1985), ligule-like structures (Kirschner 2002a), and ligules (Dahlgren et al 1985). When present they show much variation in size, shape and arrangement between species (Buchenau 1906), and they may therefore be useful to determine relationships within Juncaceae and between Juncaceae and Cyperaceae. However, the variation in the morphology is rarely referred to in taxonomic or cladistic works, although it is sometimes used to distinguish species within Juncus sect. Steirochloa (Balslev 1996; Kirschner 2002c).

The term 'ligule' used here follows the common usage for Poaceae, referring to a membranous structure on the adaxial side of the leaf, at the junction between the leaf sheath and leaf blade. In Cyperaceae it may take the form of a continuous membranous appendage (Fig. 4.1A) or it may be reduced to a line of hairs (Camelbeke & Goetghebeur 1999). The ligule referred to here is not to be confused with the contraligule of some Cyperaceae, or the abaxial ligule which occurs in some Poaceae. Although a ligule similar to that of Poaceae is present in some Cyperaceae species, none of the structures in the species of Juncaceae examined here were of very similar appearance to those of Cyperaceae, so the homologies of the structures were not clear. Figs. 4.1 and 4.2 represent the range of variation within Juncaceae observed during an external morphological examination, and include one species of Cyperaceae for comparison.

In Juncaceae it is difficult to determine whether the structures originate from the true adaxial surface using external observations; if they do not, then they are not homologous to the ligules of Poaceae or Cyperaceae. Initial observations indicated that in some of the genera where a ligule appeared to be present, e.g. *Distichia muscoides* (Fig. 4.1B), it was associated with a unifacial leaf blade on the upper side. This meant that it was not clear whether the 'ligule' is an outgrowth of the adaxial surface, as although an adaxial surface is present below it, it is not present above it.

In some species, e.g. Juncus bufonius (Fig. 4.2A) there are no structures present at the junction between the sheath and the blade, but in others, such as *Rostkovia* and some Juncus species, two membranous lobes are present. These may appear as outgrowths of the top of the sheath, and not originating from the adaxial surface, for example in Juncus tenuis Willd. (Fig. 4.2B), or they may appear to occur on the adaxial surface, for example in *Rostkovia magellanica* (Fig. 4.2C). However, intermediate forms can also be seen in some species of Juncus where the lobes extend part way across the adaxial surface, but do not meet in the middle of the leaf (Emberger 1960). A further type of structure, different in appearance to any of the above structures, was apparent in Juncus maritimus (Fig. 4.1C), where a bifacial leaf sheath joins a unifacial leaf blade, as it does in *Distichia muscoides*, but the junction does not possess a continuous membranous outgrowth as it does in *Distichia*.

It was considered that a study of the anatomy of the leaves at the junction between the leaf sheath and leaf blade would be necessary to establish which structures are homologous and which are not.

4.1 Introduction



Figure 4.1 Ligules and ligule-like structures. A *Carex spicata*. Ligular stipule present in the form of a membrane which crosses the adaxial surface, at the junction between the leaf sheath and blade, and is continuous with the sheath margins. B *Distichia muscoides*. A ligule-like structure in the form of a continuous membrane occurs adaxially, between the bifacial leaf sheath and unifacial leaf blade. C *Juncus acutus*. Unlike *Distichia* there is no ligule-like structure present at the junction between the bifacial leaf sheath and unifacial leaf sheath and unifacial blade.



Figure 4.2 Auricles. A *Juncus bufonius*. No structures are present at the junction between the bifacial leaf sheath and blade. **B** *Juncus tenuis*. The margins of the sheath extend upwards to form two membranous auricles. Some marginal proliferation also occurs laterally (indicated by arrowheads). **C** *Rostkovia magellanica*. The membranous auricles formed at the top of the sheath occur adaxially, and meet in the middle of the adaxial surface. The auricles overlap but they are not fused together.

4.1.1 Literature review

There is some inconsitency in the literature in what is meant by the term 'ligule'. In the anatomical literature, Fahn (1990), Mauseth (1988) and Metcalfe (1960) define it as a small, membranous flap of tissue on the adaxial surface, where the blade joins the sheath. These authors also describe projections which may occur at the margins of the leaf in the same region, which are called 'auricles' in Poaceae, where they occur from the upper side of the sheath, and 'stipules' in some other families, where they occur either side of the ligule (e.g. Hydrocharidaceae, Potamogetonaceae, Pontederiaceae).

Other authors, such as Heywood (1993) define the ligule as a 'scale-like membrane on the surface of the leaf'. It is sometimes used in this broader sense in the taxonomic literature, including in Juncaceae (e.g. Dahlgren et al 1985), and although the structures referred to tend to be adaxial in their position, they may be associated with the leaf margins or with unifacial leaves, and not necessarily with the adaxial epidermis. As a consequence, structures with different forms, in different positions on the leaf, and possibly with different origins, may sometimes be referred to as ligules. This can lead to difficulties with cladistic studies, where knowledge of the homology of structures is important.

Among Monocotyledons ligules occur frequently among Poaceae, and the ligule has been most thoroughly investigated in this family (Mohl 1845; Colomb 1887; Philipson 1934; Cusset 1965; Tran Thi Tuyet Hoa 1965). Mohl (1845) showed that in *Poa alpina* L. the ligule is formed from a membranous outgrowth from the adaxial surface at the junction between the sheath and leaf blade, and that this outgrowth sometimes fuses on either side with membranous lobes formed from the margins of the sheath. Similar observations have also been made in Cyperaceae (Guichard 1929), where ontogenic studies of the leaves have shown that an outgrowth occurs from the adaxial epidermis to form a central structure, which Guichard refers to as the ligule. This then fuses with the margins of the sheath to form a continuous membranous structure extending across the adaxial surface of the leaf, between the margins of the sheath (Fig. 4.1A). This structure can, therefore, be considered to be formed by the fusion of structures of different origins. It has been termed 'ligular stipule' by Troll (1939), which he defines as a structure formed from a fusion of

true 'ligules' (outgrowths from the adaxial epidermis) with the 'stipules' (lobes formed from the membranous edges of the sheath).

Structures with a superficially similar appearance also occur among several other Monocot families, including Hydrocharidaceae, Juncaceae, Liliaceae, Pontederiaceae, Potamogetonaceae, and Zingiberaceae (Guédès 1967). Troll (1939) also observed ligulelike structures in the Dicotyledons, in the form of two membranous lobes which lie adjacent to one another on the adaxial side of the petiole, and are fused together along the length of their inner margins. He believed that they are not equivalent to the adaxial ligule in Poaceae, and linked their presence to the loss of the adaxial surface in the petiole, which does not occur with the ligule of Poaceae. At the base of the petiole, a bifacial region sheathes the stem, but above this the margins of the sheath draw together, eventually fusing to form the unifacial petiole. The lobes occur at this point, bounded on one side by the bifacial sheath, and on the other by the unifacial petiole. Troll considered that the junction between the bifacial and unifacial structures was critical for the development of this structure, and that the two lobes were formed by an outgrowth from each margin. The lobes are fused together at the point where the two margins fuse to form the unifacial petiole, and there is no outgrowth from the adaxial epidermis. Some of the ligule-like structures in the Monocotyledons are also of this form, but occur at the junction between a bifacial sheath and unifacial leaf blade. Troll (1939) termed these structures 'median stipules', to distinguish them from the 'ligular stipules' of Cyperaceae and Poaceae.

Guédès (1967) studied a number of other Monocotyledons to discover whether they possessed true ligules (*sensu* Guichard 1929), ligular stipules or median stipules. Among these were three species of Juncaceae and two of Cyperaceae. His observations indicated that the ligular structures of the two Cyperaceae species were homologous to those of many Poaceae, consisting of an adaxial outgrowth which extends laterally, and is continuous with the edges of the sheath. Transverse sections through the junction between the sheath and the blade showed a single arc of vascular bundles in the sheath that was maintained through the junction and into the leaf blade. There were never any vascular bundles adaxially (Fig. 4.3), and they never formed a complete ring, as usually happens in unifacial leaves (Cutler 1969; see also Section 3.4.1 *Reduction of the adaxial epidermis*). He interpreted this to mean that the leaf retained its adaxial surface throughout the

transition from sheath to blade, and the ligular structure must, therefore, be at least in part derived from an outgrowth from the adaxial surface. Guédès concluded that the ligular structures of Cyperaceae are ligular stipules, following the terminology of Troll (1939).



Fig. 4.3. *Schoenus nigricans* transverse sections in the ligular region of a vegetative leaf. Illustration taken from Guédès 1967. From left to right: leaf sheath to leaf blade.

Guédès came to different conclusions regarding the structures of the three Juncaceae species. He found no evidence for any structures which originate directly from the adaxial surface, indicating that the ligular structures of Juncaceae are not homologous with the ligular stipules of Cyperaceae. The junction between the leaf sheath and blade in Juncus squarrosus possesses two membranous lobes which are continuous with the sheath margins, and which extend part way across the adaxial surface, but do not meet in the centre (Fig. 4.4 I). A series of transverse sections of this region shows that the adaxial surface is continuous from the sheath to the blade, but it is reduced in area in the blade, causing the 'true' margins to become displaced, and drawn part way across the ventral surface (marked by black arrows in Fig. 4.4). The reduction in the adaxial surface is indicated by a reduction in the area of adaxial parenchyma, the edges of which are taken to mark the edges of the adaxial surface (for further discussion see Section 3.4.1 Reduction of the adaxial epidermis). The orientation of the vascular bundles also changes at this point, adding support to this interpretation. The bundles closest to the margin rotate so that the xylem poles are orientated towards the parenchyma, and they are no longer perpendicular to the edges as they are in the sheath (Fig. 4.4 II-IX). Subepidermal strands of sclerenchyma, which do not occur in the sheath, appear at this point in the 'apparent' margins (marked by white arrows in Fig. 4.4).

The edges of the non-chlorenchymatous parenchyma occur at the inner edge of the lobes. The region between the lobes, therefore, appears to correspond to the adaxial surface of the
sheath, with which it is continuous, and the surface either side of them, which includes the apparent margin, appears to correspond to the abaxial surface of the sheath. Guédès concludes that the membranous lobes originate entirely from the margins, and not from the adaxial surface.



Fig. 4.4. *Juncus squarrosus*. I Ventral view of leaf. **II–IX** Transverse sections in the ligular region. Illustrations taken from Guédès 1967. Black arrows indicate the true margin; white arrows indicate the apparent margin. Sclerenchyma strands at apparent margin are marked in black. Vascular bundles are stippled, with a dotted line indicating the xylem–phloem pole orientation. Stippled areas with no line indicate air canals. A dotted line indicates the boundary of non-chlorenchymatous parenchyma which is present adaxially.

Guédès found a similar situation in *Juncus lamprocarpus* Rchb., in which the lobes are formed from the margins, and not from the adaxial surface. *Juncus lamprocarpus* differs, however, in that the lobes do meet in the middle of the leaf, and the leaf blade is unifacial (Fig. 4.5 I–II). Using a series of diagrams of transverse sections (Fig. 4.5 XI–XV) he shows that the margins are drawn together completely, as indicated by the arc of vascular bundles in the sheath (Fig. 4.5 XI) coming together to form a complete ring in the blade (Fig. 4.5 XV). The point of insertion of the lobes, which are continuous with the margin, remain adjacent to the ends of the arc of vascular bundles. As in *Juncus squarrosus*, the area between the lobes is interpreted as being equivalent to the adaxial surface in the

sheath, with which it is continous. Where the lobes meet, the adaxial surface is lost and the leaf becomes unifacial. Although Guédès does not indicate them in his diagrams, he describes bulliform cells being present on the adaxial surface in the sheath, and present between the membranous lobes in XI and XIII (Fig. 4.5), but absent in XIV and XV, further supporting his argument that the adaxial surface becomes lost.



Fig. 4.5. *Juncus lamprocarpus*. **I–II** Ligular regions of leaves. **XI–XV** Transverse sections through the region of passage from the sheath to the blade. Illustrations taken from Guédès 1967. **M** indicates the vascular bundle at the midrib. Major vascular bundles between the midrib and the margin are numbered 1 to 5 to show continuity through the series.

However, the two lobes are not fused together, as they are in the median stipules described by Troll (1939). Guédès concludes that the structures present in *J. squarrosus* and *J. lamprocarpus* are neither ligular stipules nor median stipules, but he does not suggest a term to describe the structure. He states that the individual membranous lobes could be considered to be stipules (the term used by Troll 1939) only in a broad sense, as they are not equivalent to the stipules of the Dicotyledons; instead, he refers to them as 'earlets' (oreillettes).

The third species of Juncaceae studied by Guédès (1967) was *Oxychloe andina*. This species has a continuous membranous structure across the adaxial side of the leaf, between a unifacial leaf and bifacial blade (Fig. 4.6 I), which Guédès concluded is a true median stipule. In this case, as in *Juncus laprocarpus*, the membranous structure also occurs at the transition from the bifacial sheath to the unifacial sheath. Diagrams of a series of transverse sections through the leaf (Figure 4.6 II–IV) show a transition from an arc of vascular bundles in the sheath to a ring in the blade. The vascular bundles make a complete ring above the membranous structure, but below it they do not. Guédès concludes that this differs from *J. lamprocarpus* in that the sheath margins are fully fused,

forming a median stipule, whereas in *J. lamprocarpus*, although they are adjacent, they are still free.



Fig. 4.6. *Oxychloe andina*. I Ventral view of leaf. **II–IV** Transverse sections through the region of passage from the sheath to the blade. Illustrations taken from Guédès 1967. **M** labels the vascular bundle at the midrib. Certain vascular bundles are labelled with numbers or letters to indicate continuity through the sections.

In conclusion, it can be seen that the structures present at the junction between the leaf sheaths and blades, in at least some Juncaceae, are not homologous to those of Cyperaceae, and a number of different types of structures are present. The structures present in each of the species examined by Guédès (1967) differ in the degree of fusion between the margins of the sheath, and an important factor in their comparison is in the degree to which the adaxial surface is lost in the leaf blade. Although some well-defined terminology has been applied to these structures (Troll 1939; Guédès 1967), it has not been used in the taxonomic literature produced since this time (Balslev 1996; Kirschner 2002a, b, c), and the different types of structures present have rarely been described in detail for species of Juncaceae other than the few described above. Consequently, the homologies of the full range of variation of these structures are still uncertain.

4.1.2 Aims

- 1. The range of variation in the structures occurring at the junction between the leaf sheath and leaf blade are examined.
- 2. Homologies are determined between these structures within Juncaceae, and between Juncaceae and Cyperaceae.
- 3. Characters and character states are determined in order to reflect the variation observed in these structures.
- 4. Data are collected in order to include these structures in analyses and a discussion of evolution in Chapter 5 *Phylogeny*.

4.2 Materials and methods

In order to investigate the nature of the ligular structures, the positions of the structures relative to the adaxial and abaxial surfaces must be determined, and for this it is necessary to examine the anatomy in transverse section. Transverse sections were made of the region of the junction between the leaf sheath and leaf blade, using methods identical to those set out in Chapter 3 *Anatomy*, but serial sections were taken through the region of the junction between the leaf blade.

The specimens selected for study were chosen to represent the range of structures found in Juncaceae, after an initial survey of herbarium specimens using a binocular light microscope. The same specimens were used as in Chapter 3 *Anatomy*, and each species examined is figured and discussed in Section 4.3 *Results*. Table A1 lists voucher specimens.

4.3 Results

The junctions between the leaf sheaths and blades are described from series of transverse sections taken through the region, from the sheath just below the junction, to either the point of the junction, or to the blade just above the junction. See Table A1 for a list of specimens examined.

4.3.1 Terminology

In the literature different terms have been used for similar structures. In order for the descriptions here to be clear and consistent, the choice of the terms used is outlined below.

Ligule will be used to refer to structures which appear to be homologous to the ligules of Cyperaceae and Poaceae. They must arise from the true adaxial surface at the junction between the sheath and the blade. The ligule has a different developmental origin to auricles, although it may sometimes be fused with auricles (see '*Ligular stipule'*).

Auricle will be used to refer to lobes which are formed from an extension of the edges of the margins. These structures were termed 'earlets' by Guédès (1967), and have also been termed 'stipules' (Troll 1939) or 'stipule-like lobes' (Dahlgren et al 1985). Since the term 'stipule' is used in Dicotyledon families to refer to different structures, it is considered inappropriate here. The use of the term 'auricle' for these structures is consistent with the use of the term in Poaceae, and it has also been used in this sense by Balslev (1996) and Kirschner (2002a, b, c).

Ligular stipule will be used to refer to a structure formed from the fusion of a ligule with two marginal auricles, as described by Troll (1939). Although the use of the word 'stipule' is not consistent with the use of the term 'auricle' above, its use here is not considered to be confusing, and as no alternative terms have been applied in the literature, the terminology suggested by Troll will be followed here.

Median stipule refers to a structure formed by the fusion of the inner margins of the two marginal auricles, associated with the loss of the adaxial surface at the junction between a bifacial sheath and a unifacial blade, as described by Troll (1939).

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4.3.2 Descriptions of the junctions between leaf sheaths and blades

Leaves with no ligule-like structures

A number of species of Juncaceae have no structures on the adaxial side of the leaf. The top of the sheath sometimes takes the form of auricles, but their point of attachment to the leaf is on the margin, and is not adaxial. *Juncus tenuis* (Fig. 4.2B) and *J. trifidus* are examples of species which have very prominent auricles, whereas *J. bufonius* (Fig. 4.1A) lacks auricles.

1. Juncus trifidus

Sections were taken of *Juncus trifidus* to provide an example of a species with bifacial leaves but with no ligule-like structures present (Fig. 4.7). *J. trifidus* has auricles which extend from the margins; this arrangement is similar to that shown in Fig. 4.2B of *J. tenuis*.

In the sheath, the margins are extended laterally to form thin (three cells thick or less) margins which sheathe the stem. In the blade the margins are acute to rounded and not membranous, and do not extend to sheathe the stem. At the junction between the sheath and the blade, the membranous edges of the sheath extend upwards to form auricles which continue to sheathe the stem, and to some extent they also extend laterally from the inner sides of the margins, as they do in *J. tenuis* (Fig. 4.2B). However, although the auricles lie next to the leaf blade, they are free from it.

The point at which the transition occurs between the sheath and the blade, and the sheath margins begin to form the auricles, may occur at slightly different heights on each side of the leaf; in Fig. 4.7 a progression is shown of the transition from sheath to blade on the right side, but on the left side this progression occurred at a lower point, and the left margin in Fig. 4.7 is that of a leaf blade, not a leaf sheath.

Throughout the transition from the sheath to the blade the adaxial and abaxial surfaces are continuous and their relative proportions do not show significant change, and the number and arrangement of vascular bundles remains constant.



Figure 4.7 *Juncus trifidus* junction between leaf sheath and blade. A The transition between leaf sheath and blade occurs at a different level on the left and right sides. The right edge has a sheathing margin, and the left does not, but an auricle is present (a). B Near the top of the sheath. Note constrictions (c). C Leaf blade. The margins no longer form a sheath. One of the two auricles which are present at the top of the sheath (a) is visible.

Leaves with unifacial blades

2. Juncus ensifolius

Juncus ensifolius Wikstr. has a laterally flattened leaf, so that the margins of the bifacial sheath lie more or less parallel to one another, and the blade is unifacial and ensiform (Fig. 4.8). Fig. 4.8A shows the leaf split longitudinally in the region of the junction between the sheath and the blade. Figs. 4.8B and C show the blade and sheath in transverse section. The adaxial surface is progressively lost during the transition from sheath to blade, as the inner surfaces of the sheathing margins become reduced in area as they become closer to the top of the sheath.

The major vascular bundles are visible as ridges on the inside of the cut leaf, and faintly visible on the inner surface of the sheathing margins as darker bands. As the sheathing margins are reduced in area and the unifacial region increases in area, the number of vascular bundles which occur in the sheathing margins is reduced, and the number occurring in the unifacial region increases. However, the bundles are continuous throughout the transition from sheath to blade, and remain parallel to one another. In the transverse sections the adaxial surface is present on the insides of the sheathing margins, and the ground tissue under the adaxial surface is parenchymatous. The ground tissue under the abaxial surface is chlorenchymatous. In the unifacial blade all of the ground tissue is chlorenchymatous. The vascular bundles are orientated towards the adaxial surface in the bifacial regions of the sheath, but in the unifacial blade they are in a 'ring' (this appears to be two parallel rows due to the lateral flattening of the leaf), with the xylem poles orientated towards the centre of the blade.

Figure 4.9 shows a series of transverse sections of the junction between the sheath and the blade. In 4.9A the sheathing margins are present but small, and most of the leaf is unifacial. The adaxial surface occurs between the margins, and in this area the ground tissue is parenchymatous, whereas in the unifacial region the ground tissue is chlorenchymatous. The vascular bundles retain their arrangement in the v-shape which occurs in the sheath and do not appear to form a complete ring, but only two vascular bundles are now present in the small sheathing margins. These two bundles are orientated

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Figure 4.8 Juncus ensifolius. A Photograph of leaf in the region of the junction between the sheath and the blade, cut in half longitudinally and showing the inner surface. B Transverse section of leaf blade. C Transverse section of leaf sheath.

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Figure 4.9 Juncus ensifolius leaf sheath below the junction between sheath and blade. A Parenchyma (p) is present adaxially. The abaxial ground tissu e is chlorenchymatous (c). The vascular bundles occur in an arc, with the bundles at each end of the arc (vb) occurring within the sheathing margins. **B** The area of adaxial surface is reduced closer to the junction, and the sheathing margins reduce in size. The vascular bundles marked vb have drawn closer together, occurring at the junction between the abaxial chlorenchyma and the adaxial parenchyma (p). **C** Just below the junction between the sheath and the blade the sheathing margins are reduced to minute ridges (indicated by arrows) with a minute area of adaxial parenchyma between them. The orientation of the vascular bundles has rotated so that the xylem poles are orientated more towards the middle of the leaf and away from the epidermis.

with the xylem poles towards the small area of adaxial parenchyma. The rest of the vascular bundles occur in the unifacial region, with the xylem poles orientated towards the centre of the leaf.

In 4.9B and 4.9C the margins are progressively reduced; they are small membranous projections in 4.9B, and minute ridges in 4.9C. The area of adaxial parenchyma is also progressively reduced. The vascular bundles which were present in the margins of 4.9A change their orientation, with the xylem poles tending to form a complete ring and to become orientated towards the centre of the leaf as the area of adaxial surface becomes lost.

3. Juncus maritimus

Juncus maritimus, like J. ensifolius, shows a transition from a bifacial sheath to a unifacial blade, with a progressive reduction in the adaxial surface in the sheath as it approaches the junction with the blade, and a corresponding reduction in size and then loss of the sheathing margins (Fig. 4.1C). Figure 4.10 shows transverse sections in the region of the junction between the sheath and blade. In Fig. 4.10A the margins are present but small (compare to Fig. 3.16A showing a transverse section taken in the mid-section of the sheath). The adaxial surface occurs in the small area between the margins, but most of the epidermis is abaxial. Fig. 4.10B shows the same area under higher magnification. The xylem poles of the vascular bundles are orientated towards the centre of the leaf throughout most of the section, but in the sheathing margins they are orientated towards the adaxial surface. Figs. 4.10C and D show a section taken higher up, at the junction between the sheath and blade. The sheathing margins are almost entirely lost, visible only as two minute ridges which are directly adjacent to one another. Since the adaxial surface occurs on the inner surface of the margins, it corresponds to the few cells between the ridges in this section, and has almost been lost. The arrangement of the vascular bundles has changed, as the bundles which were present in the sheathing margins in Figs. 4.10A and 4.10B have drawn closer together, almost forming complete rings, and the xylem poles are orientated more towards the centre of the leaf and away from the leaf surface.



Figure 4.10 Juncus maritimus junction between leaf sheath and blade. A Leaf sheath near to the leaf blade. The adaxial surface (ad) occurs between the margins and is reduced in area compared to the abaxial surface (ab). **B** The vascular bundles are arrangeed in an arc on the adaxial side, with the xylem poles orientated towards the adaxial surface (ad). **C** Junction between the sheath and the blade. A small adaxial groove indicates the point where the sheath margins meet. The unifacial blade occurs above this point. **D** The vascular bundles almost form a complete ring, with the xylem poles tending to become orientated away from the epidermis, indicating the almost complete loss of the adaxial surface at this point. Minute sheath margins are present and are adjacent to one another.

4. Juncus subulatus

Juncus subulatus also shows a transition from a bifacial sheath to a unifacial blade, associated with a progressive reduction in the adaxial surface in the sheath as it approaches the junction with the blade, and a corresponding reduction in size and then loss of the sheathing margins. J. subulatus, however, also possesses two auricles, which are continuous with the top edge of the margins of the sheath. They are adaxial in position on the leaf, with their inner margins lying adjacent to one another.

Fig. 4.11 shows transverse sections in the region of the junction between the sheath and blade. In Fig. 4.11A the sheathing margins are present, but reduced in size in comparison to the mid-section of the sheath (compare to Fig. 3.10A). The vascular bundles form a u-shape, with the bundles at the end of the u-shape occurring in the sheathing margins (in Fig. 4.11 the vascular bundles labelled vb 1 and vb 2). The xylem poles of the vascular bundles are orientated towards the centre of the leaf throughout most of the section, but in the sheathing margins they are orientated towards the adaxial surface. Fig. 4.11B shows the leaf blade, just above the junction with the sheath. The vascular bundles which were present in the sheathing margins have been drawn together and form a complete ring in the leaf blade, with the xylem poles orientated towards the centre of the leaf. A new vascular bundle has appeared (labelled vb 3 in Fig. 4.11B). No vascular bundles are present in the sheathing margins, which have become free from the leaf blade to form auricles.



Figure 4.11 *Juncus subulatus* junction between leaf sheath and blade. A Leaf sheath just below junction. The vascular bundles form a u-shape with the vascular bundles labelled vb 1 and vb 2 present in the sheathing margins. **B** Leaf blade just above junction. The vascular bundles which were present in the sheathing margins have been drawn together. A new vascular bundle has appeared (vb 3), so the vascular bundles now form a complete ring. The sheathing margins are free from the leaf blade and form auricles (a).

5. Juncus articulatus

Fig. 4.12 shows the point of transition from sheath to blade in *Juncus articulatus*. The morphology is very similar to that of *Juncus subulatus*, in this point forms the junction between a bifacial sheath and a unifacial blade, associated with two auricles whose margins lie adjacent to one another, but not fused together, in the centre of the adaxial side of the leaf. A vascular bundle, which was not present in the leaf sheath, appears in the centre of the adaxial side and between the margins of the auricles (Fig. 4.12A).

Juncus articulatus differs from *J. subulatus* in the morphology of the junction between the sheath and the blade in that distinct ridges are present on the leaf, and between these the adaxial side of the leaf has a concave surface, so that the ridges appear to indicate the leaf margins, and the concave region appears to be homologous to the adaxial epidermis. This gives the auricles the appearance of being adaxial in their position, rather than marginal. However, these ridges do not correspond to the 'true' margins. The arrangement of the vascular bundles in a ring, and the loss of the adaxial parenchyma which is present in the sheath (Fig. 3.19E) indicate that this surface is homologous to the abaxial surface of the sheath.

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Figure 4.12 *Juncus articulatus* junction between leaf sheath and blade. A Junction between sheath and blade. Note vascular bundle (vb) present near the centre of the adaxial groove. **B** Leaf blade just above the junction between sheath and blade. The auricles (a) formed from the sheath margins are free from the blade, but lie next to it.

6. Distichia muscoides

The external appearance of the junction between the sheath and blade of *Distichia muscoides* is shown in Fig. 4.1B. It possesses a continuous membranous structure across the adaxial side of the leaf, marking the division between the bifacial sheath and unifacial blade. Transverse sections of this region are shown in Fig. 4.13.

In the upper parts of the leaf sheath near to the junction, the single arc of vascular bundles present in the sheath forms a v-shape. Adaxially, between the ends of the v-shape, lies a region of parenchyma, whereas the abaxial ground tissue is chlorenchymatous. The bundles at the end of each row are orientated with the xylem poles pointing more-or-less towards one another, but also towards the adaxial parenchyma, which lies between them (Fig. 4.13A). Higher up the leaf two grooves develop abaxially, on each side, between the sheathing margins and the main body of the leaf (Fig. 4.13B). These grooves become larger closer to the top of the sheath, eventually meeting one another, and at this point the sheathing margins become separated from the leaf blade. The margins remain fused together on their inner sides, so that instead of forming two separate auricles as in *Juncus articulatus*, they form a single continuous structure.

In parallel with these changes are changes in the ground tissue. In the sheath the parenchyma only occurs adaxially, between the sheathing margins. Near to the leaf blade the margins become closer together, and the area of parenchyma is also reduced, as it remains between the margins (Fig. 4.13B). The region of chlorenchyma is correspondingly increased. Once the sheathing margins have become free from the blade, the parenchyma is lost and the ground tissue of the leaf blade is entirely chlorenchymatous.

Therefore, it can be concluded that the adaxial surface becomes reduced in area in the sheath below the junction with the blade, marked by the sheathing margins being drawn together, the adaxial parenchyma becoming reduced in area, and the vascular bundles tending to form a complete ring rather than a v-shape. At the junction between the sheath and blade the adaxial surface becomes lost entirely, so that the leaf blade above this point is unifacial; at the same point the margins, fused at their inner edges, extend outwards to form a median stipule.

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Figure 4.13 *Distichia muscoides* junction between leaf sheath and blade. A Leaf sheath just below junction with blade. Note adaxial parenchyma (p) between the points of attachment of the membranous margins of the sheath. **B** The points of attachment of the sheathing margins are closer together and the area of adaxial parenchyma is reduced. **C** Junction between leaf sheath and blade. **D** The membranous margins have become free from the leaf blade, but they are fused at their inner margins, forming a single auricle (a). The ground tissue of the leaf is entirely chlorenchymatous.

7. Distichia acicularis

The junction between the sheath and blade of *Distichia acicularis* is similar to that of D. *muscoides*, but the membranous structures on the adaxial side of the leaf are extended into two prominent auricles. Transverse sections are shown in Fig. 4.14. The progression of changes is very similar to those seen in D. *muscoides*. At the top of the sheath, just below the junction with the blade, the adaxial surface becomes progressively reduced, marked by a change in the point of attachment of the membranous, sheathing margins and by a reduction of the adaxial parenchyma. At the junction between the sheath and the blade the membranous margins become free from the leaf blade, and the leaf blade is unifacial, having lost the adaxial surface.

In the sheath the vascular bundles are arranged in a u-shape with the xylem poles orientated towards one another; the bundles at the ends of the row also being orientated towards the adaxial parenchyma, which lies between them. Closer to the junction between the sheath and the blade the adaxial parenchyma becomes reduced in area, remaining between the sheathing margins as they are drawn towards one another. The orientation of the vascular bundles at the ends of the row also changes slightly, as the vascular bundles tend to become orientated more towards the centre of the leaf as the adaxial surface becomes reduced. At the point where the margins become free from the leaf blade, the adaxial parenchyma has disappeared and is replaced by chlorenchyma, and a small vascular bundle appears in the middle of the adaxial side of the leaf. This vascular bundle was not present in the sheath. A complete ring of vascular bundles, surrounded by a complete ring of chlorenchyma, is present in the blade.

As in *D. muscoides* the inner margins of the auricles are fused together at their bases (Fig. 4.14E), so the structure is a median stipule. However, unlike *D. muscoides* they are also extended upwards into two prominent auricles.



Figure 4.14 *Distichia acicularis* junction between leaf sheath and blade. A Leaf sheath near to junction. Note adaxial parenchyma (p). **B** The points of attachment of the membranous margins are closer together, and the area of adaxial parenchyma is reduced. **D** Junction between sheath and blade. The sheathing margins are free from the body of the leaf (a), but their inner margins are fused. The adaxial parenchyma has been lost, and a small vascular bundle (vb), which was absent in the sheath, is present in the centre of the adaxial side of the leaf. **E** The sheath margins extend upwards into two prominent auricles, which are not fused at their inner margins.

8. Oxychloe andina

Vegetatively, *Oxychloe andina* is similar to *Distichia*, having a continuous membranous structure across the adaxial side of the leaf at the junction between the sheath and blade, similar to Fig. 4.1B. Transverse sections through this region are shown in Figs. 4.15–4.16.

Near the top of the sheath a projection occurs in the middle of the adaxial side (Fig. 4.15A). Above this point (Fig. 4.15B), the projection splits into two separate structures. A groove also develops on the abaxial side, between the sheathing margin and the body of the leaf (Fig. 4.15C). With increasing height, each groove extends towards the centre of the adaxial side (Figs. 4.15C–D), until the sheathing margins become free from the body of the leaf, and form auricles at the junction between the sheath and the blade (Fig. 4.15E–F).

During this progression the arrangement of the vascular bundles also changes. The vascular bundles at the ends of the arc in the sheath become drawn closer to the centre of the adaxial side, in parallel with the movement of the abaxial groove towards the centre of the adaxial side. As this happens the the vascular bundles at the ends of the arc also become rotated, so that the xylem poles become orientated more towards the centre of the adaxial side (Fig. 4.15D); this bundle was not present in the sheath. At the point where both auricles have become free from the leaf blade (Fig. 4.15F) the vascular bundles can be seen to form a complete ring, each orientated with the xylem poles towards the centre of the leaf, indicating that the leaf has lost the true adaxial surface, and is unifacial. Therefore, *Oxychloe andina* has a median stipule similar to those seen in *Distichia*, as the sheath margins project from the leaf in the region where a bifacial sheath meets a unifacial blade, and the margins are fused at their inner edges.

This series, however, differs from *Distichia*. In *Distichia* the fused inner edges of the margins occur at the point where the adaxial surface becomes entirely lost. In *Oxychloe*, however, the point at which the inner margins of the sheath fuse in the middle of the adaxial side (Fig. 4.15A) is lower than the point at which the leaf becomes entirely unifacial (Fig. 4.15F), as the vascular bundles still form a u-shape rather than a ring, and adaxial parenchyma is still present. Although the median stipule forms a continuous membranous structure projecting from the leaf, it is lower in the centre of the leaf.

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Figure 4.15 Oxychloe andina junction between leaf sheath and blade. A Leaf sheath. Vascular bundles form a u-shape with vb1a and vb1b at each end. Fused inner margins of the sheath form a projection from the middle of the adaxial side (arrowhead). B Higher up the leaf the inner margins of the sheath are no longer fused (arrowheads). C The sheathing margin on the left develops a constriction from the abaxial side (arrowhead). D A vascular bundle which was not present in the sheath (vb2) appears in the middle of the adaxial side. E The sheathing margin on the left is entirely free from the body of the leaf forming an auricle (a). F Leaf blade. The vascular bundles form a complete ring. Two auricles (a) are present.

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Figure 4.16 Oxychloe andina junction between leaf sheath and blade. A The fused inner margins of the sheath form the centre of the median stipule. The epidermis is adaxial, with tangentially elongated cells and thin outer cell walls. **B** The margins are free. The small region of epidermis between them is continuous with the abaxial epidermis higher in the leaf. **C** The tip of a projection formed by a downwards proliferation of the epidermis is visible between the margins (arrowhead). **D** As the margins become further apart higher in the leaf, the projection becomes wider. **E** The morphology of the epidermis between the inner margins is similar to the abaxial epidermis elsewhere in the leaf. **F** The epidermis on the adaxial side of the leaf is continuous with the abaxial epidermis.

Oxychloe andina also has a projection in the centre of the leaf, just above the point where the inner sheath margins are fused (Fig. 4.15C); such a projection was not seen in *Distichia*. However, although this projection may bear a superficial resemblance to a ligule, it appears to be formed from a proliferation of the the abaxial surface, not the adaxial surface. Fig. 4.16 shows a series of sections from the same regions, at higher magnification.

In Fig. 4.16A the fused inner margins project from the centre of the adaxial side of the leaf. The adaxial epidermis is the only epidermal surface visible; it is continuous with that lower in the sheath, and the morphology is consistent with the adaxial epidermis of the sheath, with thin outer walls, and cells which are wider tangentially than radially (Fig. 3.31A). In Fig. 4.16B the inner margins are no longer fused, and lie adjacent to one another. Between them is a region of epidermis which is continuous with the abaxial surface higher up the leaf. In Fig. 4.16C and D a projection occurs from this region of epidermis, which extends downwards, so that at its lowest point (Fig. 4.16C) it lies adjacent to the rest of the epidermis but is not continuous with it.

With increasing height in the leaf, the margins occur further apart (Fig. 4.16E). Between them the region of epidermis containing the projection expands, and develops a morphology which is consistent with the abaxial epidermis of *Oxychloe*, having cells which are elongated radially, and with a thickened outer wall (Fig. 3.31B). As the margins become free from the body of the leaf (Fig. 4.16F) this region can be seen to be continuous with the abaxial epidermis of the leaf, and with a similar morphology to the abaxial epidermis of the leaf, and also to the abaxial epidermis of the sheathing margins, which at this point now form free auricles. The adaxial epidermis of the auricles retains the morphology seen in the sheath, with tangentially elongated cells with thin outer walls.

Based upon the morphology of the epidermis, and observations of the continuity of the abaxial and adaxial epidermis of the sheath and blade, it is concluded that the membranous structures of *Distichia* and *Oxychloe* are homologous, but that in *Oxychloe* a downwards proliferation of the abaxial epidermis occurs, associated with the occurrence of the middle of the median stipule lower down the leaf than the rest of the median stipule.

Leaves with bifacial blades and with auricles present adaxially

9. Juncus bulbosus

Juncus bulbosus, like *J. trifidus*, has bifacial leaves, and auricles are present at the junction between the sheath and blade. They show a form intermediate in appearance between that of *J. tenuis* (Fig. 4.2B) and *Rostkovia magellanica* (Fig. 4.2C), as the auricles occur on the adaxial side of the leaf rather than at the margins, but they do not meet in the centre of the leaf as they do in *R. magellanica*.

Fig. 4.17 shows a series of transverse sections through the region of the junction of the sheath with the blade. Fig. 4.17A shows the leaf sheath near to the junction. The vascular bundles are arranged in a u-shape, and a region of adaxial parenchyma lies between the sheathing margins. The parenchyma extends from the adaxial epidermis into the centre of the leaf, so that all of the ground tissue on the adaxial side of the vascular bundles is parenchymatous. Fig. 4.17B shows the sheath directly below the junction. The sheathing margins and the ends of the row of vascular bundles have been drawn closer together, and the vascular bundles are arranged in a horse-shoe shape instead of a u-shape. The area of adaxial parenchyma has been reduced, but the area of parenchyma within the centre of the leaf has increased. The orientation of the vascular bundles has also changed, as the xylem poles are orientated slightly more towards the centre of the leaf. In Fig. 4.17D, both sheathing margins are free, and two auricles are present, adjacent to the leaf blade.

However, between Figs. 4.17B and 4.17D the arrangement of the vascular bundles, and the area of parenchyma, does not change significantly; the vascular bundles never appear to form a complete ring, and the region of parenchyma adjacent to the epidermis on the adaxial side of the leaf remains present, indicating that the adaxial surface is not entirely lost, so the leaves do not become unifacial at the junction between the sheath and the blade. However, the adaxial surface is reduced in area, thus drawing the sheath margins close together on the adaxial side of the leaf, although they do not meet.

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Figure 4.17 *Juncus bulbosus* junction between leaf sheath and blade. A Leaf sheath. The vascular bundles form a u-shape. A region of adaxial parenchyma is present, extending into the centre of the leaf. B leaf sheath just below junction with blade. The ends of the row of vascular bundles have been drawn inwards slightly, forming a horse-shoe shape. The sheathing margins have been drawn towards one another, and the area of adaxial epidermis between them is reduced. C The sheathing margin on the left side has become free from the leaf, forming an auricle (a). D Both sheathing margins are now free from the leaf blade and form auricles.

10. Juncus capillaceus

Juncus capillaceus appears to have auricles which are entirely adaxial, as their inner margins lie directly adjacent to one another on the adaxial side of the leaf. Their margins are not fused, but overlap one another. This is similar to the morphology shown in Fig. 4.2C of *Rostkovia magellanica*. Fig. 4.18 shows a series of transverse sections taken through the junction between leaf sheath and blade of *J. capillaceus*. As the leaf is bifacial both below and above the auricles, the adaxial position of the auricles cannot be explained by the loss of the adaxial surface in the leaf blade, as it can in the species described in the previous section.

Fig. 4.18A shows the leaf sheath just below the junction. A series of vascular bundles is present in an arc on the abaxial side of the leaf, and parenchyma is present on the adaxial side. As for the previous species described, the level at which the sheath becomes blade is different on each side of the leaf. In Fig. 4.18B, the margin on the right of the figure has become that of a leaf blade, with an adjacent, free auricle and a marginal sclerenchyma strand, but the margin on the left is still sheathing, and lacks a sclerenchyma strand.

In Fig. 4.18B the left side has two constrictions between the body of the leaf and the sheathing margin, one on the abaxial and one on the adaxial side. In Fig. 4.18C, taken slightly higher up the leaf, the constriction on the abaxial side extends almost to the constriction on the abaxial side, but not quite, so the margin is still attached to the body of the leaf by a thin strand of tissue. In Fig. 4.18D the margin has become free from the body of the leaf, and forms an auricle. The point which is now the leaf margin is marked by the presence of a sclerenchyma strand, which was not present in the sheath. It is also marked by a difference between the epidermal cells, which are much larger on the adaxial surface than the abaxial surface.

Throughout these sections the arrangement and orientation of the vascular bundles remains the same, forming an arc with the xylem poles orientated towards the adaxial side of the leaf, indicating that the leaf remains bifacial. The adaxial and abaxial epidermal cells also differ, so that the adaxial epidermis can be identified. In the sheath the adaxial epidermal cells are elongated tangentially, and they are much longer than the abaxial cells. They also lack a thickened outer wall, while the abaxial epidermal cells have a distinctly thickened

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Figure 4.18 *Juncus capillaceus* junction between leaf sheath and blade. A Leaf sheath near to junction between sheath and blade. **B** The point of transition between sheath and blade occurs at slightly different levels on the left and right sides. The right side now consists of a blade with marginal sclerenchyma strand (s) and adjacent auricle (a), but a sheathing margin is still present on the left. Note small constrictions on the left margin, on abaxial side (c1) and adaxial side (c2). **C** The constriction on the abaxial side (c1) has not increased significantly, but the adaxial one (c2) extends almost across the margin. **D** Leaf blade. Auricles (a) formed from extensions of the leaf sheath lie adjacent to the leaf blade, but free from it. A sclerenchyma strand (s), which was not present in the sheath, occurs at each margin of the blade. Arrowheads mark the 'true' margins of the auricle and leaf.

outer wall. In the leaf blade the adaxial cells remain wider than the abaxial cells, but they also become deeper, so they are much larger than the abaxial cells, which show no change.

There is no indication that the adaxial surface becomes significantly reduced, or lost, at any point throughout this region, so the reduction of the adaxial surface is an unlikely explanation for the presence of the auricles adaxially in *J. capillaceus*. The difference between the arrangement of the auricles in these two species can be explained by the position at which the constriction appears on the adaxial side of the sheath. In *J. trifidus* (Fig. 4.7B), at the top of the sheath, two constrictions appear opposite one another, one adaxially and one abaxially. Just above this point they meet, causing the sheathing margin to become free from the leaf blade. In *J. capillaceus* two constrictions also appear, but the adaxial one is not directly opposite the abaxial one; instead, it occurs near to the centre of the adaxial side (Fig. 4.18B).

The morphology of the auricles also differs slightly from those of *J. trifidus*. The point at which the auricle becomes free from the body of the leaf is not at the apparent margin of the auricle, but part way along the auricle. A ridge is present in the auricle which corresponds to this point; it is marked by arrowheads in Fig. 4.18D. To the left of this ridge, on the abaxial side of the auricle, the epidermis has a thickened cuticle, similar to the abaxial epidermis of the sheath. To the right of the ridge, the abaxial side of the sheath is more similar in appearance to the adaxial surface, with small cells and a thin cuticle. This ridge corresponds to the 'true' margin of the sheath, and the epidermis to the left is continuous with the adaxial epidermis of the sheath.

It is concluded that the auricles of *J. capillaceus* may occur adaxially, not as a result of the reduction of the adaxial surface, but of a proliferation of it.

11. Rostkovia magellanica

The junction between the sheath and blade in *Rostkovia magellanica* appears similar to that of *Juncus capillaceus* externally, with two auricles in an adaxial position on the leaf, but with a bifacial leaf both above and below the auricles. Transvserse sections of the region (Fig. 4.19) show that the vascular bundles do not change in their arrangement or orientation, and are continuous throughout the region. Differences between the abaxial and adaxial epidermis are smaller in the blade than in the sheath, but the adaxial cells are slightly larger than the abaxial cells. It can be seen that the relative proportions of the abaxial and adaxial surfaces do not change significantly from the sheath to the blade, so the presence of the auricles adaxially is not likely to be due to a reduction in the adaxial surface.

However, although there is a superficial resemblance with the morphology of *J. capillaceus*, the anatomy of the junction between the sheath and the blade appears to differ between these species. The entire abaxial side of the auricle (Fig. 4.19D) has an epidermis with a morphology resembling the abaxial epidermis of the sheath, with thickened outer cell walls and radially elongated cells, while the adaxial side has tangentially elongated cells and thin cell walls, similar to the adaxial epidermis of the sheath. The point at which the auricle becomes free from the body of the leaf is not reflected in the morphology of the auricle, as it is in *J. capillaceus*, where it is marked by the presence of a ridge and a change in the morphology of the epidermis.

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Figure 4.19 *Rostkovia magellanica* junction between leaf sheath and blade. A Leaf sheath. **B** The inner margins of the sheath appear to project from the adaxial surface. **C** The sheathing margin on the left is attached to the body of the leaf in a small region of its abaxial side. **D** The sheathing margin on the left is entirely free from the body of the leaf, forming an auricle (a).

12. Juncus triglumis

Fig. 4.20 shows transverse sections of the region between the sheath and the blade of *Juncus triglumis*. As for *Juncus capillaceus* and *Rostkovia magellanica*, little change is visible through this region in the arrangement of vascular bundles and ground tissue of the leaf, and the adaxial surface does not appear to become reduced or lost, the leaf remaining bifacial throughout this region. However, the auricles appear to occur adaxially, and their inner margins meet in the centre of the adaxial side of the leaf.

Although the auricles are not distinctly ridged on their adaxial side as they are in *Juncus capillaceus*, the auricles of *J. triglumis* have slight ridges (Fig. 4.20C) which appear close to the margins of the body of the leaf. In the region of the ridge the auricles are three cell-layers thick, but elsewhere they are two cell-layers thick. It is possible that, as for *Juncus capillaceus*, these ridges may mark the 'true' margins of the auricles. However, like *Rostkovia magellanica*, the morphology of *Juncus triglumis* differs from that of *J. capillaceus* in that the epidermis appears continous across the whole of the adaxial side of each auricle, and is not interrupted by a distinct adaxial ridge. In addition, *Juncus triglumis* differs from both species in that the point at which the auricles become free from the blade is in the centre of the adaxial side, and not at the margins of the leaf (Fig. 4.20C).

4.3 Results



Figure 4.20 *Juncus triglumis* junction between leaf sheath and blade. A Leaf sheath near to the junction between the sheath and blade. B Junction between sheath and blade. C leaf blade. The sheathing margins are free from the blade and form two auricles (a). Two slight ridges are present (indicated by arrowheads) where the auricles are 3 cell layers thick.

4.3.3 Character scoring for analyses

The data are given in Table A4. Absence/presence characters are scored as '0' or '1', respectively. Data were collected using observations of the external morphology of material rehydrated by boiling for approximately five minutes and examined using a binocular microscope.

Character List

67. Auricles present / absent.

68. Auricles free (0) or their inner margins fused to form median stipule or ligular stipule (1).

This character does not distinguish between a median stipule and a ligular stipule; however, a median stipule can only be present in leaves with a unifacial blade, and a ligular stipule in leaves with a bifacial blade, and the unifaciality and bifaciality of the leaf is recorded elsewhere (Character 1 in Section 3.3.3 *Character scoring for analyses*).

69. Auricles marginal (0) or auricles occurring adaxially (1).

This refers to auricles which appear to be associated with the adaxial surface instead of being marginal; i.e. they are not associated with a reduction or loss of the adaxial surface in the leaf blade. Auricles which appear to be adaxial because they are associated with a reduction of the adaxial surface in the leaf blade are scored as marginal. This character is not scored for Cyperaceae as it is not possible to determine whether the auricles are adaxial, or whether all of the adaxial growth is ligular in origin, with the methods used here.
4.4 Discussion

Among the species with unifacial leaf blades there is little variation in the auricles and their arrangement; auricles may be present or absent, and when present, they may or may not be fused to form a median stipule. However, the median stipule of *Oxychloe andina* differs from those of the other species studied in that the centre of the median stipule is lower than the rest of the median stipule, and this is associated with a small projection which occurs from the abaxial epidermis of the leaf blade. Although Guédès (1967) figures the median stipule of *Oxychloe andina* (Fig. 4.6), he does not note the presence of the abaxial projection.

The greatest anatomical variation appears to occur among species with auricles which appear in an adaxial position, but which have bifacial leaves. Guédès (1967) describes one such example of these (*Juncus squarrosus*, Fig. 4.4), in which the adaxial position of the auricles is caused by a reduction, but not total loss, of the adaxial surface. Similar observations have been made here for some species, but other species have been described here which also have adaxial auricles in which no evidence could be found for a reduction in the adaxial surface.

Guédès (1967) argues that the presence of structures in an adaxial position in Cyperaceae, with no evidence of the loss of the adaxial surface, indicates the presence of a ligule. Conversely, the lack of evidence of growth from the adaxial surface indicates that a ligule is not present in Juncaceae. However, in both *Juncus capillaceus* and *Rostkovia magellanica* adaxial structures are present that are not associated with the loss of the adaxial epidermis. This is, in appearance, a small step away from the ligule of *Schoenus nigricans* (Fig. 4.3) as it is described by Guédès; if the inner margins were fused in *J. capillaceus* or *R. magellanica* it would be difficult to distinguish them from *Schoenus nigricans* based upon his description. Although firm conclusions cannot be drawn from the serial sections obtained in this study, it is considered possible that true ligules may occur in some Juncaceae.

The anatomy of the auricles

Auricles are either present or absent in Juncaceae, and when present are formed by an extension of the margins of the sheath in the uppermost region of the sheath. The extension is mostly upwards, but there is also a degree of lateral extension towards the adaxial side, so that there may be some overlap of the auricles with the adaxial surface, e.g. *Juncus tenuis* (Fig. 4.2B). The auricles are similar in their anatomical morphology to the margins of the sheath lower down, being entirely membranous, or with membranous margins but up to approximately 10 cell layers thick in the centre. All ground tissue is parenchymatous, with no vascular tissue or sclerenchyma present.

Some of the morphological variation in the junction between the sheath and blade is explained by the reduction of the adaxial surface. The progressive reduction of the adaxial surface at the top of the sheath in some species draws the margins closer together, so that they may appear to be adaxial in their position on the leaf. However, their marginal position is confirmed by the anatomy of the leaf, in particular by the position and orientation of the vascular bundles, and the nature of the ground tissue, which is usually parenchymatous adaxially and chlorenchymatous abaxially.

Median stipules

In species with unifacial leaf blades, the adaxial surface is progressively reduced in the sheath as the junction with the blade is approached, and at the junction it is entirely lost, so that the two sheath margins meet at the junction with the blade. The loss of the adaxial surface is indicated by the transition of the u-shaped row of vascular bundles in the sheath to a complete ring in the blade, and, likewise, the abaxial chlorenchymatous ground tissue of the sheath forms a complete ring in the blade, whereas the adaxial parenchyma of the sheath is absent in the blade. Frequently, the loss of the adaxial surface may also be marked by the appearance of a vascular bundle in the centre of the adaxial side of the unifacial leaf blade, but which was not present in the sheath.

If no auricles are present, then a median stipule is not present at the point where the leaf becomes unifacial, but if the margins are extended into auricles, then the inner margins may become fused at this point, forming a single membranous structure extending across the adaxial side of the leaf from one margin to the other, e.g. in *Distichia* and *Oxychloe*

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(Figs. 4.1B; 4.13-4.16). This is a median stipule as described by Troll (1939). However, this is not always the case. Some species, e.g. *Juncus subulatus* (Fig. 4.11), show complete loss of the adaxial surface at the junction between the sheath and the blade, but although the sheath margins are extended into auricles, the inner margins of the auricles are not fused, so no median stipule is present.

Some morphological variation in the median stipule is associated with a downwards proliferation of abaxial surface, e.g. in *Oxychloe andina* (Fig. 4.16). This causes the centre of the median stipule to occur lower in the leaf than the rest of the median stipule, instead of at the same level or higher, as would be expected with the progressive loss of the adaxial surface, and as seen in *Distichia acicularis* and *D. muscoides* (Figs. 4.13–4.14). This is also accompanied by a small projection in the centre of the adaxial side (Fig. 4.16C–D). This projection occurs just above the point where the inner margins of the sheaths become fused together (Fig. 4.16A), and in a region which is continuous with the abaxial epidermis of the leaf. It cannot be considered to be homologous with the ligule of other families, as it is not adaxial in origin.

Adaxial auricles

Although the auricles are marginal in origin, in unifacial leaves they may appear to be adaxial, due to the loss of the adaxial surface causing the margins to be drawn together. However, in the bifacial leaves of some species they also occur in an adaxial position, e.g. in *Juncus bulbosus*, *J. capillaceus* and *Rostkovia magellanica* (Figs. 4.2C; 4.17–4.19). In *Juncus bulbosus* this can be explained by a reduction in the adaxial surface. At the junction between the sheath and the blade the arrangement and orientation of the vascular bundles changes. The ends of the arc of bundles become drawn together, and the xylem poles are orientated more towards the centre of the leaf, but they are not drawn into a complete circle as they are in leaves which become unifacial at this point. This species has previously been described as having bifacial leaf blades but with a significantly reduced adaxial epidermis (Adamson 1925; Cutler 1969). In *Juncus bulbosus* the adaxial surface becomes progressively lost in the leaf blade, accompanied by similar changes in the position and orientation of the vascular bundles and changes in the ground tissue to those seen in species with entirely unifacial leaves, but the complete loss of the adaxial surface

occurs part way along the leaf blade, and not at the junction between the sheath and the blade (Section 3.4.1 *Reduction of the adaxial epidermis*). Therefore, although the sheathing margins are drawn close together on the adaxial side of the sheath, their inner margins do not quite meet.

This explanation cannot be used for *Juncus capillaceus*, *J. triglumis* and *Rostkovia magellanica*. The serial transverse sections examined here show no evidence of a reduction or loss of the adaxial surface at this point, and the leaves can be seen to be bifacial both below and above the point of insertion of the auricles, so the auricles give the appearance of being truly adaxial. However, the margins of the auricles remain continuous with the margins of the sheath, and apart from their adaxial position, they are morphologically similar to the marginal auricles of other species, e.g. *Juncus trifidus* (Fig. 4.7).

There are several explanations which could be suggested, and the presence of adaxial auricles may have a different explanation in different species, as the serial sections of *Juncus capillaceus*, *J. triglumis* and *Rostkovia magellanica* show different progressions of changes to one another.

The adaxial auricles may be true outgrowths from the adaxial surface. This implies the presence of a ligule, as occurs in some Cyperaceae and Poaceae; the extension of a structure from the margins and onto the adaxial surface is formed by a fusion of the adaxial ligule with the inner margins of the auricles. However, in most species there is little evidence for the presence of an additional structure between the auricles, as the inner margins of the auricles are directly adjacent to one another in *Rostkovia magellanica* and *Juncus triglumis*, and they overlap one another in *Juncus capillaceus* (Fig. 4.18D). It is possible, however, that two separate outgrowths from the adaxial surface have fused with the margins of the auricles.

Of the three species, this seems most likely to be the case with *Juncus capillaceus*. The points at which the sheath becomes free from the blade are identifiable as the true leaf margins (Fig. 4.18C–D), as they are marked by the ends of the arc of vascular bundles, and a change in the ground tissue. The morphology of the auricles of *J. capillaceus* is also different from those of the other species studied, as distinct ridges are present on the

surface of the auricles which appear to correspond to the 'true' margins, i.e. the junction between the epidermis which is adaxial and that which is abaxial in origin. If this is so, then the inner parts of the auricles are adaxial in origin, possibly formed by an outgrowth from the adaxial part of the leaf and then fused with the margins of the sheath; therefore the 'auricles' would be compound structures consisting of two ligules, each fused to the sheath margins. Similarly distinct ridges are figured by Guédès (1967) on the ligular stipule of *Schoenus nigricans* of Cyperaceae (Fig. 4.3). The anatomy of *Juncus capillaceus* in this region appears to differ only in that the innermost margins are not fused.

Another possible explanation is that the adaxial position of the auricles may be caused by an extension of the auricles laterally, accompanied by fusion of the abaxial side of the auricle with the adaxial surface of the leaf blade. Lateral proliferation of the margins of the auricles can be seen in other species, e.g. *J. tenuis* (Fig. 4.2B), where a small area of the auricle lies adjacent to the adaxial surface, but remains free from it, and it can be seen to be present in *Rostkovia*, as the inner margins of the auricles overlap with one another (Fig. 4.2C). In *Rostkovia magellanica*, the entire abaxial side of the auricles has an epidermis which is morphologically identical to the abaxial epidermis of the leaf blade, including the point at which the auricle has become free from the blade, indicating that this is not the true margin of the leaf; whereas on the adaxial side of the auricles the epidermis is morphologically identical to the adaxial side of the auricles the epidermis consistent with a lateral extension of the auricles followed by fusion of the abaxial side of the auricles with the adaxial side of the leaf.

Alternatively, it is also possible that the epidermis is drawn inwards only at the point of the junction between the sheath and the blade, but that this is not reflected in the internal morphology, so that the junction between the sheath and the blade is unifacial, but the sheath and blade are both bifacial.

Ligules

The ligule is known to be a structure of a different origin to the auricles (Mohl 1845; Guichard 1929), but it may sometimes fuse with the auricles; when fused, a continuous membranous structure may be formed, extending across the entire adaxial surface from one margin to the other and forming a ligular stipule, e.g. in *Carex spicata* (Fig. 4.1A).

However, in all of the species of Juncaceae that were examined, whenever a continuous membranous structure is present extending from one margin to the other it is always associated with loss of the adaxial surface, and it remains entirely marginal, not adaxial.

In species with bifacial leaves which have auricles present adaxially, e.g *Rostkovia magellanica* (Fig. 4.19), the margins are never fused to form a single continuous structure projecting from the leaf surface, as in *Carex spicata*. If their margins are fused, as they may be at the base of the auricles of *Rostkovia*, the fusion appears to occur between the inner margins of the auricles, and no additional, central structure can be identified which could be considered to be a ligule, so there is no evidence for the presence of a ligule in most of the species of Juncaceae examined here.

In *Juncus capillaceus*, however, the adaxial position of the auricles may be associated with growth from the adaxial surface which has become fused to the margins. If this is the case, then there are two separate outgrowths, each fused with one margin but not with each other, so that the external appearance remains similar to that of *Rostkovia*. Whether this is homologous to the ligules of Cyperaceae is unclear, as the ligules of Cyperaceae do not take this form.

4.5 Conclusions

The aims of this chapter were to investigate the range of variation in the morphology of the junction between the leaf sheath and blade to discover characters which may be taxonomically informative, and to determine the homologies of these structures among Juncaceae and Cyperaceae, so that they can be included in cladistic analyses.

A wide range of variation has been described, but due to the difficulties in determining homologies not all of it has been recorded for analysis. Three characters have been identified which summarise the range of variation observed here where it has also been possible to determine homologies, and which can be measured from the external morphology of the leaves (Table A4). Some of these characters have not been used previously in cladistic analyses, but the presence or absence of auricles has been included in cladistic analyses by Drábková *et al.* (2007). The presence or absence of ligules has been used in cladistic studies by Munro & Linder (1998) and Simpson (1995), and scored as present in some Juncaceae; but it is not used here. Instead, this character has been modified to score for fused or free auricles.

For the most part, the conclusions from this study are in agreement with Guédès (1967), confirming that most of the ligule-like structures which occur in the Juncaceae species examined here are not homologous to the ligules which occur in Cyperaceae and Poaceae. They are homologous to the marginal auricles which are sometimes present in both Cyperaceae and Juncaceae, and they may sometimes be associated with a reduction or loss of the adaxial surface, so that although the remain marginal, they may appear adaxial in their position on the leaf.

However, more species are described here, with a wider range of anatomical variation, than have been described previously, and it can be seen that although a median stipule is always associated with unifacial leaf blades, unifacial leaf blades are not always associated with the presence of a median stipule, even when auricles are present. For example, in *Juncus articulatus* and *J. subulatus* the auricles remain free, usually overlapping one another at their inner margins.

Among a small number of species (*Juncus capillaceus*, *J. triglumis* and *Rostkovia magellanica*) the homologies remain unclear. The adaxial auricles of these species may, or may not, be homologous to the ligular stipules of Cyperaceae. They do not form a continuous structure across the adaxial surface as the ligules of some Cyperaceae do, but instead they retain the appearance of two free auricles. It is not clear whether they are marginal in origin or arise in part from the adaxial epidermis.

Guédès (1967) has argued that the lack of evidence of growth from the adaxial surface indicates that a ligule is not present in Juncaceae. However, in *Juncus capillaceus, J. triglumis* and *Rostkovia magellanica* structures are present in an adaxial position which do not appear to be associated with the loss of the adaxial epidermis. This is, in appearance, a small step away from the ligular stipule of some Cyperaceae, e.g. that of *Carex spicata* (Fig. 4.1A); if the inner margins were fused in *J. capillaceus* or *R. magellanica* it would be difficult to distinguish them morphologically. Different explanations have been offered here for the adaxial auricles of these species, and it is suggested that a different explanation may be applied to each species; but it is difficult to prove or disprove the possible explanations for the presence of adaxial auricles using the serial sections shown here. Developmental studies would be necessary to determine the origins of these structures.

Chapter 5 Phylogeny

5.1 Introduction

5.1.1 Selection of materials and methods

DNA regions to be sequenced

This thesis is concerned with evolutionary relationships of Juncaceae at several taxonomic ranks, from family to subgenus and to section. The choice of DNA regions for phylogenetic analysis must be based upon an assessment of whether they are likely to have a rate of substitution that will prove phylogenetically informative at these levels. However, at the time of planning this work, very few molecular phylogenetic studies had been carried out upon Juncaceae, so the choice was based upon a recognition of those regions which have proved informative at similar taxonomic ranks in related families.

The chloroplast genome has been extensively used to infer plant phylogenies (Palmer *et al* 1988; Clegg *et al* 1991). The chloroplast genome is highly conserved with respect to gene content and arrangement, allowing the use of 'universal' PCR primers for sequencing, and it is also high-copy, occurring in multiple copies per chloroplast, so that PCR amplification is relatively easy (Small *et al* 2004). The chloroplast genome is haploid and non-recombinant, and usually uniparentally inherited in angiosperms, so the pattern of relationships inferred by phylogenetic analysis is likely to be bifurcating and not reticulated, which matches one of the major assumptions of phylogenetic analysis (Small *et al* 2004).

The gene encoding the large subunit of RuBisCo (rbcL) has been widely used in plant phylogenetic studies at generic and higher taxonomic levels (Chase *et al* 1993; Duvall *et al* 1993; Givnish *et al* 1999; Muasya *et al* 1998; Munro & Linder 1998). However, rbcLdoes not always provide enough variation between taxa to resolve relationships between closely related genera. For instance, intergeneric relationships among some genera of Poaceae are poorly resolved by rbcL sequences (Doebley *et al.* 1990; Hodkinson *et al* 2007). Non-coding regions tend to evolve more rapidly than coding regions, and are therefore more likely to be useful at taxonomic levels within families and between closely related taxa. Gielly & Taberlet (1994) have shown that the trnL intron and trnL-F intergenic spacer are more variable than rbcL, and more informative at generic level among Poaceae, so it was decided to sequence these regions, although they had not previously been used in Juncaceae.

There are some disadvantages to using only plastid DNA in phylogenetic studies. Plants have a potential for hybridisation and polyploidisation, but plastid DNA is uniparentally inherited so only half the parentage is revealed. Introgression is known to occur frequently among plants (Rieseberg & Soltis 1991), for example chloroplast capture may occur as a result of hybridisation and subsequent backcrossing to one of the parents, so that the chloroplast genome of one species has become transferred to a second species, without this event being reflected in the nuclear genome of the second species. Phylogenies inferred by chloroplast DNA may, therefore, not always be equivalent to phylogenies inferred by nuclear DNA in the same group of species (Soltis & Soltis 2000).

The internal transcribed spacer (ITS) region of 18S-26S nuclear ribosomal DNA was sequenced in addition to the plastid DNA regions, as it has been shown to be useful in phylogenetic analyses (Baldwin *et al* 1995; Hodkinson *et al* 2002), with a high degree of variability likely to be informative at the taxonomic levels of interest in this thesis. For example, it was used by Hodkinson *et al* (2002) to study infratribal, infrasubtribal and infrageneric relationships in Andropogoneae, Saccharinae (Poaceae).

5.1.2 Aims

- 1. DNA sequences are obtained for phylogenetic analyses, sufficient to resolve interrelationships of genera and sections in Juncaceae and close allies.
- 2. Cladistic analyses are carried out to generate phylogenetic hypotheses, using both molecular and morphological data.
- 3. Morphological characters are mapped onto the phylogenetic hypothesis and their distribution and evolution discussed.
- 4. The taxonomy of Juncaceae is revised in the light of the results of this thesis.

5.2 Materials and Methods

5.2.1 Materials

Material for DNA extraction

The material for DNA extraction was taken from samples collected in the field and dried in silica gel, or from herbarium specimens where this was not possible. Table A1 lists voucher specimens for the material used.

Wherever possible, the specimens studied were the same as those included in the macromorphology investigations in order to maintain consistency. It was not always possible to use the same specimens, as the DNA extraction was found to be difficult for specimens older than 10 years; although DNA was extracted from these specimens it was shown to be very degraded when analysed by agarose gel electrophoresis (1% agarose, stained with ethidium bromide and illuminated with UV light), and no sequences could be obtained. In addition, for very small species it was often difficult to find specimens with sufficient quantities of material so that samples could be removed for DNA extraction without excessively damaging the specimen. In many cases the specimen used for DNA collection was one which did not contain fully mature flowers or fruits, and was not, therefore used to collect morphological data.

Morphological data

The data collected in Chapters 2 *Morphology*, 3 *Anatomy* and 4 *Auricles or ligules*? and presented in Tables A2, A3 and A4 were intended for use in cladistic analyses. The algorithms used for cladistic analyses are only able to use categorical data, but some of the data is continuously variable, so it was necessary to either exclude the continuous data, or to convert it into categorical form. Although it is possible to divide continuous data into categories, this results in a loss of information, as the categories do not show the full range of variation. In addition, with most distributions of data any division of the data is arbitrary, and possibly misleading, as it introduces artificial distinctions in the degree of similarity between taxa. The continuous data were examined to determine whether any

divisions occurred in the distributions that would allow conversion to categorical form non-arbitrarily.

Histograms of each of the continuously variable characters are shown in Fig. A5. The majority of the characters were found to have either normal distributions, or were more commonly skewed right, and it was considered that these should not be divided into categories. One character (29: length of gynoecium divided by length of perianth) was found to have a bimodal distribution, with modes of non-exserted stigmas and exserted stigmas. This character was divided into two states: Stigmas not exserted from perianth (values below 1.0), scored as '0'; and stigmas exserted from perianth (values above 1.0), scored as '1'.

The morphological data matrix includes all morphological characters which were scored categorically in Chapters 2, 3 and 4, and also character 29, converted from continuous to categorical form.

5.2.2 Protocols

DNA Extraction

The DNA extractions were based on a modified 2x CTAB procedure of Doyle & Doyle (1987), as follows.

Approximately 0.1 - 0.8 g of leaf material (dried in silica gel or taken from herbarium specimens) was cut into pieces of 1 cm in length or less. Where only very small quantities of material were available all quantities in this protocol were halved.

10 ml of 2xCTAB buffer (100mM Tris-HCL pH8, 1.4M NaCl, 20mM EDTA, 2% Hexadecyltrimethyl-Ammonium Bromide) and 40 μ l of 2-Mercaptoethanol were placed in a capped centrifuge tube, and heated to 65 °C.

The leaf material was ground with a preheated pestle and mortar with a small quantity of the pre-heated CTAB buffer. When the material was ground to a fine pulp the remainder of the CTAB buffer was added, returned to the centrifuge tube and incubated at 65 °C for

10 minutes. 10 ml of CI (24 chloroform:1 isoamyl alcohol) were added, and the mixture placed on a shaker for at least 30 minutes. The samples were centrifuged at c. 3500 g for 10 minutes. The aqueous phase was retained and an equal volume of chilled isopropanol added, and the mixture stored in a freezer for approximately one week to precipitate the DNA.

The samples were centrifuged at c. 1800 g for 10 minutes to pellet the DNA. The DNA was washed in 70% ethanol. The DNA was resuspended in 50 μ l of TE buffer (10 mM Tris-HCl pH 8.0, 1mM EDTA) and cleaned using the Concert Rapid PCR Purification System (Life Technologies, Paisley, Scotland), following the protocol given with this system. The DNA extractions were stored at -21 °C or -80 °C.

Amplification

All DNA regions to be sequenced were amplified using PCR. The DNA samples and reagents were thawed, and all tubes were subsequently kept on ice. 3 μ l of each DNA sample was placed in 0.5 ml centrifuge tubes. A master mix was prepared and appropriate quantities added to each sample, containing the following reagents, given in quantities per DNA sample:

Reagent	Details	Volume
Ultra pure water		35.75 µl
Promega 10x Buffer	500 mM KCl, 100 mM Tris-HCl (pH 9.0), 1.0% Triton X-100	5 μΙ
dNTPs	Each dNTP at 10mM	1 μl
Primers	100 ngµl ⁻¹	0.5 µl each
Magnesium Chloride	25mM	4 μl
Taq polymerase	5 units μl ⁻¹	0.25 μl

The *trnL-F* region was amplified in either one part using primers c and f, or in two parts using the primers c and d, and e and f described in Taberlet *et al.* (1991). The *rps16* region was amplified by the F and 2R primers described in Oxelman *et al.* (1997). The ITS region was amplified using the ITSF primer described by White *et al* (1990), and 26SE of Sun *et al* (1994).

The PCR and cycle sequencing reactions were carried out using a 9700 thermal cycler (Applied Biosystems, Warrington, Cheshire, UK). Thermal cycling comprised 30 or 35 cycles, with 1 min. denaturation at 97 °C, 1 min. annealing at 50 °C, extension of 3 min. at 72 °C, and a final extension of 7 min. at 72 °C. The PCR products were incubated at 4 °C.

Amplified DNA fragments were purified using Concert PCR purification columns (Life Technologies, Paisley, Scotland) following the protocols of the manufacturer. Cleaned PCR products were stored at -21 °C or -80 °C.

Sequencing

The PCR products and reagents were thawed and kept on ice. $3 \mu l$ of each PCR product was placed in 0.5 ml centrifuge tubes. A master mix was prepared and appropriate quantities added to each sample, containing the following ingredents, given in quantities per sample:

Reagent	Concentration	Volume	
Big Dye TM Sequencing Mix		1 µl	
v.1.1 (Applied Biosystems)			
Ultra pure water		1.8 µl	
Sequencing Buffer (70 mM Tris,		3.5 µl	
1.75 mM MgCl ₂)			
Primer	$5 \text{ ng}\mu\text{l}^{-1}$	0.7 μl	

The same primers were used as for the initial amplificaton but diluted 20 times.

The cycle sequencing reactions were run on an Applied Biosystems 310 Genetic Analyzer or 377 Automated DNA sequencer. Thermal cycling consisted of 29 cycles, with 10 s denaturation at 96 °C, 8 s annealing at 50 °C, and 4 min extension at 60 °C. The products were incubated at 4 °C.

Purification of the cycle sequencing products was then carried out. The cycle sequencing products and reagents were initially thawed and kept on ice. A master mix was made and added to each sample, consisting of the following reagents given in quantities per sample:

Reagent	Concentration	Volume	
Sodium acetate (NaOAc)	3M	2 µl	
Ethanol	100%	50 µl	

The tubes were mixed thoroughly and left to precipitate for 5 min at room temperature, and then 10 min on ice.

After incubation the tubes were centrifuged for 25 min at c. 12 000 g, and drained by briefly placing upside down on tissue. 300 ml of 70% ethanol was added, the tubes inverted to mix, and centrifuged for 15 min at c. 12 000 g and drained, and this process repeated once. The tubes were then thoroughly dried by placing them upside down on tissue to drain excess liquid and then placed on their sides on tissue and covered in tissue in a fume hood overnight. The dry tubes were stored at -21 °C or -80 °C.

The cycle sequencing products were prepared for sequencing by adding 25 μ l of template suppression reagent (TSR, Applied Biosystems), vortexing and then heating for 5 min at 95 °C.

The sequencing reactions were run on an Applied Biosystems 310 Genetic Analyzer or 377 Automated DNA sequencer using module SEQ POP6 RAPID (1.0-mL) E. The sequences were then processed using ABI PrismTM DNA Sequencing Analysis Software, v. 2.1.1.

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Data Analysis

Sequence editing and assembly was carried out using Autoassembler 2.1 software (Applied Biosystems, 1998). The DNA sequences were then imported in to PAUP vers. 4.0b10 (Swofford 2002) and aligned by eye. Gaps were coded as missing data.

The matrices were analysed by maximum parsimony using heuristic search options with 1000 replicates of random stepwise addition, with tree bisection-reconnection (TBR) swapping, saving no more than 50 trees per replicate.

The plastid DNA regions *trn*L-F and *rps*16 were analysed in a combined matrix, as the plastid genome is almost always uniparentally inherited and not recombining. Analyses of the plastid DNA regions and of the ITS region showed that there were no major conflicts between the results (if there was conflict it was not supported by high bootstrap values), so a combined analysis of both nuclear and plastid DNA data was carried out. Individual analyses of the plastid genomic data and the nuclear genomic data are given in Appendix A Figs. A2 and A3; the result of the analysis of the combined molecular data is presented in this chapter (Fig. 5.1).

Analysis of the morphological data showed little resolution and support (Fig. 5.2), but where groups were resolved they showed no conflict with the results from the molecular data, so a combined molecular and morphological analysis was also carried out (Fig. 5.3).

Branch support was assessed using 1000 bootstrap replicates (Felsenstein 1985) with simple addition sequence, saving no more than 50 trees per replicate, and TBR swapping. Groups with frequencies greater than 50% were retained in the final consensus tree. The trees based on molecular data were rooted on *Typha minima* Funck ex Hoppe as this has been shown to be closely related to Cyperales but outside the study group (Chase *et al.* 1993, Plunkett *et al.* 1995, Munro & Linder 1998, Givnish *et al.* 1999). Trees based on the morphological data were rooted on *Prionium serratum*, as this has been shown to be closely related to Linder 1998, Givnish *et al.* 1999). Jones *et al.* 2007; Plunkett *et al.* 1995, Munro & Linder 1998, Givnish *et al.* 1999).

5.3 Results

5.3.1 Results of analyses of molecular data

The combined matrix of the ITS, *trn*L-F and *rps*16 regions was 3714 base pairs long, 462 bp of which were excluded due to difficulty in aligning the sequences. Of the included characters, 1706 were variable and 1138 of these were potentially parsimony informative. Analysis of the matrix produced 54 trees of length 4169, with a CI of 0.61 and RI of 0.80. The resulting strict consensus tree is shown in Fig. 5.1.

A clade consisting of *Prionium* and *Thurnia* (100 BP) was found to be sister to a monophyletic Juncaceae and Cyperaceae group (100 BP). Juncaceae and Cyperaceae were each found to be monophyletic (100 BP).

Within Juncaceae four major clades were identified. A clade consisting of all species of *Luzula* and *Juncus trifidus* (91 BP) is sister to the rest of Juncaceae (100 BP), and within this clade, *Luzula* forms a monophyletic group (100 BP) sister to *Juncus trifidus*.

The remaining species of Juncaceae form three major clades united with low support (63 BP). Clade A (100 BP) consists of *Juncus* subgen. *Agathryon*, except for *Juncus trifidus*. Two of the four sections (sects. *Steirochloa* and *Juncotypus*) are paraphyletic with respect to one another and to sect. *Tenageia*, which is only represented by a single species. Sect. *Forskalina* is not represented.

Of the five representatives of sect. *Steirochloa*, four occur in clade A, while *Juncus trifidus* is in a clade with *Luzula*. *Juncus squarrosus* is sister to all other members of clade A (100 BP). Three further species of sect. *Steirochloa* form a clade which also contains *J. balticus* ssp. *andicola* (sect. *Juncotypus*) (100 BP), and this clade is sister to the remaining two representatives of sect. *Juncotypus* and *J. bufonius* (sect. *Tenageia*) (100 BP).

Clade B (100 BP) consists of several sections of *Juncus* subgen. *Juncus* (sects. *Iridifolii*, *Ozophyllum* and *Stygiopsis*). Sect. *Stygiopsis* is represented by three species which form a clade (86 BP) sister to a clade consisting of sects. *Iridifolii* and *Ozophyllum* (100 BP). Sect. *Ozophyllum* is paraphyletic with respect to sect. *Iridifolii*.

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Figure 5.1 Strict consensus of 54 maximum parsimony trees based on ITS, rps16 and trnL-F intergenic spacer. Numbers above branches indicate bootstrap values. Tree length 4169, CI = 0.61, RI = 0.80.

Clade C (96 BP) consists of the single-flowered genera (*Distichia*, *Marsippospermum*, *Oxychloe* and *Rostkovia*), and several species of *Juncus* subgen. *Juncus* representing sects. *Juncus* and *Graminifolii*. Although this clade is fully resolved in the strict consensus tree, the support for most nodes is low. *Oxychloe* and *Distichia* form a clade (100 BP) which is sister to *Rostkovia* (53 BP), and these three genera are sister to *Marsippospermum*, but with support of only 62 BP. All of these genera are sister in the strict consensus, but lacking support, to a clade (57 BP) which consists of section *Juncus* and two representatives of section *Graminifolii*. *Juncus falcatus* (sect. *Graminifolii*) is the most outlying species in Clade C according to the strict consensus tree, but with less than 50% bootstrap support.

Although the four major clades are well supported, the relationship between these clades is unclear, as bootstrap support for the nodes joining them is low.

5.3.2 Results of analysis of morphological data

The matrix of the morphological data was 47 characters long. Analysis of the matrix produced 3372 trees of length 165, with a CI of 0.32 and RI of 0.68 (Fig 5.2).

The strict consensus tree is not well resolved, and several of the nodes have very low bootstrap support. Several clades arise from within a large polytomy. *Luzula* forms a monophyletic group (54 BP). The species of *Luzula* are partially resolved but with less than 50 BP for the nodes, but *L. multiflora* and *L. campestris* form a clade (55 BP).

A clade consisting of *Juncus* sects. *Forskalina*, *Iridifolii*, *Juncus*, *Ozophyllum* and *Stygiopsis* is resolved in the strict consensus tree but has bootstrap support of less than 50%. Within this, a clade (54 BP) consisting of *Juncus maritimus* and *J. subulatus* (sects. *Forskalina* and *Juncus* respectively) is sister in the strict consensus tree, but without bootstrap support, to a group containing sects. *Iridifolii*, *Ozophyllum* and *Stygiopsis* (70 BP). The second clade is equivalent to Clade B that resolved in the analysis of the molecular data (Fig. 5.1).

Distichia, *Oxychloe* and *Patosia* form a monophyletic group (88 BP), within which a clade consisting of *Oxychloe* and *Patosia* (65 BP) is sister to *Distichia* (91 BP).



Figure 5.2 Strict consensus of 3372 maximum parsimony trees based on morphological data. Numbers above branches indicate bootstrap values. Tree length 165, CI = 0.32, RI = 0.68.

Juncus capillaceus forms a clade with *Rostkovia* (88 BP) as part of the large polytomy and is therefore not resolved. *Marsippospermum* and all the remaining representatives of *Juncus* sects. *Graminifolii*, *Steirochloa* and *Tenageia* are also unresolved.

5.3.3 Results of analysis of combined data

The combined matrix of the *trn*L-F, *rps*16 and ITS regions and morphological data was 3761 characters long, 462 characters of which were excluded due to difficulty in aligning DNA sequences. Of the included characters, 1252 were variable and 763 of these were potentially parsimony informative. Analysis of the matrix produced 10 trees of length 2454, with a CI of 0.67 and RI of 0.81 (Fig. 5.3).

The results are similar to the results from the molecular data alone (Fig. 5.1), with four major clades resolved within Juncaceae. *Luzula* is a monophyletic group (100 BP). *Juncus trifidus* is sister to *Luzula* (74 BP). In the strict consensus but with no bootstrap support this clade is sister to a clade consisting of the rest of Juncaceae (64 BP). The rest of Juncaceae forms three well supported major clades, but the relationships between them are unresolved in the strict consensus tree.

Clade A (100 BP) consists of *Juncus* subgen. *Agathryon*, with the exception of sect. *Forskalina* which is not represented. Sect. *Steirochloa* is paraphyletic with respect to sects. *Juncotypus* and *Tenageia*, as *J. squarrosus* is sister to the rest of the clade (100 BP). Sect. *Juncotypus* is paraphyletic with respect to both sections: *J. effusus* and *J. inflexus* form a clade with *J. bufonius* (100 BP), and *J. balticus* forms a clade with three members of sect. *Steirochloa* (*J. capillaceus*, *J. compressus* and *J. gerardii*) (100 BP).

Clade B (100 BP) consists of three sections of *Juncus* subgen. *Juncus*, sects. *Iridifolii*, *Ozophyllum* and *Stygiopsis*. Sect. *Stygiopsis* forms a clade (93 BP) which is sister to sects. *Iridifolii* and *Ozophyllum* (100 BP). Sect. *Ozophyllum* is paraphyletic with respect to sect. *Iridifolii*, as *J. scheuchzerioides* is sister to the rest of the clade (97 BP), and *J. bulbosus* sister to the remaining taxa (65 BP).



Figure 5.3 Strict consensus of 10 maximum parsimony trees based on ITS, rps16, trnL-F intergenic spacer and morphological data. Numbers above branches indicate bootstrap values. Tree length 2454, CI = 0.67, RI = 0.81.

Clade C (97 BP) consists of two sections of *Juncus* subgen. *Juncus*, sects. *Graminifolii* and *Juncus*, and all of the remaining genera (*Distichia*, *Marsippospermum*, *Oxychloe* and *Rostkovia*). The four species of *Juncus* form a clade (66 BP) which is sister to a weakly supported clade consisting of all the other genera (57 BP). Within this clade, *Rostkovia* and *Marsippospermum* are unresolved with respect to a well supported *Distichia* and *Oxychloe* clade (100 BP).

5.3.4 Discussion of the phylogenetic hypotheses

Monophyly of the families

The results of the molecular analyses (Fig. 5.1) clearly support the monophyly of Juncaceae including the genus *Oxychloe*, and the sister group relationship of Juncaceae with Cyperaceae (both 100 BP). This is in contrast to some previous molecular studies (Chase *et al.* 1993; Muasya *et al.* 1998; Munro & Linder 1998; Givnish *et al.* 1999), in which Juncaceae resolves as paraphyletic with respect to Cyperaceae. The monophyly of Cyperaceae is in agreement with previous studies (Chase *et al.* 1993; Plunkett *et al.* 1995; Simpson 1995; Muasya *et al.* 1998; Munro & Linder 1998; Givnish *et al.* 1999), and is supported by its specialised morphology. The relationship of *Prionium* and *Thurnia* as a monophyletic sister group to a Cyperaceae and Juncaceae group is also in agreement with previous studies (Chase *et al.* 1993; Duvall *et al.* 1993; Plunkett *et al.* 1995; Muasya *et al.* 1998; Munro & Linder 1998; Givnish *et al.* 1999), and it is consistent with the inclusion of *Prionium* within Thurniaceae.

Luzula

Within Juncaceae, the monophyly of *Luzula* is supported by both the molecular and morphological data, as *Luzula* is resolved in all of the analyses (Figs. 5.1–5.3).

Analyses of the molecular data place *Luzula* as sister to the rest of Juncaceae, but include *Juncus trifidus* within this clade, as sister to *Luzula*. The association of *J. trifidus* with *Luzula* has 91% BP support in the molecular analysis and 74% in the combined molecular and morphological analysis, but it is not supported by the morphological analysis, as the positions of *J. trifidus* and the *Luzula* clade are unresolved. The DNA sequences of *J.*

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trifidus are quite highly divergent from those of all the other species, including *Juncus* and *Luzula*, as they contain many short insertions and deletions, particularly in the two plastid regions sequenced, whereas in the ITS region there are an unusually large number of substitutions in comparison with other species of Juncaceae. The placement of *J. trifidus* within the trees is inconsistent between analyses of individual DNA regions. Analysis of the *trn*L-F region (data not shown) places *J. trifidus* sister to Clade A instead of *Luzula*, resulting in a monophyletic subgenus *Agathryon* (83 BP). Analysis of the *rps*16 region places *J. trifidus* as sister to Clade A, but with bootstrap support of less than 50% (Fig. A2). Analysis of ITS places it sister to *Juncus* sections *Juncus* and *Graminifolii* (Fig. A3). It seems therefore that this taxon is treated as *incertae sedis* until further analyses provide stronger evidence for its position.

Juncus

Most species of *Juncus* are divided into two clades, with the exception of the two sections *Graminifolii* and *Juncus*. One of these clades (Clade A, Figs. 5.1, 5.3) consists of all of the representatives of subgen. *Agathryon*, except for *J. trifidus*. This clade is strongly supported in both the molecular analyses and the combined molecular and morphological analysis (100 BP), but it is not resolved in the analysis of the morphological data (Fig. 5.2).

Within *Juncus* subgenus *Agathryon*, too small a number of species are represented to draw strong conclusions about the monophyly of the sections within the subgenus. Only three of the four sections are represented, with five species of section *Steirochloa*, three of *Juncotypus* and one of *Tenageia*. However, even with the exception of *Juncus trifidus*, there is no support for the monophyly of sections *Steirochloa* and *Juncotypus*. Both are paraphyletic with respect to other sections within the subgenus, with well supported clades (100 BP), but further study with a larger number of species of each section would be necessary to make the relationships between the sections clear.

The second clade (Clade B, Figs. 5.1–5.3) consists of three of the sections of *Juncus* subgen. *Juncus* (sects. *Iridifolii*, *Ozophyllum* and *Stygiopsis*). This clade is strongly supported in the molecular and combined morphological and molecular analyses (100 BP), and it is also moderately well supported in the morphological analysis (70 BP). A similar

clade to this one is resolved in Drabkova *et al.* (2003), but it also contains a single nonseptate species (*Juncus covillei* Piper), not included in this study.

Within Clade B *Juncus* sect. *Stygiopsis* is strongly supported as a sister group to the other two sections, but sect. *Ozophyllum* is paraphyletic with respect to sect. *Iridifolii*. Clade B has not been recognised as a single group by previous authors, but its division into two clades reflects the classification of Buchenau (1906), who recognised these two groups as subgenera *Alpini* (Engelm.) Buchenau and *Septati* Buchenau.

Other genera of Juncaceae

The genera *Distichia, Marsippospermum, Oxychloe* and *Rostkovia* associate into Clade C (Figs. 5.1, 5.3). *Patosia* is only represented in the analysis of morphological data (Fig. 5.2), but it is strongly supported within a clade containing *Distichia* and *Oxychloe*. These genera are distinguished from *Juncus* and *Luzula* by having a single flower per culm. The position of some *Juncus* species also within this clade is surprising, as their inflorescence morphology is closer to *Juncus* than to these genera. These data therefore suggest there has been a convergence in form. In Kirschner's (2002b) classification these species represent *Juncus* sects. *Juncus* and *Graminifolii* Engelm.

Drabkova *et al.* (2003) also recognised a clade consisting of all single-flowered Juncaceae genera, with the exception of *Oxychloe*. However, the sequences of *Oxychloe* were those used in previous studies, and may be contaminated (see Section 1.3.2 *Juncaceae and Cyperaceae*). As was found here, the clade also contained some species of *Juncus* sect. *Graminifolii*, but the position of sect. *Juncus*, also placed in Clade C in this study, was not resolved in Drabkova *et al.* (2003).

The placement of the two species of *Oxychloe* within Juncaceae and sister to *Distichia* is not surprising from a morphological point of view. *Oxychloe* has a floral structure which is typical of Juncaceae, and it shares several characteristics with *Distichia*, such as a cushion-forming habit. The placement of *Oxychloe* within Juncaceae is in agreement with the recent work by Kristiansen *et al* (2005). Kristiansen *et al* suggest that the presence of some material of Cyperaceae in the specimens of *Oxychloe* used in previous analyses resulted in contamination of the DNA sequences.

5.4 Character distribution and evolution

5.4.1 Character distribution

The phylogenetic hypotheses in Section 5.3 Results divide Juncaceae into four clades: Luzula, Clade A (equivalent to Juncus subgen. Agathryon), Clade B (Juncus sects. Iridifolii, Ozophyllum and Stygiopsis), and Clade C (all other genera, plus Juncus sects. Juncus and Graminifolii). The morphology of Juncaceae is discussed in relation to these four clades, and in relation to taxonomic classifications. Characters which are recorded in categorical form are mapped onto the phylogenetic hypothesis resulting from the analysis of the combined morphological and molecular data (Fig. 5.3) in Figs. 5.4-5.15, and characters which are not categorical are displayed in Table 5.1, showing their range and mean for each major clade. Only characters that are discussed in the text are displayed in this chapter; the raw data is in Appendix A Tables A2–A3.

Table 5.1 Range and mean of morphological characters

Data is displayed as

Mean [range]

in each cell.

Cha	iracter	Luzula	Clade A	Clade B	Clade C
7	Total leaf length	118 [55–197] 9	120 [67–137] 5	253 [47–943] 8	100 [10–565] 12
8	Leaf blade length / sheath length	3.2 [1.7–5.0] 9	4.1 [2.5–6.8] 5	3.5 [0.6–14.5] 8	1.6 [0.5–7.1] 12
16	Inflorescence bract length / inflorescence length	0.7 [0.2–1.4] 9	2.9 [0.6–8.4] 8	0.6 [0.2–1.2] 7	1.1 [0.0–3.4] 10
17	Inflorescence bract blade length / sheath length	16.7 [1.3–60.0] 9	9.9 [2.1–21] 8	1.2 [0.1–3.8] 7	21.7 [0.1–190] 9
27	Anther length / filament length	2.4 [0.51–7.64] 9	2.2 [0.55–7.57] 8	0.6 [0.21–1.14] 8	6.7 [1.30–15.96] 8
42	Leaf blade width at broadest point	5.6 [2.7–16.0] 8	0.8 [0.6–1.1] 4	3.3 [0.2–8.8] 7	1.3 [0.7–2.1] 8
43	Leaf blade shape (width at broadest point / width at narrowest point)	27.16 [8.3–72.7] 8	1.63 [1.3–2.0] 3	4.12 [1.3–9.6] 5	1.31 [1.0–2.2] 12

n



Figure 5.4 Distribution of plant habit (character 1). Strict consensus of 10 maximum parsimony trees based on ITS, rps16, trnL-F intergenic spacer and morphological data. Numbers above branches indicate bootstrap values. Tree length 2454, CI = 0.67, RI = 0.81. Plant habit mapped onto tree: Blue indicates rhizomatous, green indicates non-rhizomatous, and red indicates cushion-forming habit.



Figure 5.5 Distribution of distichous leaf insertion (character 3) and closed leaf sheaths (character 4). Strict consensus of 10 maximum parsimony trees based on ITS, rps16, trnL-F intergenic spacer and morphological data. Numbers above branches indicate bootstrap values. Tree length 2454, CI = 0.67, RI = 0.81. Distichous leaf insertion is mapped onto the tree in red; closed leaf sheaths are mapped onto the tree in blue. Black branches indicate non-distichous leaf insertion and open leaf sheaths.



Figure 5.6 Distribution of leaf rosettes (character 5). Strict consensus of 10 maximum parsimony trees based on ITS, *rps*16, *trn*L-F intergeneric spacer and morphological data. Numbers above branches indicate bootstrap values. Tree length 2454, CI = 0.67, RI = 0.81. Rosette of leaves mapped onto tree in blue. Red indicates the absence of this character.



Figure 5.7 Distribution of cauline leaves (character 6). Strict consensus of 10 maximum parsimony trees based on ITS, rps16, trnL-F intergeneric spacer and morphological data. Numbers above branches indicate bootstrap values. Tree length 2454, CI = 0.67, RI = 0.81. Cauline leaves mapped onto tree in blue. Red indicates the absence of this character.

Clade A

Clade C

Clade B



Figure 5.8 Distribution of pseudolateral inflorescences (character 14). Strict consensus of 10 maximum parsimony trees based on ITS, rps16, trnL-F intergeneric spacer and morphological data. Numbers above branches indicate bootstrap values. Tree length 2454, CI = 0.67, RI = 0.81. Pseudolateral inflorescence mapped onto tree in red. Black indicates that absence of a pseudolateral inflorescence.



Figure 5.9 Distribution of racemose and cymose inflorescences (character 15). Strict consensus of 10 maximum parsimony trees based on ITS, *rps*16, *trn*L-F intergeneric spacer and morphological data. Numbers above branches indicate bootstrap values. Tree length 2454, CI = 0.67, RI = 0.81. Racemose or cymose inflorescence mapped onto tree. Blue indicates racemose and red cymose inflorescences. Black indicates that the character is unknown.



Figure 5.10 Distribution of bracteoles (character 19). Strict consensus of 10 maximum parsimony trees based on ITS, rps16, trnL-F intergeneric spacer and morphological data. Numbers above branches indicate bootstrap values. Tree length 2454, CI = 0.67, RI = 0.81. Presence of bracteoles mapped onto tree in blue. Red indicates the absence of this character.



Figure 5.11 Distribution of placentation types (character 31). Strict consensus of 10 maximum parsimony trees based on ITS, *rps*16, *trn*L-F intergeneric spacer and morphological data. Numbers above branches indicate bootstrap values. Tree length 2454, CI = 0.67, RI = 0.81. Placentation types mapped onto tree. Blue indicates axile placentation. Green indicates parietal placentation. Red indicates basal placentation. Black indicates lack of data.


Figure 5.12 Distribution of reduction and loss of adaxial surface of leaves (character 41). Strict consensus of 10 maximum parsimony trees based on ITS, *rps*16, *trn*L-F intergeneric spacer and morphological data. Numbers above branches indicate bootstrap values. Tree length 2454, CI = 0.67, RI = 0.81. Reduction or loss of leaf adaxial surface mapped onto tree: Blue indicates no reduction of the adaxial surface, greed indicates a reduced adaxial surface, and red indicates unifacial leaves. Black indicates lack of data.



Figure 5.13 Distribution of transverse septae in the leaf blades (character 52). Strict consensus of 10 maximum parsimony trees based on ITS, rps16, trnL-F intergeneric spacer and morphological data. Numbers above branches indicate bootstrap values. Tree length 2454, CI = 0.67, RI = 0.81. Presence of transverse septae in the leaf blades mapped onto tree in red. Blue indicates the absence of this character. Black indicates that the character is unknown.



Figure 5.14 Distribution of multi-layered vascular bundles (characters 54 and 61). Strict consensus of 10 maximum parsimony trees based on ITS, *rps*16, *trn*L-F intergeneric spacer and morphological data. Numbers above branches indicate bootstrap values. Tree length 2454, CI = 0.67, RI = 0.81. Vascular bundles in more than one layer in the leaf blades (character 54) mapped onto tree in green. Red indicates vascular bundles in more than one layer in the sheaths but not the blades (character 61). Blue indicates vascular bundles in a single layer only. Black indicates that the character is unknown.



Figure 5.15 Distribution of transverse septae in the leaf sheaths (character 63). Strict consensus of 10 maximum parsimony trees based on ITS, rps16, trnL-F intergeneric spacer and morphological data. Numbers above branches indicate bootstrap values. Tree length 2454, CI = 0.67, RI = 0.81. Presence of transverse septae in the leaf sheaths mapped onto tree in red. Blue indicates the absence of this character. Black indicates that the character is unknown.

<u>Luzula</u>

There are several morphological characters that support *Luzula*, in particular the presence of multicellular hairs and basal placentation; closed leaf sheaths are also shared with *Prionium* (Fig. 5.5) and *Cyperaceae*, although they are generally absent from the rest of Juncaceae. Apart from these characters, the morphology of *Luzula* possesses many characteristics that appear to be plesiomorphic within Juncaceae, such as a well-developed basal rosette (Fig. 5.6), a racemose inflorescence (Fig. 5.9) of many flowers, and broad, flat leaves, as indicated by the leaf blade width and shape (Table 5.1), with no tendency for a reduction in the adaxial surface.

Within *Luzula*, however, there is little morphological variation. The shape of the leaf tip and of the capsule apex, and the presence or absence of apical or basal appendages on the seeds shows some variation. The leaf anatomy of Juncaceae as a whole is particularly variable, but within *Luzula* the variation is largely confined to the presence or absence of abaxial or adaxial sclerenchymatous girders. Other variable characters in the leaf anatomy are the presence of frequent stomata on the leaf sheath, observed only in *L. luzuloides*; and transverse septae, associated with stellate chlorenchyma cells. However, the septae were very infrequent, and observed only in *L. sylvatica*. It is possible that they are present in other species, but many sections would have to be examined due to the lack of frequency of their occurrence.

The lack of resolution in the trees resulting from cladistic analysis of the molecular, morphological and combined data makes it difficult to comment on the distribution of these characters in relation to groupings within *Luzula*, or to current or previous taxonomic classifications. The few groups that are resolved do not appear to correspond to sections of *Luzula*, with the exception of section *Alpinae*. The characters which are observed here to be variable within *Luzula* do not correlate well with one another, with the sections of *Luzula*, or with the clades which have been resolved; the cladistic analyses of the morphological data shows poor resolution within *Luzula*, with very poor bootstrap support of the clades resolved, indicating either a high degree of homoplasy in the morphology of *Luzula* or insufficient characters in the analyses.

Clade A

Juncus subgen. *Agathryon* is well supported as a monophyletic group by the molecular data in Chapter 5 *Phylogeny* (with the exception of *Juncus trifidus*), but it is not supported by the morphological data. A number of morphological characters are associated with this group, but relatively few define it clearly within Juncaceae. A cymose inflorescence is present only within this group (Fig. 5.9), while all other Juncaceae included in this study have either have a racemose inflorescence, or a single flower per inflorescence. Kirschner 2002a). The distribution of this character across the whole tree suggests that the ancestral state would have been racemose. Clade A has, therefore, evolved cymose inflorescences from the racemose state.

Juncus subgen. *Agathryon* can also be distinguished from other species of *Juncus* by the presence of bracteoles subtending the inflorescences, but this is not a unique character to this group, as it shares the presence of bracteoles with most other genera of Juncaceae, except for *Rostkovia* (Fig. 5.10). These two characteristics have been used in previous classifications (e.g. Buchenau 1906; Kirschner 2002b, c) to define this section. It is not clear whether this is a derived or ancestral character state because both character states are widespread in clades derived from its ancestral nodes.

In addition to these, there are other morphological characters which tend to unite the clade, but which are not sufficiently distinct to define it clearly. The inflorescences have a tendency towards being pseudolateral (Fig. 5.8), with a well-developed lower inflorescence bract which continues the line of the culm, pushing aside the inflorescence. This character is not universal within the clade, and *Juncus* sect. *Tenageia* (represented here by *J. bufonius*) does not have a pseudolateral inflorescence. *Juncus* sect. *Steirochloa* is variable for this character; of the species included here only *J. squarrosus* lacks a pseudolateral inflorescence, but other species within the section also lack it (Kirschner 2002b).

The pseudolateral inflorescence tends to be associated with other characters, such as a very well-developed inflorescence bract which has a sheath which is reduced relative to the blade. This is reflected in the measurements of the inflorescence bract, which show that the size of the inflorescence bract relative to the inflorescence among Clade A is unusually

5.4 Character distribution and evolution

large for Juncaceae. The average ratios of inflorescence bract length to inflorescence length shows only a small degree of overlap with *Luzula* and Clades B and C (Table 5.1). The ratio of the inflorescence bract blade to sheath is also greater for Clade A than for the Clades B and C (Table 5.1). Only *J. squarrosus* of Clade A overlaps with the range of Clade B, and there is no overlap with Clade C. *Luzula*, however, has a very large range which overlaps with all of the other clades.

There is also a tendency for the leaves to become thickened, and the adaxial epidermis reduced in area relative to the abaxial surface. In *Juncus* sects. *Steirochloa* and *Tenageia* the leaves are thickened and never entirely flat, as they are in *Luzula* and *Juncus* sect. *Graminifolii*. The adaxial surface is always at least slightly smaller in area than the abaxial side (Figs. 3.12–3.13). In some species the reduction of the adaxial surface is very significant, e.g. in *J. squarrosus*, and the blade becomes unifacial in its upper part. Other species in sect. *Steirochloa* which are not included in the analyses here are known to have terete and unifacial leaves, e.g. *Juncus uruguensis* (Balslev 1996). The tendency for the adaxial epidermis to become reduced is not unique to this group, however; Clades B and C also show a tendency to lose the adaxial surface.

Juncus sects. *Steirochloa* and *Tenageia* are distinguished from sect. *Juncotypus* by the presence of foliar leaves; i.e. the leaf blades are large in comparison to the size of the sheath. In Section *Juncotypus* the leaf blades are reduced to a mucro at the tip of the leaf sheath, and they have not been examined here. However, some species may have a small leaf blade on the basal one or two sheaths, for example *Juncus balticus* ssp. *mexicanus* Snogerup (Balslev 1996), and these leaf blades are terete and unifacial.

Juncus trifidus is the only representative of subgen. Agathryon which does not appear within Clade A. However, its morphology does indicate an association with Clade A, although it also differs in some respects. It lacks the characters which define Luzula as a group (hairs are absent from the leaves of J. trifidus, and the leaf sheaths are open), but it shares the unusually large ratios of bract length to inflorescence length, and inflorescence bract blade length to sheath length with Clade A (7.8 and 22, respectively). It has a leaf anatomy which is similar to the rest of sect. Steirochloa, with dorsiventrally flattened, but small, thick leaves, with an adaxial surface that is slightly reduced relative to the abaxial surface. However, the inflorescences differ from those of the rest of subgen. Juncus (and

also *Luzula*), in that they often consist of only one flower, sometimes with up to three flowers per inflorescence. It also has slightly papillose leaves, as does *Luzula*; whereas both of these are uncommon characteristics among *Juncus*.

Clade B

Clade B is not equivalent to any taxonomic groups previously recognised, consisting of three sections of *Juncus* subgen. *Juncus*. It is, however, easily identified morphologically, as all of these sections have transverse septae in the leaf blades, which are either absent or infrequent and indistinct in all other Juncaceae (Fig. 5.13), except for *Juncus subulatus*. This character has been used to define *Juncus* sect. *Ozophyllum* previously, but only in combination with other characters, such as leaf cross-sectional shape, to distinguish it from the other two sections with transverse septae (e.g. Kirschner 2002b).

This clade also lacks bracteoles subtending the flowers, but this does not serve to define the clade morphologically, as the species of *Juncus* which occur in Clade C also lack bracteoles, as does *Rostkovia*; otherwise, bracteoles are present throughout the family (Fig 5.10).

A character which does appear to be unique to Clade B is the arrangement of the vascular bundles in the sheaths (Fig. 5.14); this character has not previously been identified in the literature (Section 3.4.6 *Vascular bundle arrangement*). The vascular bundles in the sheaths of most species occur in a single row, alternating with a single row of air canals, but in Clade B additional rows of smaller vascular bundles are present. In small individuals they may only be recognisable as smaller vascular bundles occurring abaxially of the air canals, but in the larger species (e.g. *J. subulitepalus*), and particularly in large individuals, they can be seen to alternate with small air canals in a row, with some of the vascular bundles occurring directly abaxially of the vascular bundles in the larger row. This arrangement is only seen in the sheaths, which have not previously been the subject of detailed investigation. In the blades the air canals alternating with the vascular bundles are lost, so additional rows of vascular bundles can only be identified when small bundles occur directly abaxially of the larger ones; this tends to occur only in the largest species (e.g. *J. subulitepalus*).

Juncus sect. *Juncus*, which does not occur in Clade B, also has vascular bundles occurring in more than one row, but it differs in that the vascular bundles are scattered throughout the section, and do not alternate with air canals.

Among the continuously variable characters that were not included in the cladistic analysis, the ratio of anther to filament length tends to be very low in Clade B, in comparison to the rest of the family (Table 5.1). Clade B is also unusual within Juncaceae in having primarily parietal placentation (Fig. 5.11). The rest of Juncaceae usually has axile placentation, except *Luzula*, which has basal placentation.

Clade C

The genera *Distichia*, *Marsippospermum*, *Oxychloe* and *Rostkovia* are all distinguished from the rest of Juncaceae in having only a single flower per inflorescence, and these all occur in a clade within Clade C. This seems to be a derived character state.

The position of some *Juncus* species also within Clade C is more surprising. These species are classified within subgen. *Juncus*, on the basis of the presence of a racemose inflorescence and lack of floral bracteoles, but the rest of this subgenus forms Clade B. These three species do, however, differ from both of the *Juncus* Clades A and B in some respects. They lack bracts subtending the flowers, and they also lack septae in the leaves. This combination of characters associates them morphologically with neither of the two *Juncus* clades, as Clade A possesses bracteoles subtending the flowers, and Clade B possesses septate leaves. These characters do not unite Clade C very clearly, however. Most of the other species in Clade C (as in *Luzula* and Clade A) possess bracteoles, but *Rostkovia* does not. The presence of well-defined transverse septae in the leaf blades is unique to Clade B, and they are missing in all other Juncaceae, including Clade C.

The species of *Juncus* subgen. *Juncus* in Clade C also lack other characteristics which have been identified here as belonging to the rest of the subgenus in Clade B. *Juncus* sect. *Juncus* does not possess the same arrangement of vascular bundles in more than one row, as the scattered arrangement of vascular bundles in sect. *Juncus* differs in several respects. These species also have axile placentation, which is frequent in Clades A and C, but infrequent in Clade B. The ratio of anther to filament length is very low in Clade B in comparison to the rest of the family (Table 5.1), but in *J. maritimus* it is 1.63, which lies outside the range of Clade B, and within that of Clade C. These measurements were not taken of the species of Sect. *Graminifolii* which are included in the cladistic analysis. However, it should be noted that other species of sect. *Graminifolii* were measured for this character, and they lie within the range of Clade B.

5.4.2 Character evolution

Several trends in the distribution of characters have been noted within Juncaceae, and are either distinct enough to be used to define clades within the phylogenetic hypothesis, or they indicate trends within clades. Their distribution and evolution within the family is discussed here.

Reduction of the adaxial epidermis

Within Juncaceae there is a tendency towards the reduction of the adaxial epidermis of the leaf blade, and only *Luzula* and *Juncus* sect. *Graminifolii* have blades which are entirely flat and have adaxial surfaces which cover a similar area of the leaf to the abaxial surface. It is likely that this is the ancestral state for the family, as it is common in Cyperaceae, and *Prionium* and *Thurnia*, which are sister to Cyperaceae and Juncaceae also have similarly flat and bifacial leaves. This adds support to the position of *Luzula* as sister to the rest of Juncaceae; *Luzula* is placed in this position in the phylogenetic hypothesis based on molecular data (Fig. 5.1) but this is poorly supported by bootstrap percentages.

Most species of *Juncus*, however, show varying degrees of the reduction of the adaxial surface. This is least developed in Clade A, in which the leaves tend to be thickened in comparison to those of *Luzula* (see leaf blade shape Table 5.1), and the area of adaxial surface is only a little smaller than the abaxial surface in most species, remaining clearly bifacial throughout the length of the leaf in most species. *Juncus squarrosus*, however, has a significantly reduced adaxial epidermis, which is entirely lost near the tip of the leaf.

Clade B shows a tendency to lose the adaxial surface, and the blades of the species of this group are often entirely unifacial. *Juncus* sect. *Stygiopsis* is the exception within this group, with leaves which are bifacial but with a reduced adaxial epidermis at the base of

the blade, tending to become unifacial closer to the tip of the blade; these species are sister to the rest of the clade.

Clade C shows a high degree of variation. *Juncus* sect. *Graminifolii* has flat, bifacial leaves similar in their morphology to those of *Luzula*, and *Juncus* sect. *Juncus* has entirely terete and unifacial leaf blades. Also within this clade, most of the other genera of Juncaceae have terete, unifacial blades, but *Rostkovia* does not; although the leaves are thickened, they remain bifacial. *Marsippospermum*, also, has a very small area of adaxial surface which occurs within a small groove on the otherwise terete leaf.

It is difficult to determine relationships within Clade C, as several nodes in the phylogenetic hypotheses have very poor bootstrap support, so it is difficult to conclude, with any confidence, how the different leaf forms may have evolved within this group. However, Clades A and B may both have had an ancestral state of a leaf blade with a reduced adaxial surface, tending to become unifacial near to the tip of the leaf, as species with this morphology occur sister to the rest of the species within both groups.

Transverse septae

The presence of well-defined transverse septae in the leaf blades has been considered taxonomically useful within *Juncus* by previous authors (e.g. Buchenau 1906; Kirschner 2002b, c), and it has been found here to be unique to one of the four major clades (Clade B; Fig. 5.13) within Juncaceae. The transverse septae are associated with transverse vascular bundles which cross the air canals in the leaf, within a plate of chlorenchyma consisting of lobed to stellate cells. These septae occur regularly throughout the length of the leaf, and they can often be seen externally as slight ridges on the surface of the leaf; when they are not visible, they can still often be felt when the leaf is handled.

Although no other members of Juncaceae appear to have a similar morphology, such septae have been observed here in a number of other species, although extremely infrequently, usually in the sheaths, and usually in only one or two sections among a large number of sections examined. They usually occur across a single air canal within the row of small air canals that occur in the sheaths, and have never been observed in the large central air canal of the blades if a central air canal is present, as they do in Clade B. They have been observed in three of the four major clades identified, but they were not observed in Clade A (*Juncus* subgen. *Agathryon*).

As is true of most Monocotyledons, the majority of the vasculature in the leaves runs parallel to the length of the leaf, with additional smaller vascular bundles running perpendicular to the major ones and interlinking them. In Juncaceae, particularly in the sheaths, these cross the air canals, as the air canals lie directly between the main vascular bundles. Therefore the canals are partially blocked by the small amount of ground tissue which is associated with the transverse vascular bundle, but as large intercellular air spaces are present between the lobed or stellate cells of this tissue, it is not entirely blocked.

It seems likely, therefore, that this character is present generally throughout the family, but it has become highly developed only among Clade B, where the frequency is increased and the plates are larger, often crossing a central air canal rather than individual air canals between each vascular bundle. Their function is unclear, but it is possible that it confers mechanical support to the leaf. The leaves of Clade B are hollow, and frequently large, as indicated by the measurements of leaf length and leaf blade width (Table 5.1). *Luzula* and the species of Clade A never have a large central air canal; the majority of the volume of the leaf consists of ground tissue. Among Clade C some species have hollow leaves. The cushion-plant genera *Distichia, Oxychloe* and *Patosia* have hollow leaves, but they are very small, and also heavily sclereified; *Oxychloe* tends to have the largest leaves among these genera, but it has frequent, large sclerenchymatous girders. *Marsippospermum* also has relatively large, hollow leaves, but has no very obvious means of additional support. However, the ground tissue consists of more cell layers than in even the larger members of *Juncus* sect. *Ozophyllum* of Clade B, and the vascular bundle sheaths are much more heavily sclereified.

Arrangement of vascular bundles in the leaves

Most species of Juncaceae have a simple arrangement of a single row of vascular bundles in the leaves, but most species of *Juncus* subgen. *Juncus* have vascular bundles in more than one layer. These species can be divided into two groups, each with a different vascular bundle arrangement, and this division corresponds to the division of these species between Clades B and C in the phylogenetic hypotheses. The species occurring in Clade B

5.4 Character distribution and evolution

(Juncus sects. Iridifolii, Ozophyllum and Stygiopsis) have vascular bundles in distinct layers, with the vascular bundles of the largest row alternating with air canals, and further rows of smaller vascular bundles, sometimes also alternating with smaller air canals, occurring abaxially of the largest row; this is only seen clearly in the sheaths, as the vascular bundles do not alternate with air canals in the leaves, but surround a single central air canal. In contrast, *Juncus* sect. *Juncus*, which occurs in Clade C, does not have distinct rows alternating with air canals; the vascular bundles are scattered throughout the section, which has a relatively small number of indistinct air canals in the sheaths and entirely lacks air canals in the blades.

These two groups also represent the species of *Juncus* which have the largest leaves; although small species occur within these groups, many species are very large (see the ranges of leaf length and width in Table 5.1). These large leaves are also usually terete or nearly so, so that increases in volume are proportionally greater than the circumference of the leaves, and the number of vascular bundles that could occur within a single ring would not increase in proportion to the volume of the leaf. Only sect. *Iridifolii* has flat leaves, which could allow a greater number of vascular bundles due to the increased circumference relative to the volume. However, these are unifacial, laterally flattened leaves, which are probably derived from terete leaves similar to those seen in sect. *Ozophyllum*. As is the case with the transverse septae in Clade B, the multiple layers of vascular bundles, which tend to be heavily sclereified, may confer structural support, as well as supplying sufficient capacity for transport within the leaf.

It is likely that the different arrangements of multi-layered vascular bundles in the species of *Juncus* in Clade B and C are uniquely derived in each group, to solve similar problems associated with transport and structural support in very large leaves.

5.4.3 Discussion

The morphological characters which have been investigated in this thesis show a high degree of homoplasy, and this creates some difficulty in using the morphology to support taxonomic classifications. Some of these characters have been considered important in defining genera, subgenera and sections by previous authors, and among these, most have been shown to correlate well with the clades resolved in the phylogenetic analysis, whereas a small number have not. Among the characters which have been used to define genera and subgenera previously are closed or open leaf sheaths, the presence or absence of multicellular hairs, the presence or absence of floral bracteoles, racemose or cymose inflorescences, and placentation; the results of this study confirm that these are informative characters at these taxonomic levels.

Characters such as the presence or absence of transverse septae, the vascular bundle arrangement, and the presence or absence of pseudolateral inflorescences have previously been used to define sections within *Juncus* and these, also, are confirmed here as informative at this taxonomic level, even when their distribution does not entirely support previous classifications; for example, pseudolateral inflorescences occur in both *Juncus* sects. *Juncotypus* and *Steirochloa*, and these sections appear to be paraphyletic with respect to one another, but are associated within a clade. Vascular bundle arrangement has been used previously to identify *Juncus* sect. *Juncus*, but here it has also been seen to have an arrangement which is unique to Clade B. Other characters which have been used at this taxonomic level include the leaf cross-sectional shape and the presence or absence of foliar leaves; *Juncus* sects. *Juncotypus* and *Steirochloa*, for example, are defined by these characters. These characters appear to remain taxonomically informative at sectional level according to the phylogenetic hypothesis.

Of the four clades which are resolved in Section 5.3 *Results*, it is possible to find apomorphic characters associated with most of these clades and which can be used to identify them. *Luzula* is the most distinct morphologically, and is recognised as a separate genus within the family in all classifications of Juncaceae (e.g. Buchenau 1906; Kirschner 2002a). Clade A consists of *Juncus* subgen. *Agathryon*. This group has been recognised in many classifications of Juncaceae (e.g. Buchenau 1906; Kirschner 2002a) although the

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taxonomic rank has varied according to the author. It was recognised by these authors by its cymose inflorescences, which are unique to this group within Juncaceae (and also to *Luzula* subgen. *Marlenia*). It has also been recognised by the presence of floral bracteoles, which are lacking in other species of *Juncus*; however, these are also present in most other genera. It is observed here that it also possesses other characteristics which are unusual within the family, such as pseudolateral inflorescences, and a long inflorescence bract blade, although these are not universal within the clade.

Clade B can also be identified morphologically, and although it has not previously been recognised taxonomically, the characters which serve to identify it have been used previously to define subgenera or sections (e.g. Buchenau 1906; Kirschner 2002a) in combinations with other characters. In particular, it has distinct, frequent transverse septae in the leaves, and vascular bundles in more than one row in the sheaths, both uniquely derived within Juncaceae.

Among the four major clades of Juncaceae, Clade C shows the most morphological variation; it is also the only one which combines more than one genus. For example, the variation includes the presence or absence of bracteoles; one to many flowers; flat and bifacial leaves to entirely unifacial leaves; vascular bundle arrangement scattered or in a single row; pith present or absent; hollow leaves or not hollow; cushion-plants and plants with well defined rhizomes and culms; and placentation axile or parietal. It is difficult to find any morphological characters which are unique to this group, other than the lack of the characters which define *Luzula*, Clade A and Clade B.

5.5 Classification of Juncaceae

The genus *Juncus* is paraphyletic according to the phylogenetic hypothesis presented here, based on data from two plastid genomic regions, one nuclear genomic region and morphology; and the clades which indicate this have very high bootstrap support.

Of the three clades between which the genus *Juncus* is distributed, two are also welldefined morphologically. Clade A is equivalent to one of the two currently recognised subgenera of Juncaceae, *Agathryon*, and it is identified by characters already identified for this group (Buchenau 1906; Kirschner 2002b). The second group has not previously been recognised taxonomically, but it consists of most of the species of subgenus *Juncus*, and can be defined by several apomorphic morphological characters, in particular, the presence of distinct, frequent transverse septae in the leaves and vascular bundles in more than one layer in the leaf sheaths.

Two further sections of subgenus *Juncus* appear to be more closely associated with other genera of Juncaceae, in Clade C. This clade is very well supported in the phylogenetic hypothesis, but there is a very high degree of morphological variation within the clade; each of the genera within it are easily recognised morphologically, but there is no clear apormorphic morphological character uniting them, and in the absence of such a character it is not possible, on a morphological basis, to recognise Clade C as a single taxon.

Relationships within Clade C are poorly resolved, and although *Juncus falcatus* appears sister to the rest of the clade according to the phylogenetic hypothsis based on molecular data (Fig. 5.1), the bootstrap support is less than 50%, so the relationship between *Juncus* sects. *Graminifolii* and *Juncus*, and the other genera, is unclear, and it cannot be concluded on the basis of this that *Juncus* sect. *Graminifolii* is paraphyletic in relation to the other genera in this clade. Further work needs to be done to determine the status of *Juncus* sect. *Graminifolii* within Juncaceae.

It is considered here that the genus *Juncus* should no longer be recognised in the current sense. One option to restore monophyly to the group is to include the genera *Distichia*, *Marsippospermum*, *Oxychloe*, *Patosia* and *Rostkovia* within *Juncus*. However, the degree of morphological variation between these taxa is large, particularly in the anatomy, and the

recognition of different genera based on such a high degree of variation does not seem inappropriate. However, Clades A and B show a similar degree of difference between one another morphologically as can be seen between several of the genera, and they are both easily defined morphologically.

It is suggested here that Clades A and B should be raised to genus level, and it is proposed that the following two genera should be recognised. As a result of this treatment, most species have been removed from the genus *Juncus*, and *Juncus* contains only two sections, *Graminifolii* and *Juncus*. These two sections have not received further treatment here, as it is considered that the relationship between them, and between *Juncus sensu stricto* and the other genera, is unclear, and further investigation is required.

Tenageia (Dumort.) Fourr.

Type Tenageia vaillantii Rchb. Ic. Fl. Germ. IX. 22 (Syn. Juncus tenageia Ehrh. ex L.f.)

Syn. Juncus subgen. Agathryon Raf., Autik. Bot. 196 (1840)

Leaf blades dorsiventrally flattened or canaliculate, bifacial (rarely unifacial in the upper portions of the blade), or the only leaf blades present are reduced to rudimentary blades on a well-developed sheath. Central air canals and transverse septae are absent from the leaves. Inflorescences cymose, frequently pseudolateral. Floral bracteoles present.

Stygiopsis (Dumort) E.Jones

Type: Stygiopsis acutiflorus Ehrh. ex Hoffm. (Syn. Juncus acutiflorus Ehrh. ex Hoffm.)

Leaf blades terete, with or without a concave adaxial surface, or laterally flattened (ensiform). Rarely dorsiventrally flattened. Leaf blades unifacial throughout the blade, or bifacial only at the base of the blade, with a reduced adaxial epidermis, and unifacial in the upper portions. Leaf blades with one or more central air canal, regularly interrupted by transverse septae characterised by the presence of small transverse vascular bundles surrounded by lobed or stellate chlorenchyma cells. Inflorescences racemose, never pseudolateral. Floral bracteoles absent.

5.6 Conclusions

The aims of this thesis were to investigate the monophyly and relationships of the three families Cyperaceae, Juncaceae and Thurniaceae, and to investigate character evolution and relationships of the taxa within Juncaceae, concluding with a review of the taxonomy of Juncaceae.

A large set of morphological and molecular data was collected. The morphological data presented here includes many characters which had not previously been used in cladistic analyses, although some of these had been used in classifications of Juncaceae; and of some of those characters which had been used previously, for example the auricular and ligular structures, a new understanding of the homologies has been gained. In addition, new descriptions of the anatomy of Juncaceae were presented, highlighting a number of taxonomically informative characters which had not previously been recognised.

The cladistic analyses of these data showed that each of the individual genetic regions sequenced (ITS, *trnL* intron, *trnL*-F intergeneric spacer and *rps*16) gave good resolution of the genera of Juncaceae, Cyperaceae and Prioniaceae, but with low bootstrap support of outlying groups within Juncaceae. The combined molecular data were, however, sufficient to give good resolution, with strong bootstrap support, of the relationships between taxa from family to genus level, and between sections of *Juncus*. There was a high degree of congruence between the nuclear and plastid DNA datasets, but the variability of the ITS region caused problems with alignment of the sequences between genera so that some parts of the sequences had to be excluded from the analysis.

There is insufficient variation within *Luzula* in the regions analysed here for good resolution between the species. However, the highly variable regions which were excluded from the analysis, particularly from the ITS region, may prove useful in resolving relationships between species of *Luzula*.

The morphological data has a high degree of homoplasy, as indicated by the low CI and RI values in the analysis (0.32 and 0.68 respectively in Section 5.3 *Results*; see also Section 5.4 *Character distribution and evolution*). As a result, there was poor resolution in the

phylogenetic tree, but the groups that were resolved were also identified in the results of the analysis of the molecular data, providing it with some additional support.

The molecular data clearly supports the monophyly of these families, and the sister-group relationship between Juncaceae and Cyperaceae is well-supported, as is the sister-group relationship of Thurniaceae to the Juncaceae–Cyperaceae clade.

Within Juncaceae, the monophyly of *Luzula* is supported by both molecular and morphological data, and it is likely to be sister to the rest of Juncaeae, but the lack of resolution at this level of the phylogenetic hypothesis makes it difficult to confirm the position of *Luzula* in relation to the rest of the family. However, *Juncus* is paraphyletic with respect to all the other genera. Among these genera, the cushion-plant genera *Distichia*, *Oxychloe* and *Patosia* form a monophyletic group, confirming the position of *Oxychloe* within Juncaceae.

Subgeneric and sectional classifications of *Juncus* are partly supported, as major divisions within *Juncus* correspond, in part, to the subgeneric classifications of Kirschner (2002b, c). However, two sections of *Juncus* subgen. *Juncus* are associated in a clade with the single-flowered genera of Juncaceae. There is an insufficient number of species to draw strong conclusions about the relationships between sections of *Juncus*, but some, particularly sects. *Steirochloa* and *Juncotypus* are paraphyletic.

To restore monophyly to *Juncus*, it has been proposed that it should be divided into three genera, and descriptions of two additional genera *Steirochloa* and *Tenageia* are presented here.



Table A	A1 V	<i>oucher</i>	specimens	and	data c	obtained

Species	Voucher specimen	Location	Data ob				
			TrnL-F	Rps16	ITS	Anatomy	Macro-morphology
Carex sylvatica Huds.	Simpson 2667	TCD	Yes	Yes	Yes		
Chorizandra cymbaria R.Br.	Wilson KLW 9738	NSW	Yes		Yes		
Cladium mariscus (L.) Pohl	Simpson 2669	TCD	Yes	Yes	Yes		
Distichia acicularis Balslev & Laegaard	Laegaard 53240	K, A					Yes
	Jorgensen, Laegaard & Steere 56213	K					Yes
	Laegaard, Dhooge & Jones 21503	TCD	Yes	Yes	Yes	Yes	Yes
	Laegaard 55099	K					Yes
Distichia muscoides Nees & Meyen	Rob. & Fries. 725						Yes
	Holm-Nielsen 20866	A, RNG					Yes
	Laegaard 55727	BM, C, A					Yes
	Chaipin & Novara 20775	BM					Yes
	Asplund 11553	K					Yes
	Laegaard, Dhooge & Jones 21490B	TCD				Yes	Yes
	Laegaard, Dhooge & Jones 21476	TCD	Yes	Yes	Yes	Yes	
	Iltis & Ugent 1285	K					Yes
Eriophorum angustifolius Honck.	Simpson 2672	TCD	Yes	Yes	Yes		
Hellmuthia membranacea (Thunb.)	Werdermann et al 269	K	Yes		Yes		
Haines & Lye							
Hypolytrum testui Cherm.	Apemu 163	K	Yes	Yes	Yes		

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Table A1 Continued

			Data ob	btained						
Species	Voucher specimen	Location	TrnL-F	Rps16	ITS	Anatomy	Macro-morphology			
Juncus articulatus L.	Hodkinson 1	TCD	Yes	Yes		Yes				
	Townsend s.n.	K					Yes			
	Shaw 1948	K					Yes			
	Turrill 2054	K					Yes			
Juncus balticus Willd. ssp. andicola	Laegaard, Dhooge & Jones 21479	TCD					Yes			
(Hook.) Snogerup	Laegaard, Dhooge & Jones 21536	TCD	Yes	Yes	Yes		Yes			
	Laegaard, Dhooge & Jones 21518	TCD					Yes			
	Blytt s.n.	K					Yes			
	Lester-Garland s.n.	K					Yes			
Juncus bufonius L.	Bor 165						Yes			
	Hubbard 9200	K				Yes Y				
	Bullock 390	K					Yes			
	Webb s.n.	TCD					Yes			
	Hodkinson 2	TCD	Yes	Yes	Yes	Yes	Yes			
Juncus bulbosus L.	Jones 43	TCD				Yes	Yes			
	Simpson ?	K	Yes		Yes					
	Britton 2013	K					Yes			
	Montford s.n.	K					Yes			
Juncus caespiticius E.Mey	Hodgon 747		Yes			Yes				
	Melville 2762	K					Yes			
	Johnson 95305	K					Yes			

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Table A1 Continued

			Data ob	tained							
Species	Voucher specimen	Location	TrnL-F	Rps16	ed ITS Anatomy ps16 ITS Anatomy es I I I I	Anatomy	Macro-morphology				
	Villagrán & Meza 1062	SGO	Yes	Yes		Yes					
	Meyer 9630	K					Yes				
Juncus capillaceus Lam.	Schinini 7715	K					Yes				
Juncus castaneus Sm.	Chase 13771	K	Yes								
	Hayden 1	TCD	Yes	Yes							
	Misolenko 216	K					Yes				
Juncus compressus Jacq.	Seregin U-40	K					Yes				
	Laegaard, Dhooge & Jones 21515	TCD				Yes	Yes				
	Gardner, Knees & De Vore 4431	K					Yes				
Juncus cyperoides Laharpe	Mexia 7882	K				-	Yes				
Juncus dregeanus Kunth	Muasya 2681	K	Yes	Yes	Yes						
Juncus ecuadoriensis Balslev	Laegaard, Dhooge & Jones 21498	TCD	Yes	Yes	Yes	Yes	Yes				
	Simpson 2665	TCD	Yes	Yes	Yes						
	Gilliat-Smith 3168	K					Yes				
	Montford 9032	K					Yes				
Juncus effusus L.	Turrill s.n.	K					Yes				
	Jones 49	TCD	Yes	Yes	Yes	Yes					
	Thompson 3645	K					Yes				
Juncus ensifolius Wikstr.	Hitchcock & Martin 7618	K					Yes				
Juncus falcatus E.Mey.	Hodgon 954		Yes	Yes							

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Table A1 Continued

			Data ob	ata obtained						
Species	Voucher specimen	Location	TrnL-F	Rps16	ITS	Anatomy	Macro-morphology			
	Simpson 2668	TCD	Yes	Yes	Yes					
	Pearson 27/30	TCD								
	Turril s.n.	К				Yes	Yes			
	Frazer s.n.	K					Yes			
	Tayler 1002	K					Yes			
	Levan s.n.	K					Yes			
Juncus gerardii Loisl.	Seppala & Kemppainen 207	К					Yes			
	Pearson s.n.	TCD					Yes			
	Hodkinson 13	TCD	Yes	Yes	Yes		Yes			
	Webb s.n.	TCD					Yes			
	Lester-Garland s.n.	К					Yes			
Juncus inflexus L.	Turril 4374	K					Yes			
	Jones 45	TCD	Yes			Yes				
	Jones 47	TCD	Yes	Yes	Yes	Yes				
	Webb s.n.	TCD					Yes			
	Ohl s.n.	K					Yes			
Juncus maritimus Lam.	Pidner s.n.	K					Yes			
Juncus oxycarpus E.Mey. ex Kunthe	Muasya 2690	К	Yes	Yes						

			Data ob	ita obtained							
Species	Voucher specimen	Location	TrnL-F	Rps16	ITS	Anatomy	Macro-morphology				
	Jones 46	TCD	Yes	Yes	Yes						
	Hodgon 589					Yes					
	Wilson 9038	K					Yes				
Juncus planifolius R.Br.	Constable 6230	K					Yes				
Juncus scheuzerioides Gaudich.	McAdam 5		Yes								
	Jones 34	TCD	Yes	Yes	Yes	Yes	Yes				
	Webb s.n.	K					Yes				
	Gamble s.n.	K					Yes				
Juncus squarrosus L.	Gamble 20157	K					Yes				
	Chase 14363	K	Yes	Yes							
	Nilsson 148	K					Yes				
Juncus stygius L.	Blytt s.n.	K					Yes				
	Sandwith 5444	TCD				Yes	Yes				
	Lindeberg 9.7	K					Yes				
Juncus subulatus Forskk.	Liseoferton 795	K					Yes				
Juncus subulitepalus Balslev	Laegaard, Dhooge & Jones 21499	TCD	Yes	Yes	Yes	Yes	Yes				

Table A1 Continued

Table A1 Continued

Species Juncus trifidus L. Juncus triglumis L. Juncus xiphioides E.Mey.			Data ob	ta obtained						
Species	Voucher specimen	Location	TrnL-F	Rps16	ITS	Anatomy	Macro-morphology			
Juncus trifidus L.	Jones 40	TCD	Yes	Yes	Yes	Yes	Yes			
	Sousler 1426	K					Yes			
	Worsdell 718/23	K					Yes			
		K					Yes			
	Jackson s.n. K						Yes			
	Jackson s.n.	K					Yes			
Juncus triglumis L.	Jones 41	TCD	Yes	Yes	Yes	Yes	Yes			
	Clarke 46842B	K					Yes			
Juncus xiphioides E.Mey.	Jones 48	TCD				Yes				
	Coville & Funston 969	K					Yes			
Lepironia articulata (Retz.) Domin	Simpson 1236	K	Yes		Yes					
Luzula arcuata (Wahlenb.) Sw.	Jones 37	TCD	Yes	Yes	Yes	Yes	Yes			
	Mackechnie s.n.	K					Yes			
	Shoolbred s.n.	K					Yes			
Luzula campestris (L.) DC.	Jones 35	TCD	Yes	Yes	Yes	Yes				
	Bullock 1	K					Yes			
	Wyatt 87	K					Yes			
Luzula gigantea Desv.	Laegaard, Dhooge & Jones 21514	TCD				Yes	Yes			
	Balslev 24189	K					Yes			
Luzula johnstonii Buchenau	Muasya 2682	K	Yes							
	Tebrum 7416	K				Yes				

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Table A1 Continued

			Data ob	obtained						
Species	Voucher specimen	Location	TrnL-F	Rps16	ITS	Anatomy	Macro-morphology			
	Hodkinson 11	TCD	Yes	Yes	Yes	Yes				
Luzula luzuloides (Lam) Dandy &	Robertson 1935	K					Yes			
E.Willm.	Jackson s.n.	K					Yes			
	Hodkinson 12	TCD	Yes	Yes	Yes	Yes				
	Jones 42	TCD					Yes			
	Jones 39	TCD				Yes	Yes			
	Hubbard 1540	K					Yes			
Luzula multiflora (Ehrh.) Lej	Smith s.n.	K					Yes			
	Bowen 3607	RNG	Yes		Yes					
	Heldreick s.n.	K					Yes			
Luzula nodulosa E.Mey.	Atchley 1343	K					Yes			
	Jones 28	TCD	Yes	Yes		Yes	Yes			
	Holm-Nielsen 24238	K					Yes			
Luzula racemosa Desv.	Balslev 24186	K					Yes			
	Jones 36	TCD	Yes	Yes	Yes	Yes	Yes			
	Souster 1428	K					Yes			
Luzula spicata (L.) DC.	Taylor 0994	K					Yes			

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Table A1 Continued

Snecies			Data ob	ta obtained						
Species	Voucher specimen	Location	TrnL-F	Rps16	ITS	Anatomy	Macro-morphology			
	Pearson s.n.	TCD					Yes			
	Davis 21564	BM	Yes	Yes	Yes					
	Jones 33	TCD				Yes	Yes			
	Summahayis 4078	K					Yes			
Luzula sylvatica (Huds.) Gaudin	Gobert a Bouaye	K					Yes			
Mapania cuspidata (Miq.) Uitt.	Marsh 4	K	Yes	Yes	Yes					
Mapania lorea	Uitt.	K	Yes	Yes	Yes					
Mapania meditensis D.A.Simpson	Simpson 2515	K	Yes	Yes	Yes					
Mapania tenuiscapa C.B.Clarke	Simpson 2661	K	Yes	Yes	Yes					
	Godley 546b	SGO					Yes			
	Pisano 5951	RNG	Yes	Yes						
	McAdam 4		Yes	Yes	Yes					
	Godley 922a	SGO				Yes				
Marsinnospermum grandiflorum (I_f)	Coppinger s.n.	K					Yes			
Hook.	Vervoorst 4602	K					Yes			
	Billiet & Jadin 5429	BR	Yes		Partial		Yes			
	Moreira & Muñoz 272	SGO	Yes	Yes		Yes				
	Werdermann 453	K					Yes			
	Wickens, Luna Nina 95	K					Yes			
Oxychloe andina Phil.	Johnston 4825	K					Yes			

Table	A1	Continued

			Data ob	btained						
Species	Voucher specimen	Location	TrnL-F	Rps16	ITS	Anatomy	Macro-morphology			
Oxychloe bisexualis Kuntze	Muñoz, Moreira, Meza & Arriagada 3558	SGO	Yes	Yes		Yes				
	Hauman I/1408	К					Yes			
Patosia clandestina (Phil.) Buchenau	Villagran, Villa & Hinojosa 8515	SGO				Yes	Yes			
	Castellanos 36631	BA					Yes			
Prionium serratum (Thunb.) Drege	Galpin 4780	GH					Yes			
	Burchell s.n.	K					Yes			
	Muasya s.n.	K	Yes	Yes	Yes					
	Pearson 3516	BM					Yes			
	Codd 9704	BM					Yes			
	Rudatis 1114	BM					Yes			
Rhynchospora alba (L.) Vahl	Simpson 2671	TCD	Yes	Yes	Yes					
Rostkovia magellanica (Lam.) Hook.f.	Pisano 6048	RNG	Yes	Yes	Yes					
	Laegaard, Dhooge & Jones 21516	TCD	Yes	Yes	Yes	Yes				
	Laegaard, Dhooge & Jones 21586	TCD	Yes	Yes	Yes	Yes				
	Laegaard 55422	K					Yes			
	Greene 3057	K					Yes			
Scirpodendron bogneri S.S.Hooper	Simpson 2560	K	Yes	Yes	Yes					
Scirpodendron ghaeri Merr.	Lye 34	K	Yes	Yes	Yes					
Scirpoides holoschoenus (L.) Soják	Parnell s.n.	TCD	Yes	Yes	Yes					
Thurnia sphaerocephala Hook.f.	Jones 2	TCD	Yes	Yes	Yes					
Typha minima Funck ex Hoppe	Hodkinson 5	TCD	Yes	Yes						

Table A2 Morphological data

Species		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Distichia acicularis	F	2	0.7	1	1	0	1	15	1.1	0	0	0	n	n	0	n	n	n	2	1	1
	М	1															0.6	0.4			
Distichia muscoides	F	2	0.7	1	1	0	1	10	0.5	2	0	0	n	n	0	n	1.2	0.2	2	1	1
	М																0.6	0.1			
Juncus arcticus		1	1.2	0	1	1	0	37	0.0	0	0	0	111	16	1	1	4.4	6.4	0	1	0
Juncus articulatus		1	0.	0	1	0	1	130	2.5	0	0	0	264	12	0	0	1.2	3.8	0	0	0
Juncus bufonius		0	0.2	0	1	1	1	67	3.3	0	0	0	114	34	0	1	0.7	5.5	1	1	0
Juncus bulbosus		0	0.3	0	1	1	1	83	3.3	0	0	0	96	26	0	0	0.5	2.3	0	0	0
Juncus caespiticius		0	0.4	0	1	1	0	205	n	0	0	0	271	9	0	0	0.9	n	0	0	0
Juncus capillaceus	2.1	1	0.8	0	1	1	0	115	6.8	0	0	0	131	10	1	1	3.5	18.5	1	1	0
Juncus compressus		1	0.5	0	1	1	1	108	2.5	0	0	0	314	17	1	1	1.4	5.2	0	1	0
Juncus cyperoides		1	1.4	0	1	0	1	88	4.1	0	0	0	166	49	0	0	0.5	7.9	0	0	0
Juncus ecuadoriensis		1	0.6	0	1	1	1	363	2.7	0	0	0	475	17	0	0	0.5	0.3	0	0	0
Juncus effusus		1	0.9	0	1	1	0	111	0.0	0	0	0	852	3	1	1	8.4	21.0	0	1	0
Juncus ensifolius		1	0.8	0	1	0	1	155	1.3	0	0	1	307	6	0	0	0.3	0.6	0	0	0
Juncus gerardii		1	1.5	0	1	1	1	172	2.9	0	0	0	404	14	1	1	0.6	4.2	0	1	0
Juncus inflexus	i.	1	8.3	0	1	1	0	79	0.0	0	0	0	741	10	1	1	2.9	16.5	0	1	0
Juncus maritimus		1	0.8	0	1	1	0	565	7.1	0	0	0	886	10	1	0	1.7	190	0	0	0
Juncus planifolius		0	0.4	0	1	1	0	185	n	0	0	0	325	11	0	0	0.8	28.8	0	0	0
Juncus scheuchzerioides		2	n	0	1	1	1	n	n	0	0	0	n	n	0	0	n	n	0	0	0
Juncus squarrosus		0	1.1	0	1	1	0	137	5.3	0	0	0	257	22	0	1	1.1	2.1	0	1	0
Juncus stygius		1	0.2	0	1	1	1	65	2.2	1	0	0	185	13	0	0	0.5	1.2	1	0	0
Juncus subulatus		1	0.5	0	1	1	1	261	2.3	0	0	0	582	13	0	1	0.2	0.5	0	1	0

Table A2 Continued

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Juncus subulitepalus	1	1.3	0	1	1	1	943	14.5	0	0	0	1176	6	0	0	0.5	0.2	0	0	0
Juncus trifidus	1	0.3	0	1	1	1	63	14.3	0	0	1	67	8	0	1	7.8	22.2	1	1	0
Juncus triglumis	1	0.1	0	1	1	0	47	1.0	1	0	1	89	5	0	0	1.3	0.1	1	0	0
Juncus xiphioides	1	0.6	0	1	0	1	237	0.6	0	0	1	463	18	0	0	0.2	0.3	0	0	0
Luzuola arcuata	1	0.2	0	0	1	1	55	3.0	0	1	0	137	47	0	0	0.2	1.4	0	1	0
Luzula campestris	0	0.1	0	0	1	1	63	2.5	2	1	1	147	16	0	0	0.8	38.0	0	1	0
Luzula gigantea	1	0.2	0	0	1	1	157	3.0	0	1	1	596	59	0	0	0.3	3.1	0	1	0
Luzula johnstonii	2	n	0	0	1	1	n	n	2	1	n	n	n	0	0	n	n	0	1	0
Luzula luzuloides	0	0.3	0	0	1	1	174	4.1	0	1	1	745	17	0	0	1.1	13.8	0	1	0
Luzula multiflora	0	0.2	0	0	1	1	102	3.6	2	1	1	187	16	0	0	1.1	60.0	0	1	0
Luzula nodulosa	1	0.2	0	0	1	1	197	3.7	0	1	1	730	8	0	0	0.5	1.3	0	1	0
Luzula racemosa	1	0.2	0	0	1	1	106	2.3	0	1	1	194	31	0	0	1.4	23.4	0	1	0
Luzula spicata	0	0.2	0	0	1	1	56	1.7	0	1	1	153	25	0	0	0.8	2.1	0	1	0
Luzula sylvatica	1	0.2	0	0	1	1	159	5.0	0	1	0	355	23	0	0	0.3	7.3	0	1	0
Marsippospermum grandiflorum	1	1.1	0	1	1	0	292	2.9	0	0	0	354	8	0	n	0.0	0.0	2	1	0
Oxychloe andina F	2	n	0	1	0	1	34	0.7	0	0	0	n	n	0	n	1.3	0.8	2	1	1
М																n	n			
Oxychloe bisexualis	2	1.0	0	1	0	1	51	0.8	0	0	0	n	n	0	n	0.8	0.4	2	1	0
Patosia clandestina F	2	n	0	1	0	1	18	0.7	0	0	1	n	n	0	n	0.9	1.8	2	1	1
М]															0.8	0.1			
Prionium serratum	1	n	0	0	1	0	834	n	0	0	1	n	n	0	0	0.3	2.9	0	0	0
Rostkovia magellanica	1	0.5	0	1	1	0	138	2.0	0	0	0	145	5	1	n	3.4	1.7	2	0	0

Table A2 Continued

Species		21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Distichia acicularis	F	8.9	1.06	10.0	9.95	n	n	n	n	1.46	0.65	1	n	n	n	n	n	n	n	n	n
CALLAR COURT	М	5.8	1.38	6.42	6.59	0.55	1	4.65	0	n	n	n	n	n	n	n	n	n	n	n	n
Distichia muscoides	F	6.5	0.94	2.86	3.62	n	n	n	n	1.37	1.64	1	6.6	1.02	2.27	0	n	1.3	2.17	0	0
	М	6.2	1.20	3.49	4.61	0.48	1	15.96	0	n	n	n	n	n	n	n	n	n	n	n	n
Juncus arcticus		4.2	1.12	3.36	3.45	0.43	1	0.94	0	0.97	1.64	1	4.1	1.17	1.48	0	6.0	0.80	1.79	0	0
Juncus articulatus		2.6	1.14	3.56	2.96	0.53	1	1.12	0	1.51	3.15	0	2.7	1.12	1.84	1	5.5	0.52	2.14	0	0
Juncus bufonius	. L	5.5	1.29	4.54	4.69	0.25	1	0.55	0	0.32	1.58	1	4.1	0.75	2.10	0	5.0	0.52	1.32	0	0
Juncus bulbosus		2.6	0.98	2.96	2.92	0.38	1	0.83	0	0.70	3.67	0	2.3	0.72	1.91	0	3.4	0.56	1.85	0	0
Juncus caespiticius		2.6	1.07	2.96	3.00	0.45	1	0.92	0	0.86	2.50	1	2.0	0.78	1.43	0	8.8	0.47	1.49	0	0
Juncus capillaceus		3.5	1.22	2.92	2.87	0.36	1	1.04	0	0.98	16.0	1	3.1	0.88	1.55	0	1.6	0.49	1.59	0	0
Juncus compressus		2.1	1.11	3.00	2.71	0.63	1	1.94	0	1.67	6.00	1	2.5	1.17	1.20	0	4.1	0.46	1.49	0	0
Juncus cyperoides		3.3	0.95	4.36	4.80	0.75	0	0.37	0	1.41	2.22	0	2.6	0.79	1.86	1	23.1	0.37	1.69	0	0
Juncus ecuadoriensis		3.2	1.08	3.28	3.26	0.58	1	0.54	0	0.79	3.00	0	1.7	0.54	1.21	1	17.6	0.69	2.46	0	0
Juncus effusus		2.3	1.90	3.17	1.65	0.59	1	1.19	0	0.67	7.67	1	2.2	0.96	1.47	0	0.0	0.55	2.18	0	0
Juncus ensifolius		2.8	1.13	3.86	4.41	0.45	0	0.65	0	0.78	5.00	0	2.8	0.99	2.35	1	10.6	0.44	2.26	0	0
Juncus gerardii		3.0	1.13	3.30	2.91	0.62	1	2.72	0	1.55	1.77	1	3.1	1.02	1.45	0	0.1	0.55	1.69	0	0
Juncus inflexus		3.6	1.36	4.29	5.14	0.38	1	1.52	0	0.67	1.25	1	2.7	0.63	1.56	1	14.8	0.52	1.67	0	0
Juncus maritimus		4.3	1.31	3.02	3.88	0.48	1	1.63	0	0.88	1.10	1	2.7	0.61	1.56	1	10.9	0.70	2.26	1	1
Juncus planifolius		2.1	0.98	2.03	2.29	0.60	0	0.43	0	0.98	2.00	1	2.0	0.94	1.33	0	10.0	0.43	1.70	0	0
Juncus scheuchzerioides		n	n	n	n	n	n	n	0	n	n	0	n	n	n	1	n	n	n	0	0
Juncus squarrosus		5.1	1.18	3.07	2.72	6.78	1	7.57	1	1.09	1.57	1	4.4	0.86	1.61	0	4.8	0.71	1.70	0	0
Juncus stygius		3.5	1.02	3.25	3.40	0.67	0	0.21	0	0.89	1.00	0	5.5	1.59	2.34	1	2.7	1.20	2.14	1	1
Juncus subulatus		3.4	1.32	3.15	2.87	0.51	1	2.48	0	0.93	0.91	1	2.5	0.73	1.56	0	8.0	0.51	1.71	1	1

Table A2 Continued1

Species		21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
Juncus subulitepalus		2.9	1.21	3.03	3.04	0.70	n	0.22	0	n	n	0	2.0	0.68	1.67	1	15.0	0.47	2.24	n	n
Juncus trifidus		3.2	1.17	2.41	3.39	0.50	1	1.31	1	1.29	1.79	1	2.5	0.78	1.46	1	25.0	1.08	1.63	0	0
Juncus triglumis		4.0	1.53	2.83	2.69	0.71	0	0.29	0	0.81	1.67	1	4.6	1.16	2.49	0	9.8	0.75	3.33	1	1
Juncus xiphioides		3.2	1.23	3.52	4.53	0.49	0	1.14	0	1.17	1.20	0	2.9	0.88	2.53	0	11.3	0.40	2.11	0	0
Luzuola arcuata		3.2	1.08	2.73	3.95	0.45	0	1.10	0	0.88	4.42	2	1.6	0.50	1.00	0	0.0	1.01	1.56	0	1
Luzula campestris		3.0	0.91	2.28	2.90	0.51	1	2.59	0	1.43	1.69	2	2.6	0.87	0.08	0	7.7	1.28	1.18	0	0
Luzula gigantea		2.4	1.40	3.48	4.50	0.50	0	1.32	0	0.51	1.33	2	2.0	0.72	1.22	0	1.3	1.18	1.76	0	0
Luzula johnstonii		n	n	n	n	n	n	n	0	n	n	2	n	n	n	1	n	n	n	1	0
Luzula luzuloides	· · · · · · · · · · · · · · · · · · ·	2.3	0.80	2.00	3.33	0.76	n	2.31	0	1.41	1.11	2	1.8	0.73	1.17	1	15.7	0.98	1.58	1	0
Luzula multiflora		3.5	1.12	3.49	3.88	0.43	0	1.28	0	1.11	3.79	2	2.0	0.63	1.25	0	3.8	1.07	0.48	0	1
Luzula nodulosa		6.0	1.13	3.20	3.13	0.51	1	3.46	0	1.35	1.40	2	3.5	0.60	1.25	1	8.6	1.72	1.43	0	0
Luzula racemosa		0.7	1.07	3.28	3.70	0.39	0	0.51	0	0.60	3.50	2	1.8	2.57	1.38	0	0.0	0.94	1.73	0	0
Luzula spicata		2.9	2.53	3.07	2.24	0.38	n	1.12	0	0.91	4.42	2	1.9	0.66	1.36	0	0.0	1.13	1.69	0	0
Luzula sylvatica		3.4	0.99	2.33	3.02	0.71	0	7.64	0	1.65	1.35	2	2.3	0.67	1.28	1	13.0	1.50	1.60	0	0
Marsippospermum grandifle	orum	29.6	1.51	11.58	7.44	0.45	1	2.95	0	0.70	2.00	0	18.0	0.66	4.33	1	4.1	1.07	1.71	1	1
Oxychloe andina	F	6.0	0.91	1.56	1.16	n	n	n	n	1.26	1.80	1	9.0	1.76	1.49	0	5.6	0.95	1.49	0	1
	M	7.5	0.98	3.01	3.88	0.65	1	7.47	1	n	n	n	n	n	n	n	n	n	n	n	n
Oxychloe bisexualis		6.2	0.89	2.43	3.30	0.61	1	5.82	1	1.22	0.96	1	n	n	n	0	n	n	n	n	n
Patosia clandestina	F	16.9	1.06	9.07	16.52	n	n	n	n	1.25	0.69	1	5.7	n	2.85	0	n	n	n	0	0
	M	5.8	1.05	2.83	3.65	0.58	1	14.11	1	n	n	n	n	n	n	n	n	n	n	n	n
Prionium serratum		3.2	1.13	2.32	2.31	0.86	1	0.58	1	0.78	11.44	1	2.1	0.66	1.24	0	8.3	0.92	3.54	0	0
Rostkovia magellanica		6.9	1.07	3.89	3.70	0.94	1	1.30	1	1.58	0.65	0	5.5	0.88	1.38	0	8.3	1.44	1.65	0	0

Species	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Distichia acicularis	2	0.7	1.2	0	n	0	0	0	0	1	0	0	0	0	0	n	0	0	0	0	0	1	0	0	0	0
Distichia muscoides	2	0.7	1.4	0	n	0	0	0	0	1	0	0	0	0	0	n	0	0	0	0	0	1	0	0	0	1
Juncus articulatus	2	0.2	1.7	0	n	0	1	1	0	1	0	1	0	0	0	n	0	0	1	1	1	1	1	0	0	0
Juncus bufonius	0	0.6	2.0	0	1	0	0	0	0	0	n	n	0	0	0	0	1	0	0	0	0	1	0	1	0	0
Juncus bulbosus	1	0.6	1.3	0	0	0	1	1	0	1	1	1	0	0	0	0	0	0	1	1	1	1	1	0	1	0
Juncus caespiticius	0	1.3	n	0	1	0	0	0	0	0	n	n	0	0	0	0	0	0	n	0	0	1	n	0	0	0
Juncus capillaceus	0	0.6	1.6	0	1	0	1	0	0	0	n	0	1	0	1	0	1	0	0	0	0	1	0	1	0	1
Juncus cyperoides	0	5.1	40.8	1	1	0	0	0	0	0	n	0	1	0	0	0	0	0	1	0	0	1	1	0	1	0
Juncus ecuadoriensis	2	3.2	n	0	n	0	1	1	0	1	0	1	0	0	0	n	0	0	1	1	1	1	1	0	0	0
Juncus ensifolius	2	5.7	6.6	1	n	1	1	1	0	1	1	1	0	0	0	0	0	0	1	1	1	1	1	0	0	0
Juncus gerardii	0	0.9	n	1	1	0	0	0	0	0	n	0	1	0	1	0	1	0	0	0	0	1	0	1	0	0
Juncus maritimus	2	2.0	1.2	0	n	0	1	0	1	0	n	0	1	1	0	n	1	1	0	1	1	0	0	0	0	1
Juncus squarrosus	1	1.1	1.3	1	0	0	1	0	0	0	n	n	0	1	0	0	1	0	0	0	1	1	0	1	0	1
Juncus subulatus	2	1.6	1.2	0	n	1	1	0	1	1	0	0	0	1	0	n	0	0	1	1	1	1	0	0	0	1
Juncus subulitepalus	2	4.3	n	0	n	0	1	1	0	1	0	1	0	1	0	n	0	0	1	1	1	1	1	0	0	0
Juncus trifidus	0	0.5	1.4	1	0	0	0	0	0	0	n	n	0	0	0	0	0	0	0	0	0	1	0	0	1	1
Juncus triglumis	1	0.6	1.4	0	0	0	0	1	0	1	1	1	0	0	0	0	0	0	1	1	1	1	1	0	1	0
Juncus xiphioides	2	8.8	9.6	1	n	1	1	1	0	1	1	1	0	0	0	0	0	0	1	1	1	1	1	0	0	0

Table A3 Anatomical data

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Lah	P	AK	(ontinued
140	10	110	Commuca

Species	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66
Luzuola arcuata	0	2.7	11.2	1	1	0	0	0	0	0	n	0	1	0	0	0	1	0	n	n	n	n	n	n	n	n
Luzula campestris	0	5.4	26.0	0	1	0	0	0	0	0	n	0	1	0	0	1	1	0	0	0	0	1	0	0	0	0
Luzula gigantea	0	16.0	72.2	0	1	0	0	0	0	0	n	0	1	0	1	1	1	0	0	0	0	1	0	0	0	1
Luzula johnstonii	0	7.8	39.8	0	1	0	0	0	0	0	n	0	1	0	0	1	1	0	n	n	n	n	n	n	n	n
Luzula luzuloides	0	4.6	25.6	0	1	0	0	0	0	0	n	0	1	0	1	1	1	0	1	0	0	1	0	0	1	1
Luzula multiflora	0	3.7	17.4	0	1	0	0	0	0	0	n	0	1	0	0	1	1	0	0	0	0	1	0	0	0	0
Luzula racemosa	0	2.8	8.8	0	1	0	0	0	0	0	n	0	1	0	0	1	1	0	0	0	0	1	0	0	0	0
Luzula spicata	0	2.5	8.3	0	1	0	0	0	0	0	n	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0
Luzula sylvatica	0	7.4	47.3	0	1	0	0	1	0	0	n	0	1	0	0	1	1	0	0	1	0	1	1	0	1	1
Marsippospermum grandiflorum	1	2.1	1.0	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	1
Oxychloe andina	2	1.4	1.3	0	n	0	0	0	0	1	0	0	0	0	1	n	0	0	0	1	0	1	1	1	0	1
Oxychloe bisexualis	2	1.3	1.1	0	n	0	0	0	0	1	0	0	0	0	1	n	0	0	0	1	0	1	0	0	0	1
Patosia clandestina	2	0.8	1.2	0	n	0	0	0	0	1	0	0	0	0	1	n	0	0	n	n	n	n	n	n	n	n
Prionium serratum	0	n	n	1	0	0	0	0	0	0	n	0	1	0	0	0	1	0	n	n	n	n	n	n	n	n
Rostkovia magellanica	0	1.1	2.2	0	0	0	1	1	0	0	n	0	1	0	1	0	1	0	0	0	0	1	0	0	0	1
Species	67	68	69																							
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Distichia acicularis	1	1	n																							
Distichia muscoides	1	1	n																							
Juncus arcticus	n	n	n																							
Juncus articulatus	1	0	n																							
Juncus bufonius	0	n	n																							
Juncus bulbosus	1	0	0																							
Juncus caespiticius	0	n	n																							
Juncus capillaceus	1	0	1																							
Juncus compressus	1	0	1																							
Juncus cyperoides	0	n	n																							
Juncus ecuadoriensis	1	0	n																							
Juncus ensifolius	1	0	n																							
Juncus gerardii	1	0	0																							
Juncus inflexus	n	n	n																							
Juncus maritimus	0	n	n																							
Juncus planifolius	0	n	n																							
Juncus squarrosus	1	0	0																							
Juncus stygius	1	0	n																							
Juncus subulatus	1	0	n																							
Juncus subulitepalus	1	0	n																							
Juncus trifidus	1	0	0																							
Juncus triglumis	1	0	1																							
Juncus xiphioides	1	0	n																							
Luzula arcuata	0	n	n																							
Luzula campestris	0	n	n																							
Luzula gigantea	0	n	n																							
Luzula johnstonii	0	n	n																							
Luzula luzuloides	0	n	n																							
Luzula multiflora	0	n	n																							
Luzula nodulosa	0	n	n																							
Luzula racemosa	0	n	n																							
Luzula spicata	0	n	n																							
Luzula sylvatica	0	n	n																							
Marsippospermum grandiflorum	1	0	0																							
Oxychloe andina	1	1	n																							
Oxychloe bisexualis	1	1	n																							
Patosia clandestina	1	1	n																							
Prionium serratum	0	n	n																							
Rostkovia magellanica	1	0	1																							
Thurnia sphaerocephala	0	n	n																							

Table A4 Data from Chapter 4 Auricles or ligules?



Figure A1 Histograms of continuous morphological characters





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Figure A1 Continued



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Appendix A



Figure A2 Strict consensus of 36 maximum parsimony trees based on rps16 and trnL-F intergenic spacer. Numbers above branches indicate bootstrap values. Tree length 3008, CI = 0.63, RI = 0.82.



Figure A3 Strict consensus of maximum parsimony trees based on ITS. Numbers above branches indicate bootstrap values. Tree length 1048, CI = 0.53, RI = 0.78.

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