A guide to the identification of Irish mammal hair

Irish Wildlife Manuals No. 92

An Roinn Ealaíon, Oidhreachta,
Gnóthaí Réigiúnacha, Tuaithe agus Gaeltachta

Department of Arts, Heritage,
Regional, Rural and Gaeltacht Affairs

A guide to the identification of Irish mammal hair

A. Cornally and C. Lawton

Zoology, School of Natural Sciences, Ryan Institute,

National University of Ireland, Galway

Citation:

Cornally, A. and Lawton, C. (2016) A guide to the identification of Irish mammal hair. *Irish Wildlife Manuals*, No. 92. National Parks and Wildlife Service, Department of the Arts, Heritage, Regional, Rural and Gaeltacht Affairs, Ireland.

Keywords: Irish mammals; Hair; Identification key; Cuticular hairs; Cross-sectional shape.

Cover photo: © A. Cornally

The NPWS Project Officer for this report was: Dr Ferdia Marnell; ferdia.marnell@ahg.gov.ie

Irish Wildlife Manuals Series Editors: F. Marnell & R. Jeffrey

ISSN 1393 – 6670

Contents

Executive Summary

The dorsal guard hairs of Irish mammals were examined to reveal diagnostic features that could be used to create a dichotomous key with a photographic reference system. The key was developed using clearly recognisable characters of cuticular scales along with the shape of cross sections, which were observed from the thickest part of the shield. The techniques employed in this study are relatively simple and can be easily, quickly and economically applied in routine investigations making it suitable for undergraduate research students, wildlife officers and non-specialists.

Acknowledgements

Thanks to a number of colleagues who contributed hair samples, especially Conor Kelleher, Neil Reid and Anthony Caravaggi. We were helped by several colleagues in the Animal Ecology and Conservation Unit in Zoology, NUI Galway; thanks to Eoin MacLoughlin for assistance in the field, Erica de Milio for help with photography, and Margaret Flaherty and Dariusz Nowak for assistance with processing the images. This study received funding from the National Parks & Wildlife Service of the Department of Arts, Heritage, Regional, Rural and Gaeltacht Affairs.

Introduction

Non-invasive monitoring

Monitoring wild animal populations is necessary for management and conservational purposes. It enables a greater understanding of the distribution, extent and status of populations, thereby facilitating the protection of threatened or rare species. Population surveys can be carried out using direct or indirect techniques. The use of direct techniques, such as the trapping of live mammals, requires knowledge, training and in many cases a licence (Bertolino *et al.*, 2009). Live trapping is useful for gathering data for population abundance estimates, whilst also providing key information on life history characteristics including age, gender, and reproductive condition. Traps such as the Sherman live trap and the British Longworth trap are popular choices when capturing small land mammals. However, live traps are often limited in terms of time and cost, and can be quite labour intensive (Bertolino *et al.* 2009). Gannon and Sikes (1998) advised that traps should be checked regularly to prevent trapped animals becoming stressed or injured. There are some risks involved when handling wild animals, and it is imperative that the researcher ensures precautions are taken to prevent bites or contamination from urine or ectoparasites (Gannon and Sikes, 1998).

Non-invasive techniques for surveying populations have been developed to minimise interfering with the natural activity of wild animals; these methods are especially important when surveying rare or elusive species. Non-invasive techniques include direct observations of the animals and other indirect methods, such as searching for tracks and signs of animals. The surveys often follow a pre-determined route or transect, or can involve the use of monitoring devices such as footprint tunnels, motion sensor cameras or hair-tubes. Searches for faeces or scats are often used, as many mammal species leave easily found, diagnostic droppings. Ruell *et al*. (2009) observed that 'scat transects' are restricted when studying species that have an uneven distribution or low population density. Sileira *et al.* (2003) found the use of recording devices such as camera trapping to be useful when studying the distribution and abundance of populations; however, they noted that this method is quite expensive initially. Often only a small number of cameras are available, and so only a small area can be examined at once, and observations rely on an element of chance.

The use of hair tube tunnels is another indirect, non-invasive technique used to study wild populations of small mammals. This is a useful method as it is inexpensive, requires little expertise and can be used to cover large areas in replicate studies in a timely manner (Bertolino *et al.* 2009). Traps can be left out for days or weeks on end and the tube can be designed to detect a specific cohort of animals, or to gather hairs from several individuals from various species. The hair tube tunnel is equipped with an adhesive substance (usually tape or glue) and food bait. The animal investigates the baited tunnel, during which the dorsal guard hairs of the animal are collected on the adhesive substance. The hairs are extracted from the tubes and the species concerned identified. If sufficient records are gathered, an index of the species' abundance can be calculated for comparison between sites (e.g. Goldstein *et al.* 2014). For larger animals a spring mechanism can be used to collect hairs, which has the advantage of allowing a single detection only, and therefore the identification of the individual through molecular techniques (Sheehy *et al.* 2013). Ausband *et al.* (2011) conducted a study in which a lure was used to attract coyotes and wolves to hair corrals and barbed wire posts; the lure

also encouraged rubbing behaviour, resulting in a hair sample being deposited by the animals. Hair samples can also be gained from dens and burrows, or snagged on barbed wire, and can also be the only means of identifying badly decomposed samples or roadkill. The identification of mammals using hair is further of use when carrying out dietary analysis of carnivore faeces or raptor pellets, particularly where diagnostic bones of the prey are not present. The examination of hair has been used in animal research in order to gather information on distribution, relative abundance, density, and translocation or reintroduction success (Waters and Lawton, 2011). Due to the benefits when working with an elusive or protected species, hair sample analysis is increasingly being used in conservation management projects. It has also been incorporated in studies of habitat occupancy, dietary composition of mammals and population genetics (Menike *et al.* 2012).

The structure of hair differs among species and has been widely developed as an adaptation to their environment; it can therefore be used to identify animals at a species level (Menike *et al.* 2012). However, the determination of species from hair samples is not always a straightforward process as only limited information can be gained from the colour and dimensions of the hair. Practitioners need to develop experience in the identification of hair, including understanding the different characteristics that could potentially cause confusion in differentiating between closely related species. Knowledge of the potential species encountered in an area, together with information on the means by which the sample was gained help to further inform the identification of the hair (Lobert *et al*. 2002).

There are two main types of hair; the under hair and the over hair. The over hair, often referred to as the guard hair, is the vital element in identifying species. They are usually long and stiff and consist of two main parts, a shaft (towards the base of the hair) and a shield (towards the tip of the hair). The under hair are usually slender and undulating and have no taxonomic relevance. There are three categories of guard hairs (GH0, GH1 and GH2), of which GH1 and GH2 are the most useful for identification purposes. GH1 hairs are usually straight and stiff while GH2 hairs are bent at the transition from the shaft to the shield (Figure 1). The overall appearance of the hairs can give some early indication of the source, based on hair colour and length. To identify to species level, microscopic examination of hairs is required to reveal the scale pattern of the cuticle and cross-sectional shape of the hairs. Cuticular scale patterns of both GH1 and GH2 hairs can be unique to species and subspecies. The shape of cross-sections can differ among species but also along the hair strand; investigations of the cross-sections should be taken from the thickest part of the shield (Teerink 1991).

Teerink's *Hair of Western European mammals: atlas and identification key* (1991) is an excellent resource, giving detailed information on a broad range of European species; it is an important text within this field of wildlife and conservation ecology. The book is quite technical, however, and contains many species not found in Ireland. The aim of this *Irish Wildlife Manual* is to provide a reference key and guide to identifying the hair of Irish mammals that is accessible to individuals with a certain amount of background knowledge, but without the specific training or considerable resources that may otherwise be required. Samples were gathered from a variety of sources to build a bank of hair from the Irish mammal community. Certain samples were available from previous ecology studies conducted in NUI Galway. Others were donated from other research institutes and further samples were collected using Longworth traps. Hair tubes of various dimensions were used to test the key against samples collected in the field. The domestic cat and dog were included in this study along with the coypu; a large rodent foreign to Ireland, which has been recorded here in recent years (Colette O'Flynn, National Biodiversity Centre, pers. comm.). The resulting key and methods used in

this study will be easily applicable for routine studies carried out by non-specialists such as early-stage undergraduate students, amateur naturalists and environmental stakeholders.

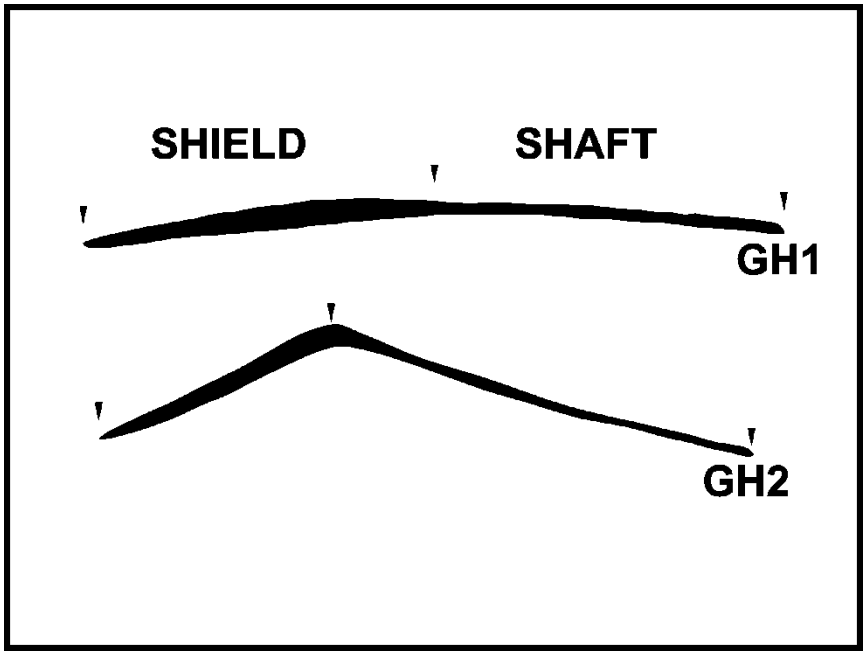


Figure 1. General shape of Guard Hairs one (GH1) and two (GH2), distinguished by the bend in GH2 where the shield and shaft meet. The shield is closest to the tip of the hair, and the shaft is towards the animal's body. GH1 shows the enlarged central portion of the shield, as evident in shrews (adapted from Teerink, 1991).

Irish mammal species

Insectivores

There are three species of primarily insectivorous mammals in Ireland (not including the bats, see below): The European hedgehog (*Erinaceus europaeus*), pygmy shrew (*Sorex minutus*) and greater white toothed shrew (*Crocidura russula*).

The European hedgehog has an overall greyish-brown appearance. Its coat consists of thickened spines which are dark brown with a pale tip. The hedgehog raises these spines when under threat as a form of defence. Its head and abdomen are coated by coarse grey hairs. Hedgehogs were introduced to Ireland, probably by the Normans, but have been present here since at least the 13th century. As a hibernating species, they will not usually be encountered between November and March. They are found across a wide range of habitats; they are prevalent in deciduous woods but have become quite common in suburban areas in recent years.

The pygmy shrew is the smallest mammal in Ireland, weighing approximately 4-6g (Harris and Yalden, 2008). Pygmy shrews are covered in dark grey fur which is lighter along the flanks and underbelly. The tail is of medium length relative to its size and is sparsely covered in fur. Their fur is velvet-like in appearance and they moult in early summer to grow a thicker coat in autumn before the winter season sets in. The pygmy shrew is common throughout Ireland and tends to favour areas with

dense vegetative cover, thereby providing ideal feeding grounds and protection. These areas include hedgerows, grasslands, woodlands and peatlands (Hayden and Harrington, 2000).

The greater white toothed shrew (GWTS) has only recently arrived in Ireland. It was first encountered in barn owl pellets and then in kestrel pellets in counties Tipperary and Limerick in 2008 (Tosh *et al.* 2008). Its presence was later confirmed by live trapping. It is currently believed that the greater white toothed shrew will have a negative impact on the prevalence of Ireland's native pygmy shrew. The pygmy shrew has been found to be absent in the presence of the greater white toothed shrew (McDevitt 2013). The coat of the GWTS is brown on the dorsal surface with a duller yellowish grey on the ventral side. Its long tail is covered in white hair. They can be found in a range of habitats, such as woodland, hedgerows, grassland and areas surrounding human settlements (Harris and Yalden, 2008).

Rodents

There are seven species of rodent in Ireland (and potentially two others); most or all were introduced to Ireland, some very recently. There are two squirrel species, the red squirrel (*Sciurus vulgaris*) and grey squirrel (*Sciurus carolinensis*). Red squirrels have been present in Ireland since the last ice age, but have probably gone extinct and been reintroduced on a number of occasions. Grey squirrels were introduced much more recently in 1911 (Lawton *et al*. 2015). As their names suggest, the hair on the two species is a different colour, however there is considerable variation in hair colour, with greys often taking on a reddish tinge on the dorsal surface, particularly in summer, and reds sometimes appearing quite grey. The ventral side of both species is white in colour. Red squirrels usually (but not always) have long ear tufts, which are typically red, but can be cream in colour. Squirrels are always found in or near wooded areas, although grey squirrels in particular can persist in urban parks and gardens.

The wood mouse (*Apodemus sylvaticus*) is one of the more common mammals in Ireland and has been present on the island for at least 8000 years, although it may have arrived with the first human settlers. It has chestnut brown fur covering its dorsal surface and whitish-grey fur on its underbelly. A yellow-brown mark is generally present on the throat. Juvenile wood mice, however, are grey, and can be confused with house mice. The optimal habitat for the wood mouse is woodland, but they can occur in all habitats, provided the ground is not saturated. House mice (*Mus domesticus*) are found throughout the world, associated with human dwellings. They struggle to compete with other small rodents such as the wood mouse, and so tend to be confined to buildings and the immediate surroundings. Fur colour can vary considerably between individuals but is generally a greyish-brown colour, with a slightly lighter ventral colour, but not as distinctive as in other species.

Two rat species are considered present in Ireland, however the only recent records of black rat (*Rattus rattus*), come from Lambay Island, Co Dublin (National Biodiversity Data Centre records). They have steadily disappeared since the 18th century introduction of the brown rat (*Rattus norvegicus*). Brown rats' coats vary in colour, but are usually a greyish brown with a paler grey ventral surface. Like the house mouse, brown rats are often associated with human settlements, but they can be found in a range of habitats including woodland, farmland and coastal areas.

The bank vole (*Myodes glareolus*) was first discovered in Ireland by Claassens and O'Gorman (1965). It continues to expand its range, and currently is found in much of the west and south of Ireland. Its fur

is usually chestnut brown with a greyish underbelly. They generally inhabit areas where there is dense undergrowth, such as woodlands with a thick shrub layer, and can be found in young coniferous woods.

Another recent introduction is the hazel dormouse (*Muscardinus avellanarius*), which was first sighted in Ireland in 2010 in county Kildare (Marnell *et al.* 2015). It is a small mouse-sized rodent that lives in thick scrub, young woodlands and dense hedgerows. They have a long hibernation, and are typically only seen between the months of April and October. They are covered in orange-brown fur with a paler coloured underbelly. Their tail is much longer and bushier than either of the mice species.

The coypu (*Myocaster coypus*) are generally not considered a part of Ireland's fauna, however they have been recorded previously and could appear again. They are large, rat-like rodents, which are often associated with waterbodies. The hairs of the coypu are long and dense with guard hairs on the dorsal side reaching a length of 80mm. The hair is brown and yellow on the dorsal surface and grey on the ventral side. They have noticeably long vibrissae, reaching up to 130mm in length.

Carnivores

There are seven carnivores in Ireland; six mustelids, and the red fox (*Vulpes vulpes*). The red colour of foxes is due to long guard hairs, which range from yellowish brown to a deep red. The shorter ventral fur has a grey colour. They have a long bushy tail with a white tip. Foxes are opportunistic hunters and have adapted to most habitats in Ireland, including quite successfully in urban landscapes. The pine marten (*Martes martes*) has a rich chocolate brown to black coat with a creamy white patch or bib at the throat. It predominantly inhabits forests and is currently in a period of recovery following a retraction in its previous range. They are found in relatively high numbers in their core range in the midlands and west of Ireland (Sheehy *et al.* 2013), but can be found throughout the island of Ireland.

The Irish stoat (*Mustela erminea hibernica*) is regarded as a separate subspecies from stoats elsewhere. The coat of the Irish stoat is covered in reddish-brown fur with a creamy white on the throat and underside, and a black tip to its tail. Unlike other subspecies of stoat the Irish stoat does not have a white coat in winter. They are an adaptable and opportunistic species and so are found in a range of habitats, although they prefer woodland and scrub cover.

The American mink (*Mustela vison*) was introduced into Ireland on a number of occasions via escapees from fur farms. Although captive mink are bred to create a variety of colour morphs, wild forms are a typical dark-brown or black colour, with a small white patch on the throat. They are semi-aquatic and prefer slow-flowing water with thick cover along the banks.

Otters (*Lutra lutra*) are another, more highly derived, semi-aquatic carnivore. They are large animals, growing up to 1m in length, with thick brown fur. They have a dense layer of a lighter under-fur, and the guard hairs serve to trap air when they dive, insulating them from the cold water temperatures.

Badgers (*Meles meles*) have a very distinctive colouration, with two black stripes running from the muzzle to the ears on a white face. The fur on the back appears grey to silver because the long guard hairs on the back and sides are white and have a tip with a dark band in the middle. The hairs on the legs and belly are quite dark in colour. Badgers often inhabit woodland and farmland mosaics and are nocturnal so are rarely encountered during the day.

Ungulates

There are three deer species in Ireland: the native red deer (*Cervus elaphus*), and two introduced species, fallow deer (*Dama dama*) and sika deer (*Cervus nippon*). The red deer is Ireland's largest wild herbivore. In the summer months, its coat has a reddish brown colour but may vary from dark brown to speckled grey. New born calves have beige spots along the flanks. In the winter the coat appears darker in colour and the stags grow a dark bushy mane. Red deer are usually found in young coniferous forests, particularly open thickets. Red deer can form hybrids with sika deer where they co-occur, which can make identification problematic. Sika deer are considerably smaller than red deer. The sika deer's coat differs depending on the season. During the summer months the coat is spotted and ranges from a dark to light brown with a dark central stripe down the back. During autumn the coat is moulted to produce a grey to almost black winter coat. Stags grow a shaggy mane on the chest during the rutting season. The tail is short and broad and is white on top and naked underneath. Sika deer inhabit mosaics of open spaces within forests and dense thickets.

The fallow deer is the most widespread species of deer found in Ireland. It was introduced to Ireland with the arrival of the Norman invaders in the 13th Century. They can be found in different colour morphs ranging from black to a yellowy white. All apart from the black and white deer have a distinctive white heart shaped rump that has a dark outline. Fallow deer inhabit deciduous and mixed woodland, preferring mosaics of open pastures and woodland. Most recently muntjac deer (*Munticus reevesi*) have been reported from the wild in a number of locations in Ireland (Dick *et al*., 2010), although it is not clear if they have established a breeding population or how widespread they are. Another wild species of ungulate in Ireland is the feral goat (*Capra hircus*), once of domestic origin and now reverted back to the wild. As with domestic goats, there is considerable variation in coat colour, pattern and shade, making any description redundant. They usually live in hilly regions and can be found sheltering in wooded areas and scrub.

Lagomorphs

Like the stoat, the Irish hare (*Lepus timidus hibernicus*) is an endemic subspecies, unique to Ireland and surrounding islands. They have a reddish brown coat that does not change colour during the winter, as can be the case with other subspecies of the Arctic hare. The tail usually has a white upper surface. Brown hares (*Lepus europaeus*) are confined to certain areas of Northern Ireland, where they were introduced for coursing. Their coat colour is very similar to that of the Irish hare, although they do have a dark tail. Irish hares live in a much broader range of habitats than Arctic hares do elsewhere, but both the Irish and brown hares are most commonly seen in grasslands.

Rabbits (*Oryctolagus cuniculus*) are greyish brown in colour, but some range from various shades of grey to black. Rabbits were introduced into Ireland by the Normans. They are an opportunistic species and inhabit many areas from the coast to uplands. They have a preference for areas of short grasses with cover and refuge areas such as bramble patches or gorse (Harris and Yalden, 2008).

Bats

There are 9 species of bat found in Ireland, split into two families, the Rhinolophidae and the Vespertelionidae. Other species make occasional appearances, but are not considered resident. Bats are nocturnal and use echolocation to seek insect prey. They are typically identified using a bat

detector, which picks up the high pitched frequencies used when hunting. It is difficult to identify most Irish bats based on appearance alone.

The lesser horseshoe bat (*Rhinolophus hipposideros*) is a member of the family Rhinolophidae and is one of Ireland's smaller species. They tend to roost in caves or in buildings such as the attics of big houses, or in stables and barns (Roche *et al.*, 2014). When hunting they fly in low circles and can manoeuvre through dense vegetation.

All other bat species belong to the family Vespertelionidae. There are three species of pipistrelle in Ireland: the common pipistrelle (*Pipistrellus pipistrellus*), soprano pipistrelle (*Pipistrellus pygmaeus*), and Nathusius' pipistrelle (*Pipistrellus nathusii*). The common pipistrelle prefers to roost in confined spaces, frequently in buildings and tends to forage around hedgerows and the edges of woodlands and parks. The soprano pipistrelle has recently been distinguished from the common pipistrelle based on a higher frequency of call. The face is noticeably darker than that of the common pipistrelle, although it takes a certain amount of experience to distinguish between the two species. In Ireland, sopranos are the more common of the two species, and are more frequently found hunting near hedgerows with some trees. The Nathusius' pipistrelle has quite a restricted range, most commonly detected in the north-east of Ireland (Roche *et al.*, 2014).

The Leisler's bat (*Nyctalus leisleri*) is the largest species of bat found in Ireland. It has limited manoeuvrability and catches all its prey in flight. Their preferred foraging areas include open deciduous and coniferous forests, scrub areas and in light density urban areas near street lamps (Roche *et al.*, 2014). The whiskered bat (*Myotis mystacinus*) is widespread in Ireland but is relatively rare. As its name suggests, it has long fine vibrissae on its face. They prefer foraging in forest areas and along hedgerows.

The Daubenton's bat (*Myotis daubentoni*) is usually found hunting over water. They are widely distributed in Ireland in small colonies close to water bodies, often making their roosts under bridges. The Natterer's bat (*Myotis natterei*) is also widely distributed but has a low density with roosts generally containing less than five members and is a rare species in Ireland (Roche *et al.*, 2014). It occurs most frequently in woodland areas and nests in trees.

Finally, the brown long-eared bat (*Plecotus auritus*) is quite distinctive with its characteristic long ears, which can measure up to 25mm in length. They have a slow, fluttering flight and usually forage in woodland and scrub areas.

Methods

The structure of guard hair was examined in twenty seven different mammal species found throughout Ireland: European hedgehog, pygmy shrew, wood mouse, bank vole, red fox, American mink, badger, pine marten, Irish stoat, European otter, red squirrel, grey squirrel, rabbit, Irish hare, Brown hare, fallow deer, the domestic dog *Canis familaris*, domestic cat *Felis catus* and the nine resident bat species. The hair samples utilised were gathered from several sources. Certain samples were already present in storage in NUI Galway from previous ecological studies. Others were donated from other institutions and some were gathered using Longworth traps for small mammals. Information for hair not included in our samples was taken from Teerink (1991).

The hairs samples were taken from the dorsal region of the body. De Marinis and Asprea (2006) noted that hairs from other body regions show similar characteristics to dorsal hair, but are often less distinct making identification more problematic. The colour and length of hair was recorded from each specimen. Hair colour and length are variable characters and their usefulness in an identification key is limited. For the most part, species identification is not possible solely on these parameters, however it can be used in combination with other features.

Several hair tube types were deployed to test the resulting key against samples gathered in the field. Some modifications were made to the identification key based on these observations.

Hair tube design

A hair tube is any device deployed with the intention of collecting animal hair in the field. The choice of design, the bait used and the location in which the hair tubes are set will all influence the animals that are detected by the hair tubes. The type of hair tube deployed must be dictated by the animal under investigation. If a general overview of mammal species present in an area is required, it may be necessary to deploy several designs in order to avoid influencing the animals positively detected.

For most species, hairs can be captured from animals visiting the hair tube by using a sticky substance. A strong adhesive glue can be applied to a rubber band stretched across the entrance to the tube (McAney 2011), or a sticky tape can be attached to the upper surface of the tube entrance or to removable blocks that fit on to the tube (Flaherty 2016). Polyethythene duct (gaffer) tape or other hardware tape has proved effective as it retains its sticky nature in the outdoor environment. For larger animals, spring-based traps can be set, which collect a sample of hair when a stretched spring closes after an animal comes into contact with it.

Hair tube dimensions vary depending on the species under investigation; the tube needs to be large enough for the animal to enter, but tight enough so that their dorsal hair will come in contact with the hair trapping device. A number of squirrel surveys have utilised hair tubes to cover a relatively large area or several areas with limited resources. The tubes are usually square in cross section, 300-mm lengths of 65x65 mm diameter PVC pipe, with removable wooden blocks attached at either entrance (e.g. Goldstein *et al.* 2014, Sheehy & Lawton, 2014). The same tubes can be used when sampling for brown rats (Figure 2). Round PVC tubes of 118 mm diameter have been used to detect pine marten (Mullins *et al.* 2010) and tubes of 52 mm diameter are used in stoat studies (McAney 2011). While collecting hair from small mammals in the current study we found no significant difference in the

number of hair samples collected using 25mm or 35mm diameter pipe. Samples were successfully collected from bank voles and wood mice with these tubes (Figure 3).

A variety of bait types can be used, with many of Ireland's mammals being omnivorous to a certain extent. Unsalted peanuts and/or porridge oats are useful baits for small rodents; squirrels also like peanuts, and also hazelnuts and maize (Waters & Lawton, 2011). Rabbit and chicken is suitable for stoat studies (McAney 2011), and pine marten investigations have also used these meats, dipped into marmalade as an extra element to draw the animal to the trap (Mullins *et al.* 2010, Sheehy *et al.* 2013). The best bait to use if the pygmy shrew is the target animal is mealworm.

Tube location can also have a big influence on the relative success of a hair tube survey, and care should be taken to choose the most likely habitat in which the animal may be found in an area, and in the placement of the trap itself. The tubes should be attached at about head height for arboreal animals such as squirrels and pine marten. Ground dwelling animals are usually more likely to investigate tubes that are concealed in cover, and placed up against the base of a tree, a hedgerow or a wall. The tube should be anchored in place using tent pegs, metal hoops and/or cable ties.

Figure 2. 65x65 mm hair tubes, with attached wooden blocks set for the detection of brown rats

Figure 3. 25 mm hair tubes set for the detection of small mammals

Hair cuticle examination

The cuticle makes up the outermost layer of hair and consists of overlapping scales. These scales can be in various patterns that are diagnostic for species and are therefore valuable for identification purposes. The cuticle was analysed based on four properties; the position of the scales in relation to the longitudinal axis of the hair, the structure of the scale margin, the distance between scales and the scale pattern. For the most part our guide was based on the scale pattern and its position on the hair.

It was difficult to observe the cuticular scale pattern directly without the use of an electron microscope, therefore the hairs required special preparation in order to view the cuticular scale pattern under a light microscope. The samples were prepared by creating a gelatine cast imprint of the hair.

A 10-20% gelatine stock solution was prepared by adding 15g of gelatine granules (widely available in supermarkets) to 100ml of heated distilled water. The mixture was heated to approximately 60°C and stirred constantly until the granules dissolved fully. A thin layer of gelatine was applied to the slide by pouring the stock down the slide which was held tilted downwards. Any gelatine remaining under the slide was wiped away. Excess run-off gelatine was collected and reused. Strands of hair were placed side by side on the slide using a pair of tweezers. A previous study conducted in the Mammal Ecology Group of NUI Galway (Quinn 2005) found that a better imprint of the cuticular scales formed when the hairs were placed on the slide immediately after the gel had been poured, rather than allowing the gel to set for 5 minutes as suggested by Teerink (1991). Pressure was applied to any hairs

that were long as they had a tendency to rise out of the gel; smaller hairs did not require pressure as they sank easily into the soft gel. The hairs were left overnight and then the following morning they were removed from the set gel using fine tweezers. The imprint of the cuticular scales in the gel cast was examined under a light microscope.

Cross-section examination

The cross-sectional shape of the hair varies along the length of the shaft, but the greatest contrast can be detected between species at the thickest part of the shield.

A stand was created using modelling clay to support a slide so that it stood vertically. A thin layer of super glue was spread over the upper edge of the slide and left for a few seconds to dry in order to hold the hair strand in position. The thickest part of the shield was placed on the glue, perpendicular to the slide using tweezers. Approximately five to six hairs were placed side by side on each slide. The hairs were left on the glue over night to ensure that they had set properly.

The following morning a fine scissors was used to cut the hairs at both sides of the slide, taking care to cut the hairs horizontally, in order to observe the cross-section. A line was marked on the slide beneath the hair to allow for fast detection. The slides were then viewed under a light microscope to reveal the shapes of the cross-sections.

Results

Key to identifying Irish mammal hairs

The key below is based on various morphological features of the hair, gained through gross examination (length and colour), and microscopic investigation (cuticular scale pattern and cross-sectional shape). On occasion it is necessary to refer back to the gross morphology of the hair after examining the microscopic features. If limited hair is available in the sample, it is therefore important to take note of these elements before preparing slides in case these features are rendered unidentifiable during the process.

1	A	Hairs are 20mm in length or less.	Go to 2
	B	Hairs are greater than 20mm.	Go to 11
2	A	The cuticular scales singular and overlapping (Figure 4).	Go to 3
	B	Cuticular scales are otherwise.	Go to 4
3	A	Normal and 'reversed K' shapes can be seen along the cuticular scales (Figure 5).	Rhinolophidae *Lesser horseshoe bat*
	B	'Reversed K' shapes are absent.	Vespertelionidae
4	A	Hair shaft may be straight, or 'zigzag', with the centre of the shield enlarged (Figure 1). Hairs are usually less than 10mm.	Go to 5
	B	Centre of shield is not noticeably enlarged.	Go to 6
5	A	Cross-section is butterfly or H-shaped (Figure 6 H).	*Pygmy shrew*
	B	Cross-section is not butterfly or H-shaped.	*Greater white-toothed shrew*
6	A	Cross-section is oval.	Go to 7
	B	Cross-section is concave on one or more sides (Fig. 6 D – G).	Go to 8
7	A	Shield has a regular wave pattern. Shaft shows broad diamond petal and elongate petal scale patterns (Figure 7).	*Hazel dormouse*
	B	Hairs are 9-13mm, light-brown with a red hue. Shield shows an irregular wave pattern (Figure 8a). The scales in the shaft show narrow diamond petal scale pattern (Figure 8b).	*Irish Stoat*
8	A	Cross-section is concave on three sides or less.	Go to 9
	B	Cross-section is concave on four sides; longitudinal scales with irregular petal pattern and rippled borders (Figure 9).	*Bank vole*
9	A	Cross-section is concave on one or two sides.	Go to 10
	B	Cross-section is concave on three sides; intermediate broad petal scale pattern, becomes more longitudinal diamond petal towards the tip, with smooth borders (Figure 10).	*Wood mouse*

10	A	Cross-section is oblong with one or both sides concave; longitudinal, broad diamond petal scales, arranged in series of V shapes (Figure 11).	*Brown rat*
	B	Cross-section is concave on one side only, resulting in a kidney shape, with overlapping elongate scales often in a single pattern.	*House mouse*
11	A	Hairs are stiffened and enlarged, becoming spines.	*European hedgehog*
	B	Hairs of regular shape, not specialised.	Go to 12
12	A	Long hair (45-90mm) with white tip, dark band at the middle. Irregular or regular wave pattern on GH1 (Figure 12), oblong cross-section.	*Badger*
	B	Hairs uniformly coloured, or with a different pattern.	Go to 13
13	A	Hairs are fine. Diameter of hair varies throughout the strand.	Go to 14
	B	Hairs are coarse, wavy and undulating. Apart from the tip, the hair strand diameter is constant throughout the strand.	Artiodactyla
14	A	Cuticular scale pattern on shaft usually diamond petal (Figures 13 - 16).	Go to 15
	B	Cuticular scale pattern on shaft is not diamond petal.	Go to 18
15	A	Cross-section is oval.	Go to 16
	B	Cross-section is oblong.	Go to 17
16	A	Hair red-brown in colour, with the shield lighter, or with a lighter section.	*Fox*
	B	Hair chocolate brown in colour.	*Pine marten*
17	A	Hairs are uniformly dark brown to black.	*American mink*
	B	Hairs are black, then white. Cuticular scales show a variable pattern on the shield, diamond pattern elsewhere (Figure 16).	*Otter*
18	A	Bright zone visible in shield. Cuticular scales in the shaft are made up of narrow longitudinal scales (Figures 17 and 18), alternating with shorter scale patterns with undulating margins.	Go to 19
	B	No bright zone in shield. Cuticular pattern otherwise.	Go to 20
19	A	Bottom half of hair is grey, especially when viewed in a clump.	*Rabbit*
	B	Bottom half of hair is white, especially when viewed in a clump.	*Irish hare OR European hare*
20	A	Cuticular pattern shows narrow, longitudinal scales in the proximal and centra shaft. The scales are V-shaped in the distal shaft and proximal shield (Figures 19).	Go to 21
	B	Scales are less elongated in the proximal shield and distal shaft.	Go to 22

21	A	Cross-section concavo-convex or biconcave (Fig. 6 D-E).	*Red squirrel*
	B	Cross-section circular to oval.	*Grey squirrel*
22	A	Oval cross-section.	Go to 23
	B	Oblong cross-section. Cuticular scales show irregular wave in shield and regular in shaft (Figure 20).	*Coypu*
23	A	Cuticular scale pattern alternates between regular and irregular wave pattern (Figure 21).	*Domestic cat*
	B	Cuticular scale pattern shows intermediate mosaic pattern (in part) (Figure 22).	*Domestic dog*

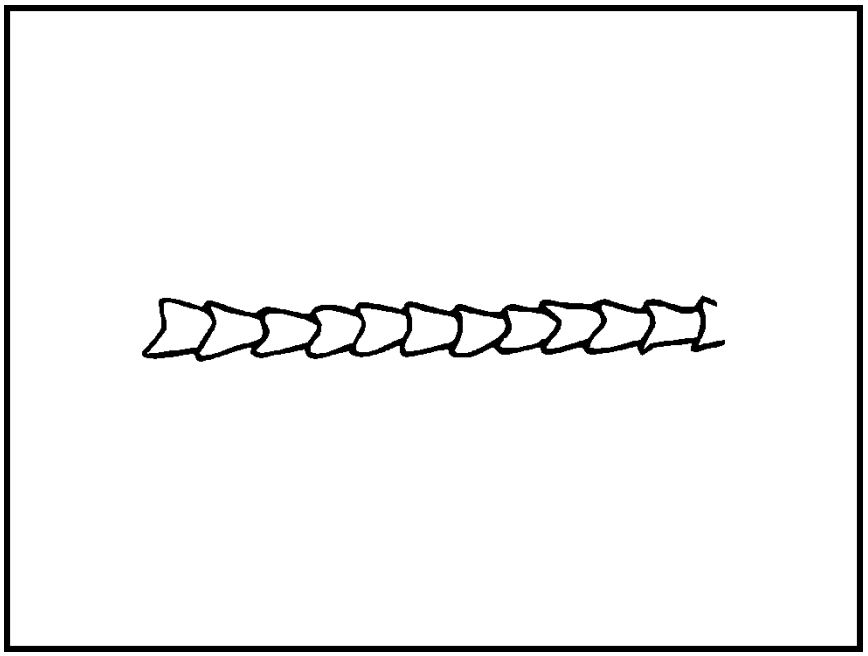


Figure 4. Overlapping single scales, as evident in bat species, most obviously in the shaft portion of the hair (adapted from Teerink, 1991).

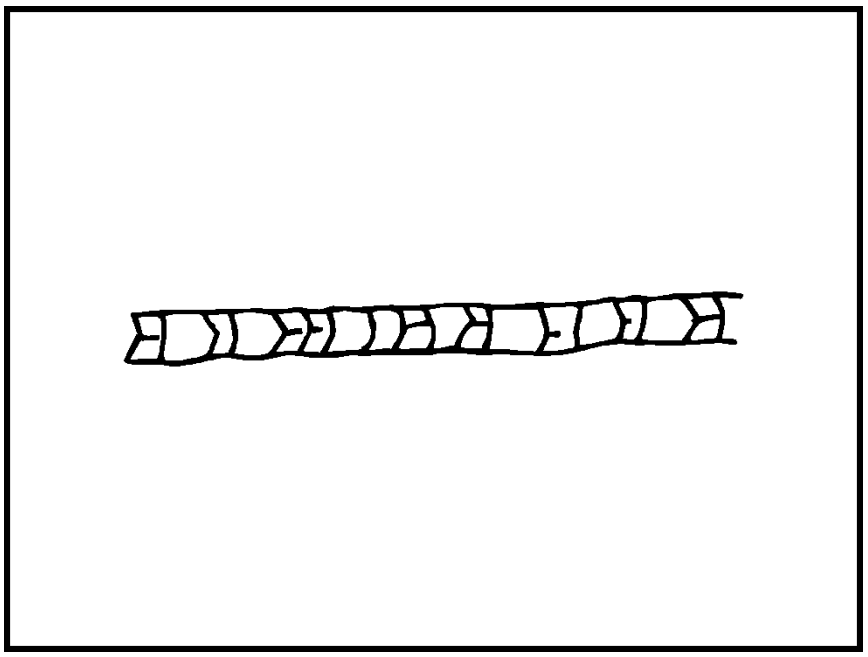


Figure 5. 'Reversed-K' shapes evident along the cuticular scales on the hair of lesser horseshoe bats (adapted from Teerink, 1991).

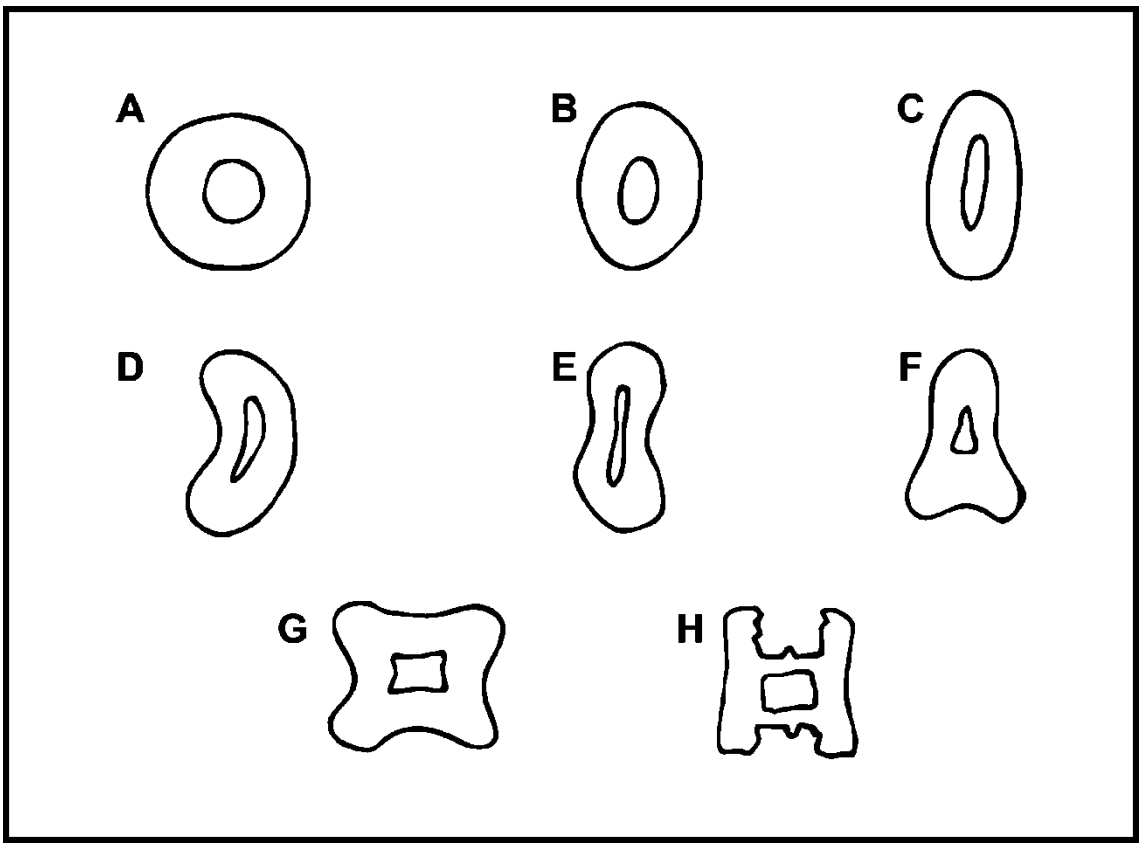


Figure 6. Cross-section shapes can be seen after cutting transversely across the thickest part of the hair shield. Shapes displayed are A) Circular, B) Oval, C) Oblong, D) Concavo-convex (kidney shaped), E) Biconcave (dumbbell shaped), F) Triconcave, G) Quadriconcave and H) H-shaped (Butterfly) (adapted from Teerink, 1991).

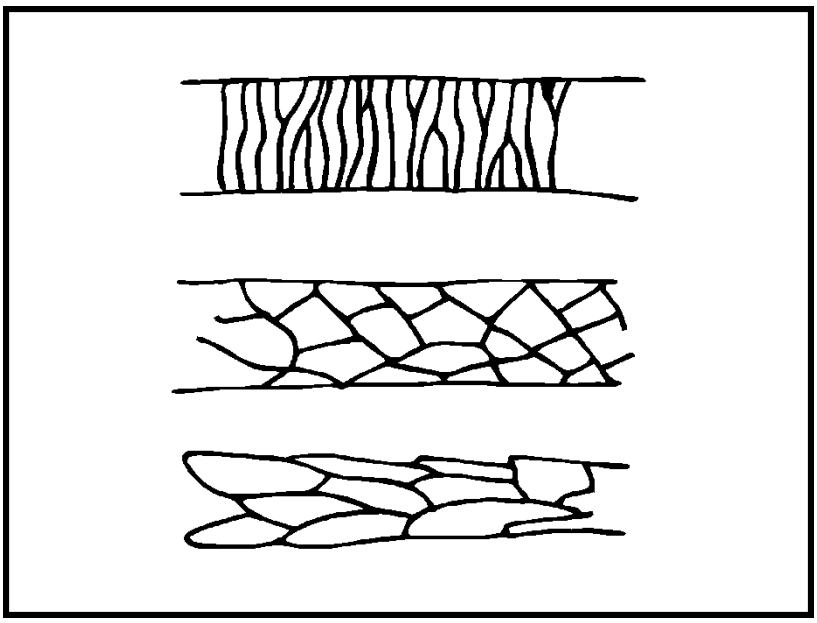
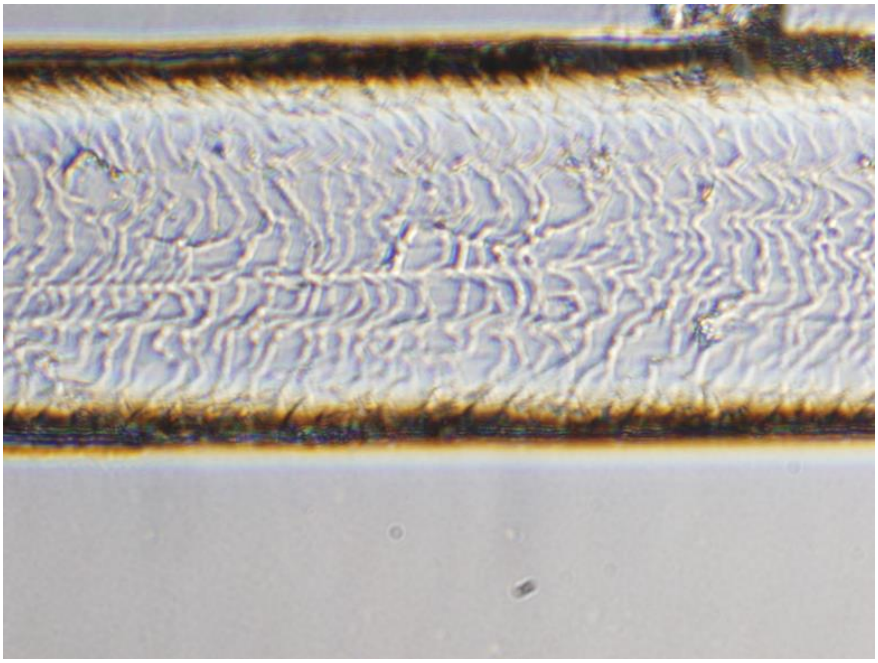
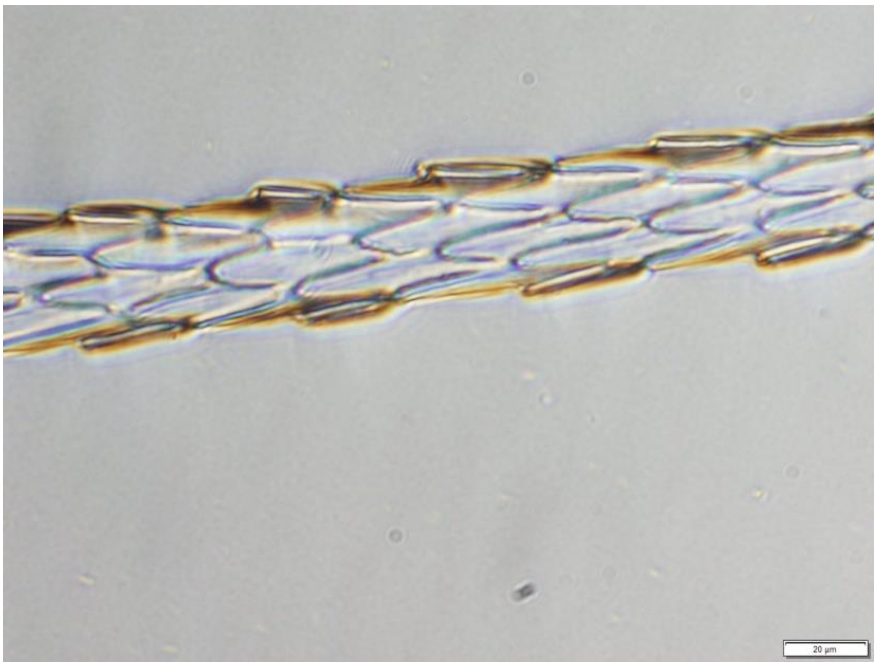


Figure 7. Regular wave (shield, top), broad diamond petal (shaft, middle) and elongate petal scale (shaft, bottom) patterns of cuticular scales in the hazel dormouse (adapted from Teerink, 1991).



(a)



(b)

Figure 8. (a) Irregular wave (shield) and (b) narrow diamond pattern petal (shaft) cuticular scale patterns of the Irish stoat.

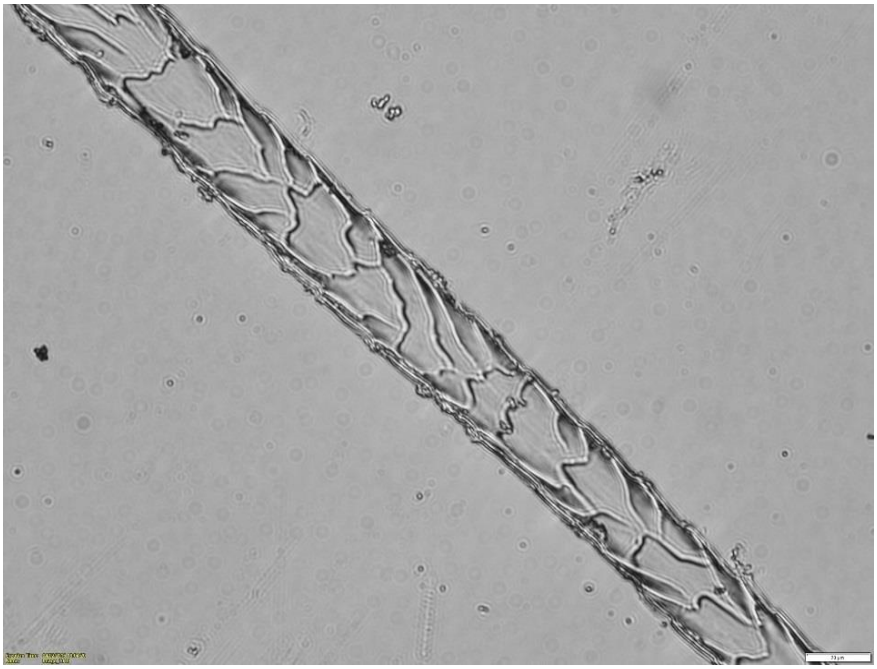
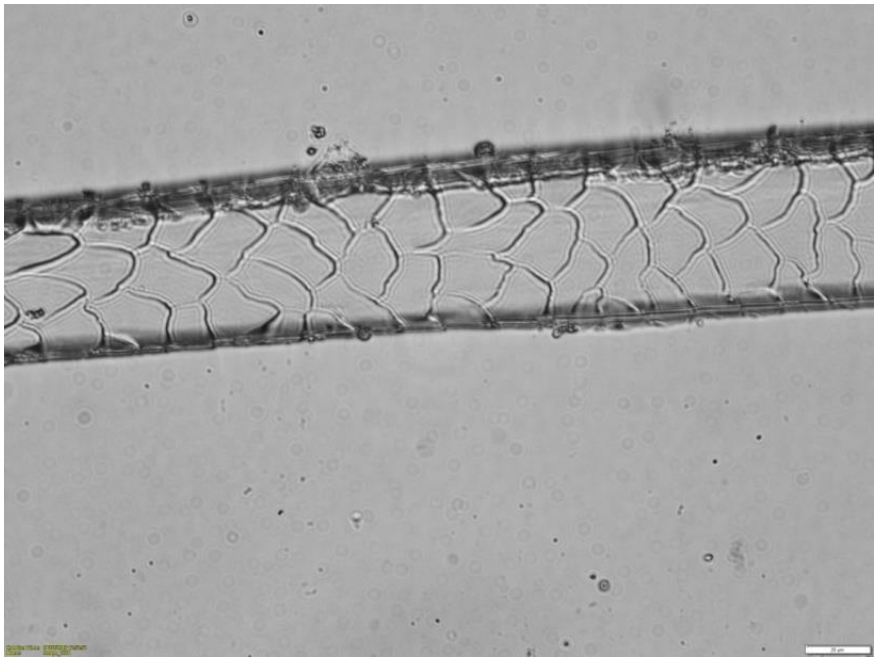
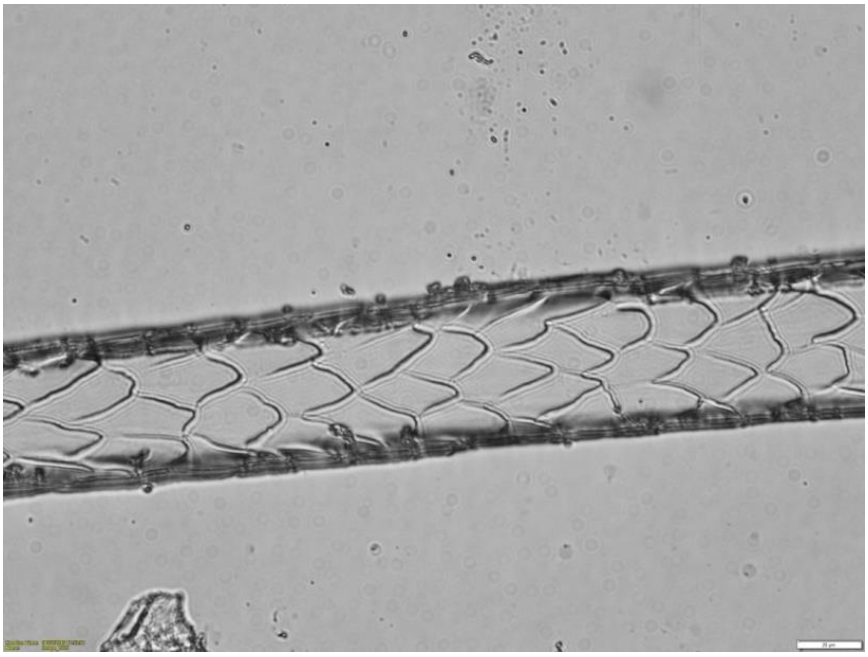


Figure 9. Longitudinal scales with irregular petal pattern and rippled borders of the bank vole.



(a)



(b)

Figure 10. (a) Intermediate broad petal scale pattern, (b) which becomes more longitudinal diamond petal towards the tip, with smooth borders, of the wood mouse.

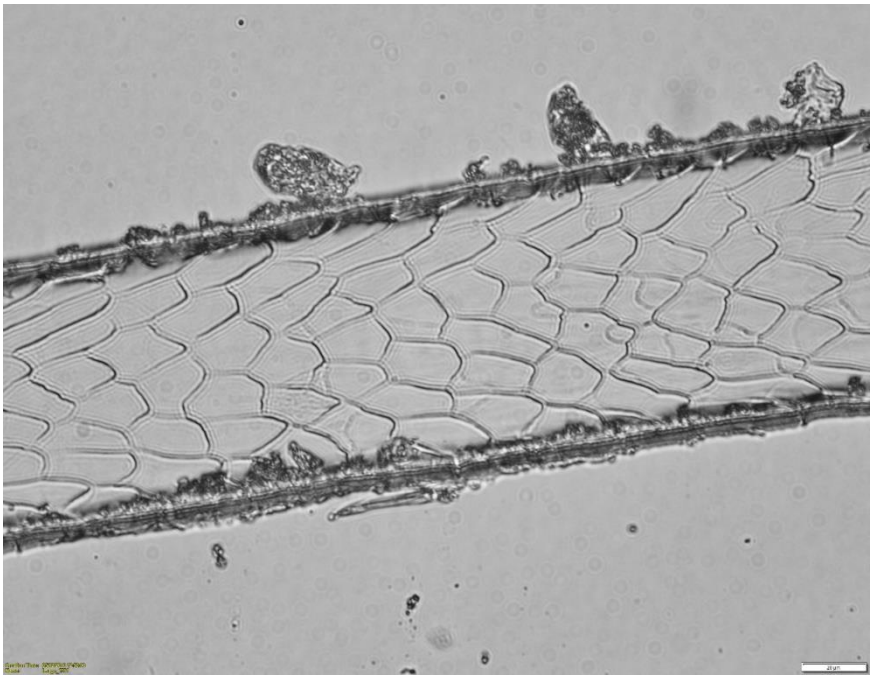
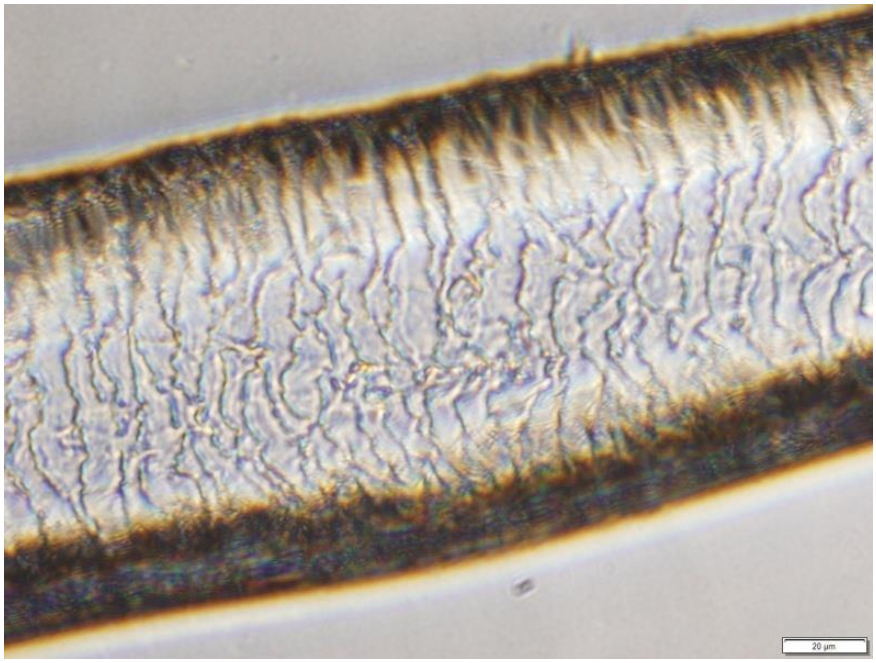
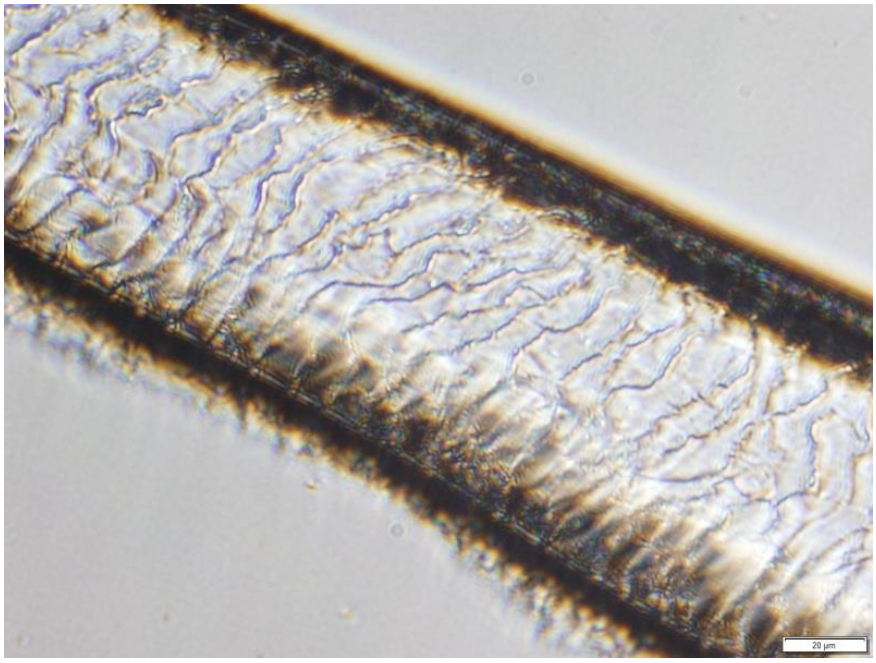


Figure 11 Longitudinal, broad diamond petal scales, arranged in series of 'V' shapes, of the brown rat.

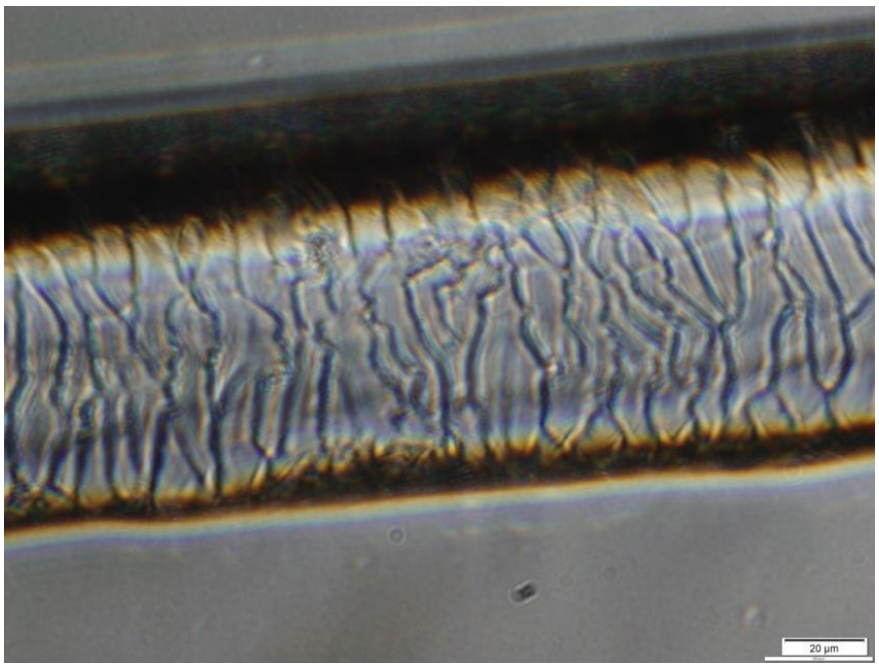


(a)

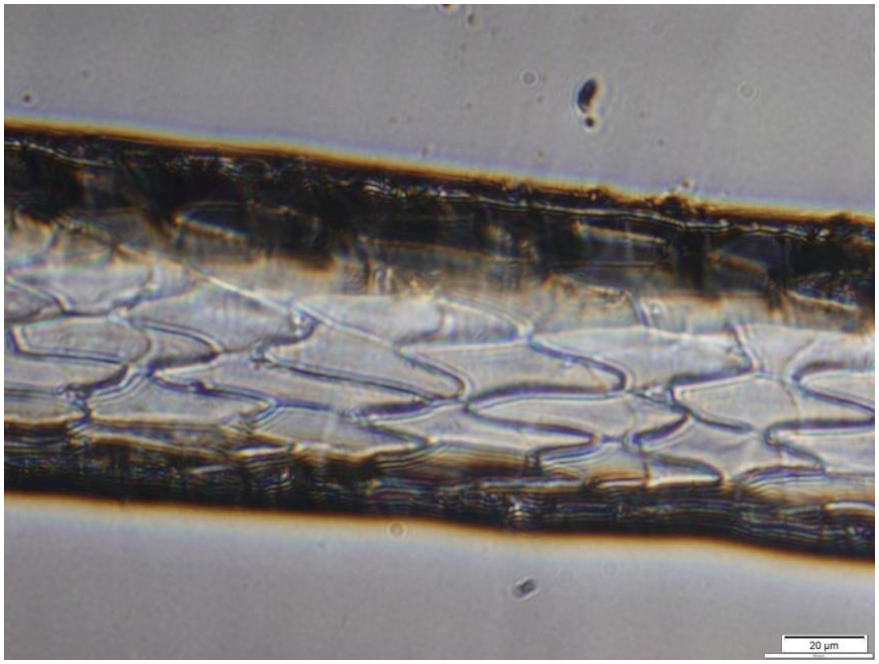


(b)

Figure 12. (a) Irregular wave and (b) regular wave cuticular scale patterns of the badger.

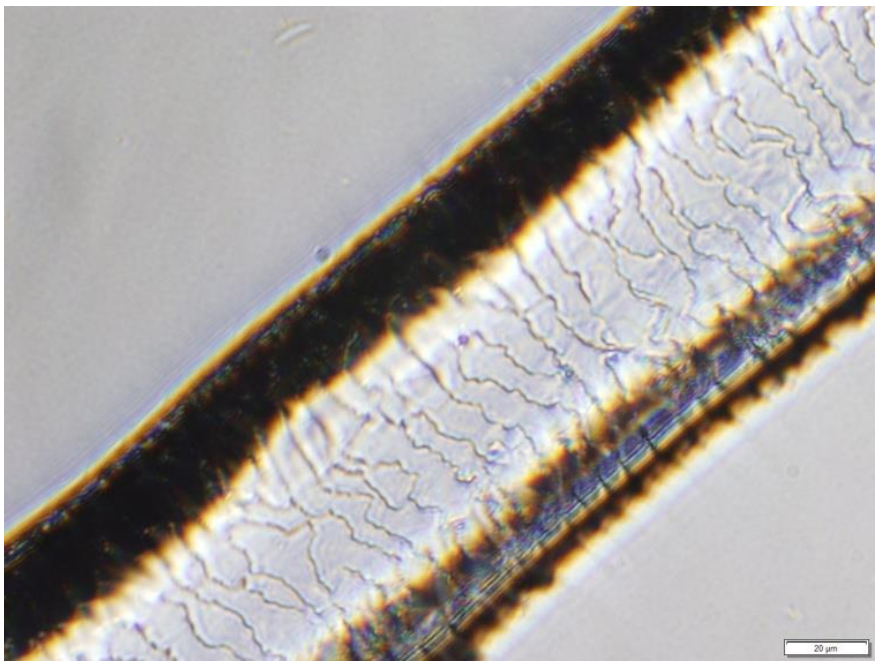


(a)

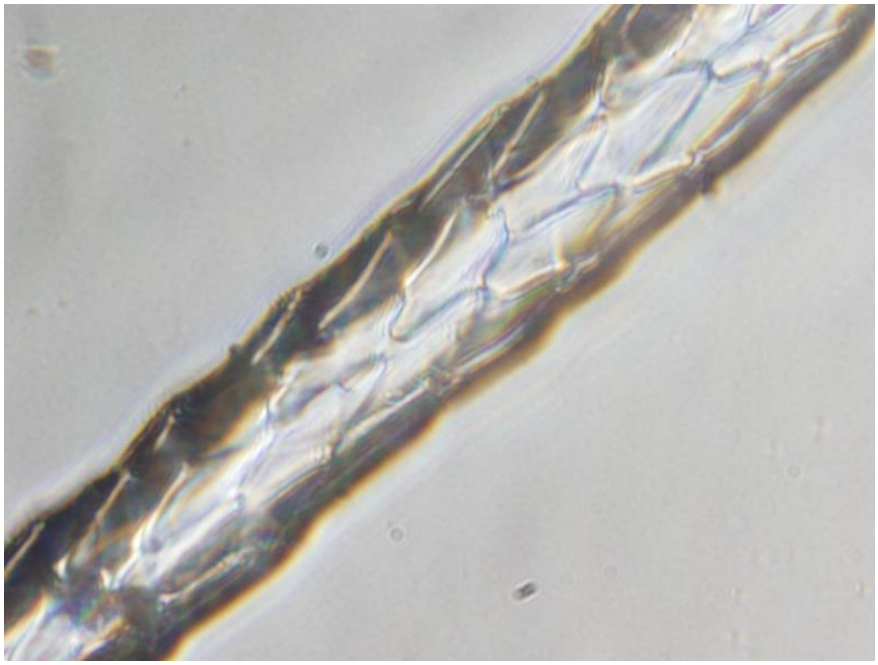


(b)

Figure 13. (a) Regular wave (shield) and (b) narrow diamond petal (shaft) cuticular scale patterns of the fox.

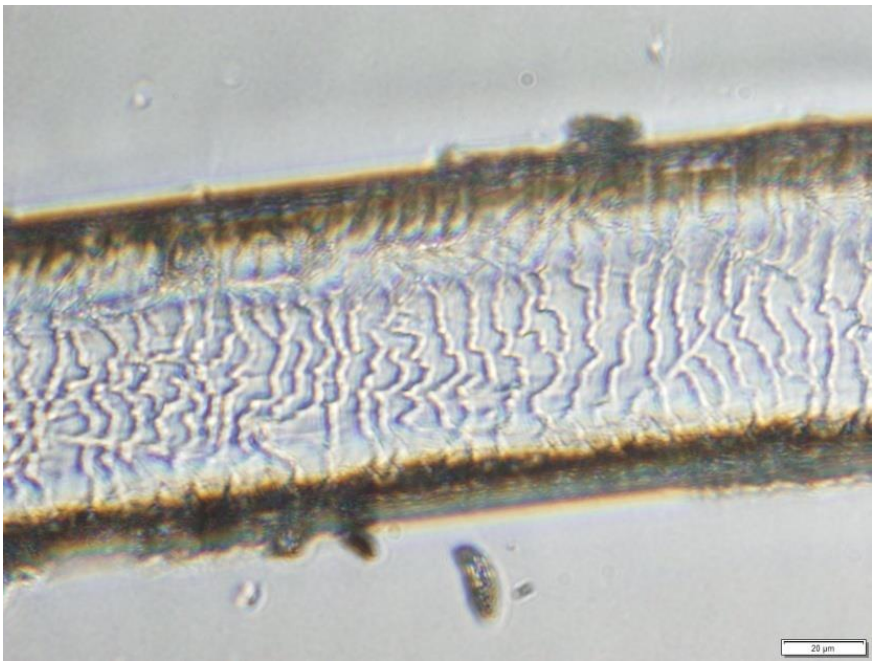


(a)

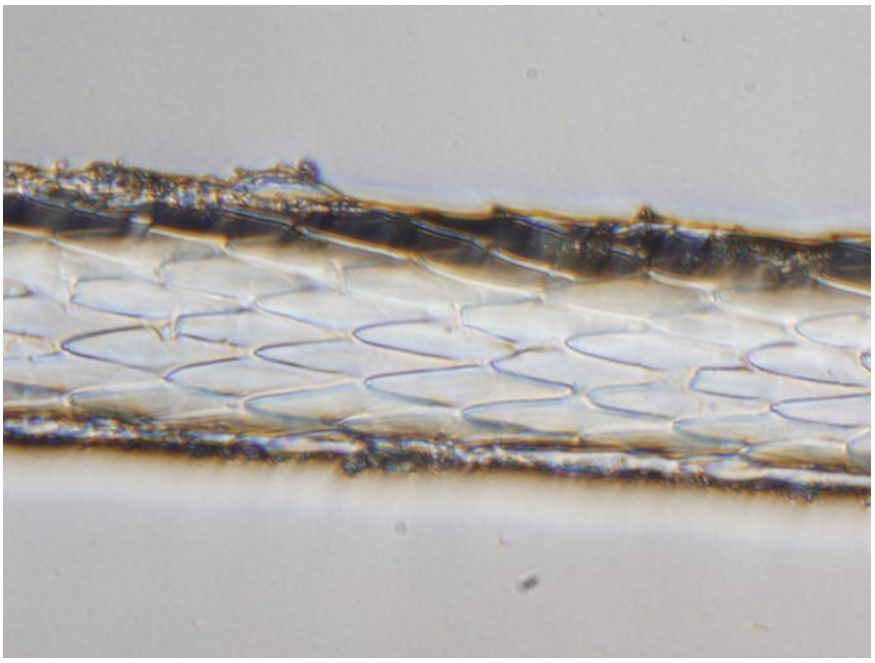


(b)

Figure 14. (a) Regular wave (shield) and (b) diamond petal (shaft) cuticular scale patterns of the pine marten.

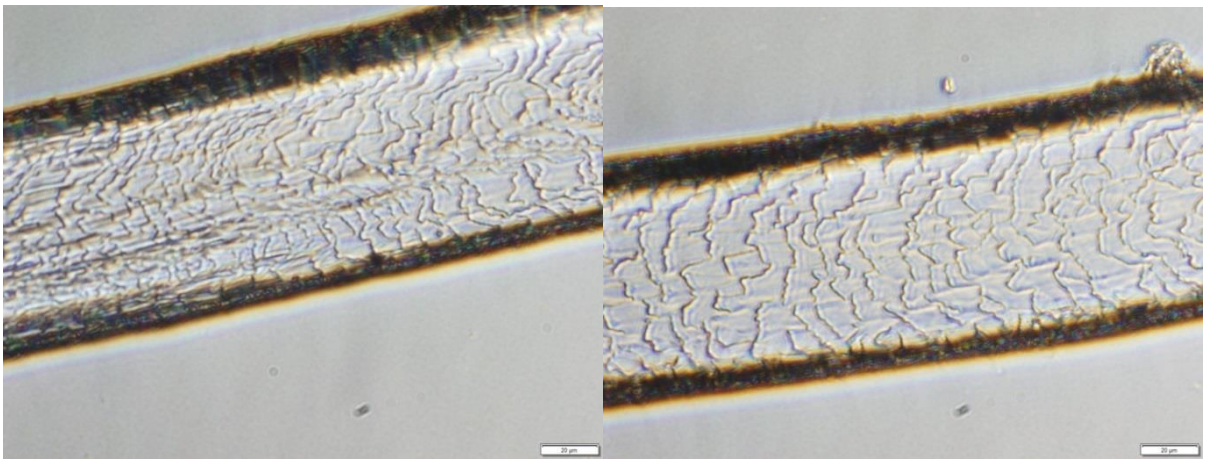


(a)

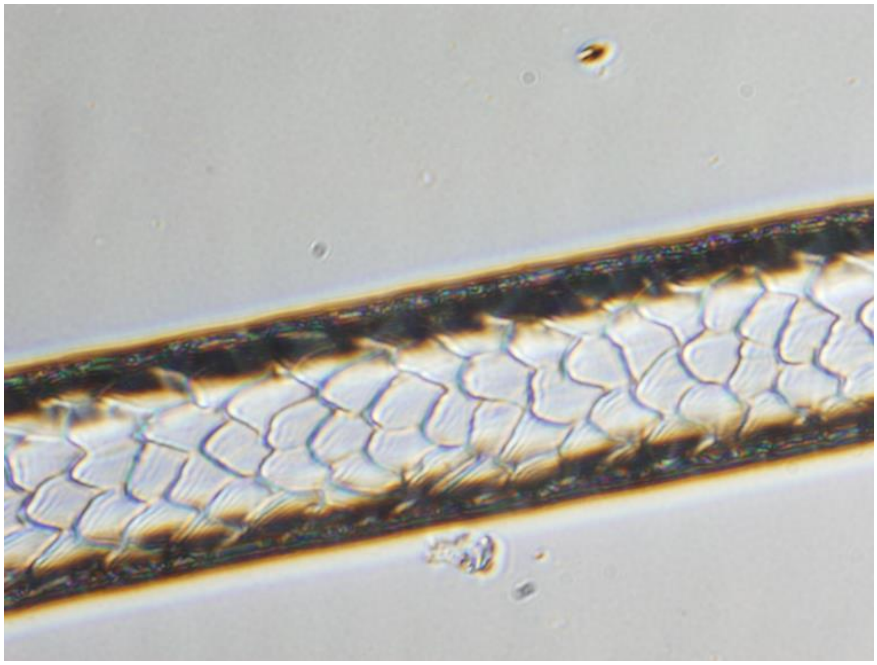


(b)

Figure 15. (a) Irregular wave (shield) and (b) diamond petal (shaft) cuticular scale patterns of the American mink.



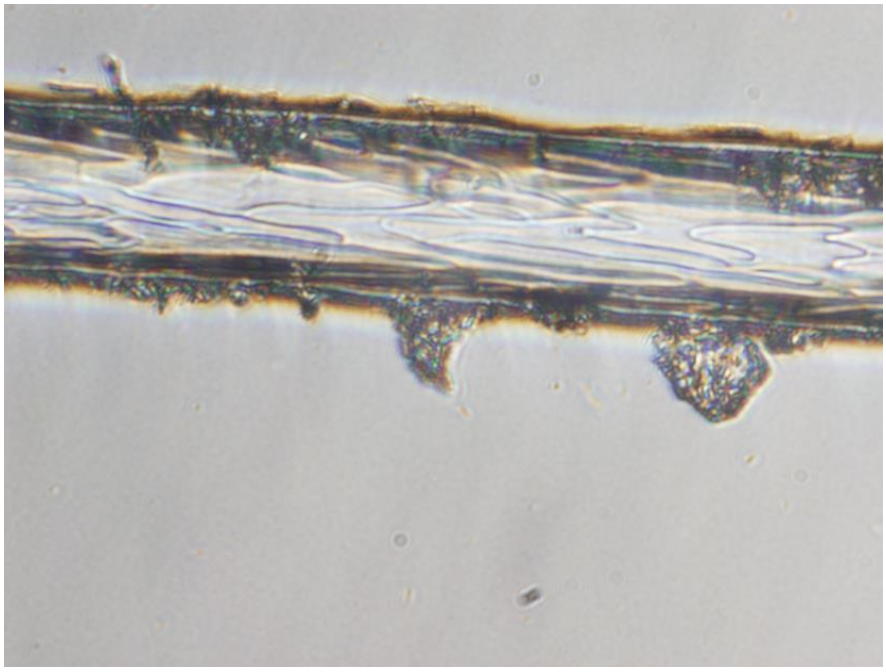
(a)



(b)

Figure 16. (a) Variable patterns evident in the shield, and (b) diamond petal cuticular scale patterns in the shaft of the hair of the otter.

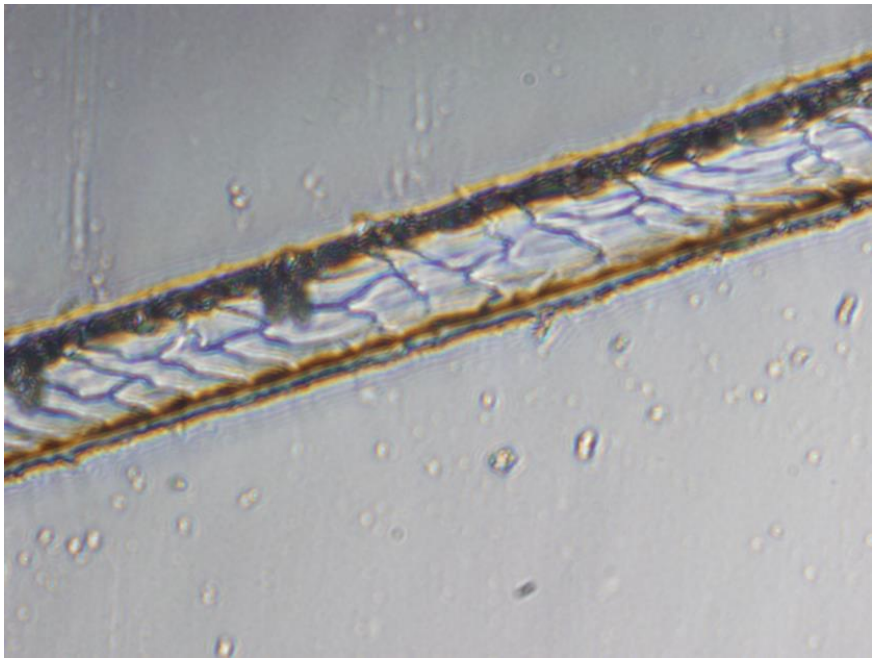
(a)



(b)

Figure 17. (a) and (b) Narrow longitudinal cuticular scales, seen in portions along the shaft of the hair of the Irish hare.

(a)



(b)

Figure 18. (a) and (b) Narrow longitudinal cuticular scales, seen in portions along the shaft of the hair of the rabbit.

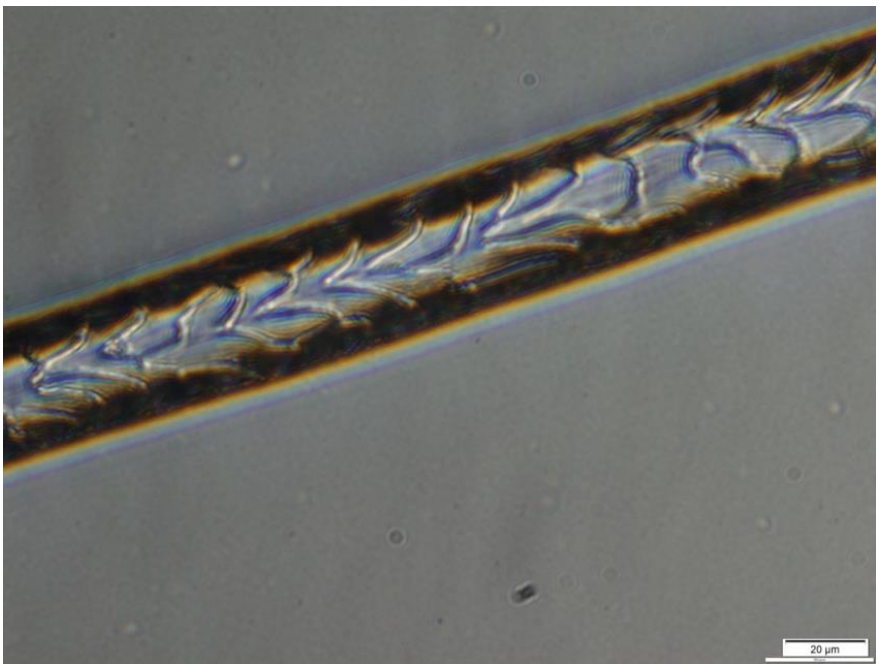


Figure 19. V-shaped cuticular scales of the proximal shield and distal shaft of the hair of the red squirrel (also evident in the grey squirrel).

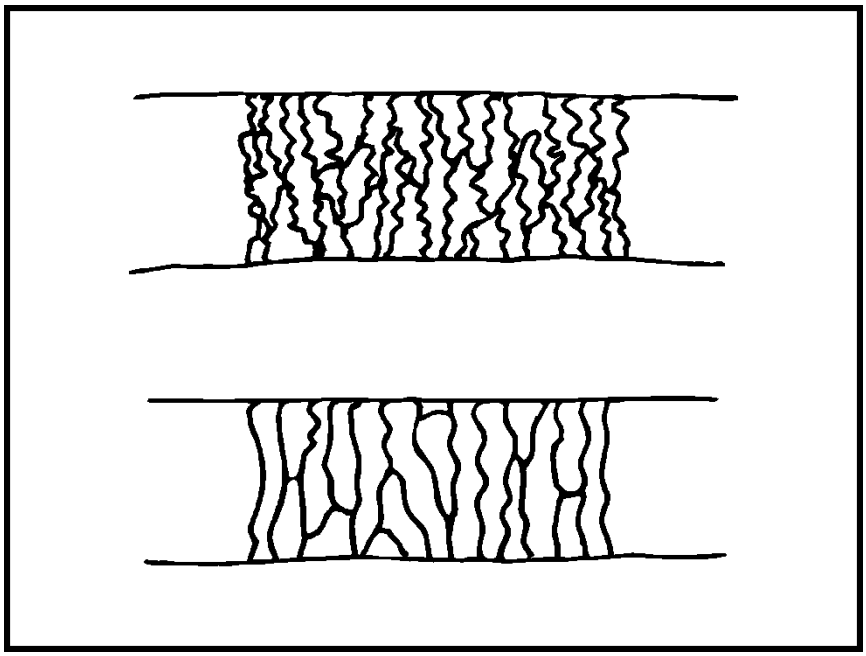
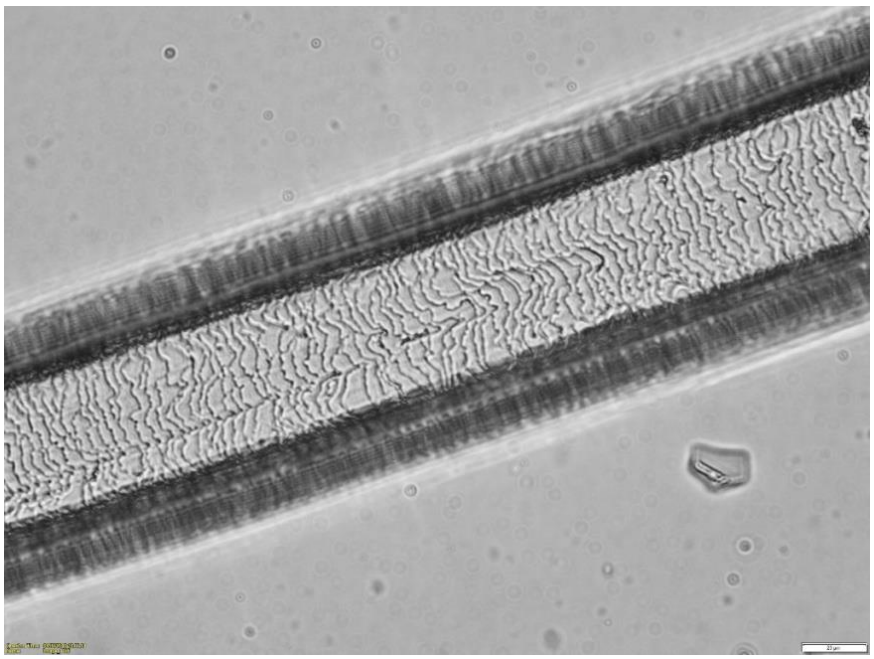
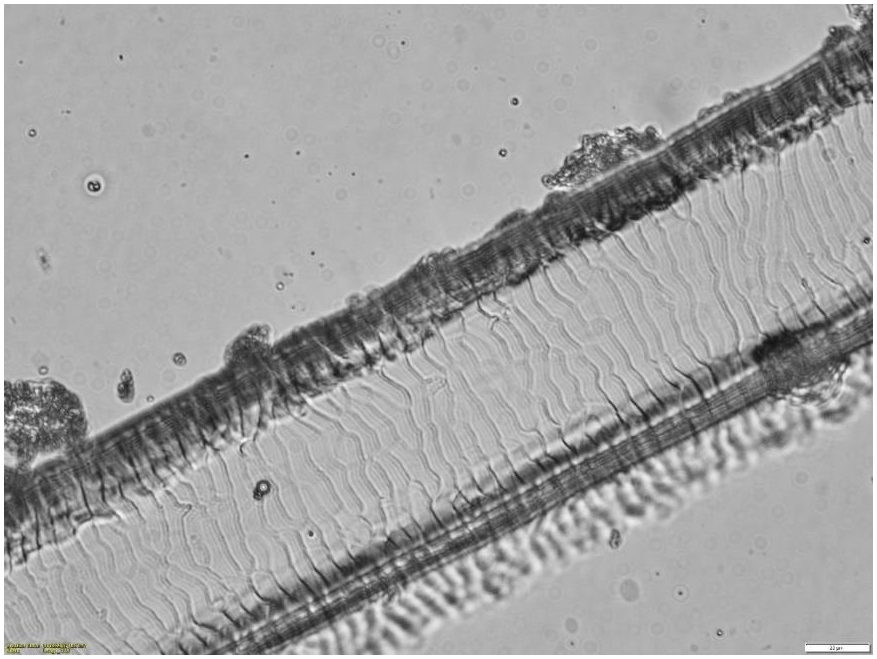


Figure 20. Irregular wave pattern (top) and regular wave pattern of cuticular scales in the shield and shaft respectively of coypu hairs (adapted from Teerink, 1991).

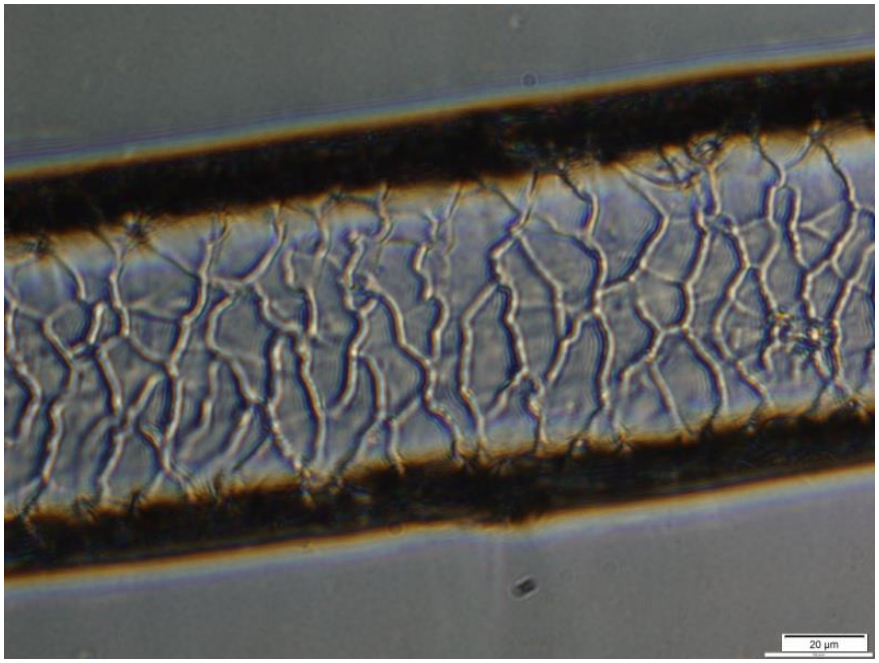


(a)

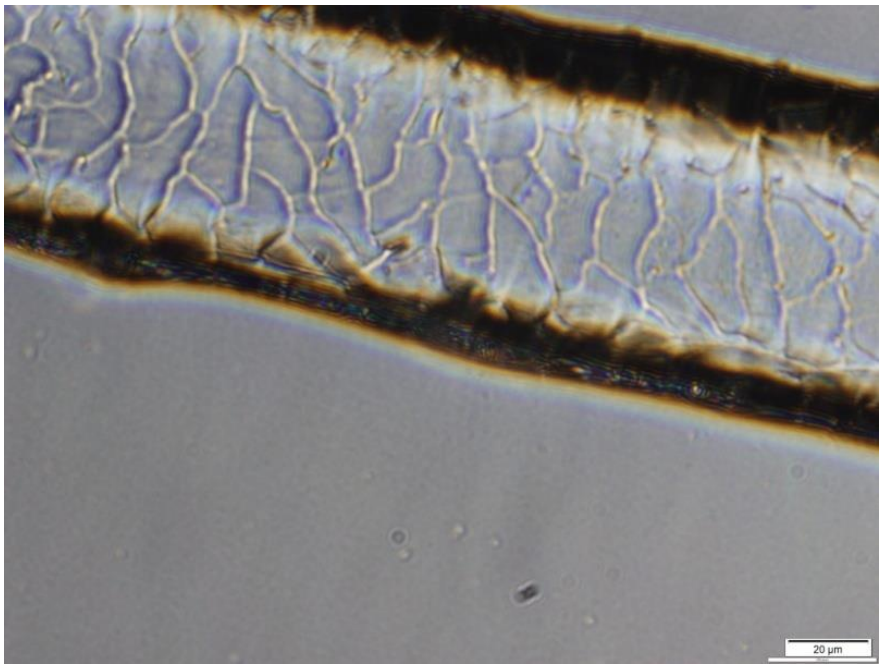


(b)

Figure 21. (a) Irregular wave and (b) regular wave cuticular scale patterns of the domestic cat.



(a)



(b)

Figure 22. (a) and (b) Intermediate mosaic cuticular scale pattern of the domestic dog.

Discussion

This guide follows on from the more detailed and diverse guide presented by Teerink (1991), which describes the hair of 73 Western European mammal species in great detail. Teerink's book remains the ultimate resource required for any in-depth investigation into mammal species using hair. Our intention was to produce a more easily accessible and quicker resource for wildlife naturalists, students and professionals concentrating on Irish species and honing in on the best diagnostic elements for identification purposes. To simplify the procedures we have not considered patterns in the hair medulla. This is the pith or central portion of the hair composed of visible dead cells and air-filled spaces, which like the cuticular scales can allow the identification of certain species. The technique required to prepare and mount the hair for investigation is advanced and requires a certain degree of expertise making it less useful for the aims of this study. We also confined our guide to the outermost guard hairs only, as these are straight forward to work with, and the most frequently found portions of hair. This study focuses mainly on GH1 which tend to be very similar to, and share many of the same characters as, GH2. This approach for the most part is suitable when working with a restricted group such as Irish mammals, as many guilds of animals are only represented by one individual species (e.g. one vole species, one field mouse species etc.). Some animals could not be separated to species level however due to similarities within the guild, such as the bats belonging to the Family Vespertelionidae and the members of the Artiodactyla. For in depth analysis of these animals it is necessary to revert to Teerink (1991).

Only two species could be identified based on macro-features such as length, colour and general form, the badger and the hedgehog. All others require some form of microscopic investigation. In saying that, the broader description of the hair, together with knowledge of the location and circumstances from which the hair was gained, can inform the investigation to help narrow down the identification process. The user of the guide can therefore consider the possible source species of the hair, and specifically look for the diagnostic features that separate them from similar animals.

One technique used to identify species examines the cross-sectional shape of the mammal's guard hair. For many species this is a round shape, varying from circular to oblong, and it may require some practice to differentiate between these categories. Some of the smaller terrestrial mammals have distinctive cross-sectional shapes to their hair. It is a major diagnostic tool in distinguishing between red (concavo-convex (kidney) or biconcave (dumbbell) shaped) and grey (circular or oval shaped) squirrels. Other small mammals can be triconcave (wood mouse) or quadriconcave (bank vole). The cross-sectional shape of the hair can vary along the hair, so it is important that the thickest part of the shield is used. In some cases, we found it difficult to view a clear cross-sectional shape of the hair. Zapponi *et al.* (2013) detail an alternative, much more accurate, method of observing cross-sectional shapes of hair, however it requires specialist equipment, which may be unavailable for the target audience of this study. We therefore recommend that cross-sectional shape is only used in conjunction with the cuticular scale pattern. Cross-sectional shape remains the best diagnostic tool for distinguishing between the newly introduced greater white toothed shrew and the pygmy shrew. Given the difficulties of using this technique, it may be necessary to consider these animals as a group, rather than separate to species level based on their hair.

The cuticular scale pattern varied significantly among species, and also along the length of the hair. It is essential that the scale pattern is examined along the length of the hair to find the diagnostic

features highlighted in this guide. It is possible to describe the patterns in much greater detail than used here. We could consider the density of the scales; they can be densely packed (close) or relatively sparse (distant). The position of the scale in respect to the shaft (longitudinal or transverse) could also be used to add detail to the description of the cuticular scales. For the purposes of identification of Irish mammal species it is sufficient to confine ourselves for the most part to the pattern of the scales, and the position of the pattern on the hair strand. The outline of the scales themselves, which can be classed as smooth, rippled or frilled, is also used when distinguishing bank voles from mice and rat species.

Every species in this key showed several scale patterns throughout the length of the hair. The cuticular scale pattern in the shield is quite similar in many species with patterns displaying close transversal regular or irregular wave patterns with rippled scale margins. It is in the medial region of the shaft where most of the interspecific variation can be seen, and therefore the most useful diagnostic features. Many closely related species share similar cuticular scale patterns. The Mustelid family, for example, are characterised by the diamond petal cuticular scale pattern in the shaft. This feature is absent in badgers, but can also be seen in fox hair. Other features, such as the gross morphology and colour of the hair must be considered to complete the identification of these animals.

It was difficult to find distinctive characteristics to separate the two hare species; Teerink (1991) makes no attempt to distinguish between them in his more detailed study. There may be a distinction in the cross-sectional shape of the hairs, with the Irish hare showing a concavo-convex shape, and the European hare occasionally showing a biconcave shape. This requires further investigation. There may also be subtle differences between subspecies such as the Irish hare, which is endemic to Ireland and some neighbouring islands, and the Arctic hare found throughout the rest of its range. The Irish hare does not turn white in winter as the rest of the species does, and further variations may also be seen in the hair at a microscopic level. Our Irish hare hair did not always match the pattern shown in Teerink, but samples of both subspecies are required before diagnostic differences can be determined.

Bat hairs were difficult to work with, because they were small, fine and fragile. We were able to separate the hairs from one another using very fine tweezers, however a complete imprint of the cuticular pattern was unsuccessful as the hairs were too light to sink into the gel fully and pressing the hairs down with a tweezers disrupted the formation of the gel cast. It was not possible to examine cross-sectional shapes of bat hairs using the technique described here. Bats were identified to family level based on cuticular scale pattern, rather than at species level due to these obstacles and because of high levels of similarities in the scale pattern shared among them. For some mammals other modes of species identification are more suitable. Using genetic analysis to identify animals to species level remains the most definitive method, however with some practice many species can be reliably identified based on the macro- and microscopic morphology of their hair.

Bibliography & relevant literature

Ausband, D.E., Young, J., Fannin B., Mitchell, M.S., Stenglein, J.L., Waits, L.P. & Shivik, J.A. (2011) Hair of the dog: Obtaining samples from coyotes and wolves non-invasively. *Wildlife Society Bulletin* 35(2): 105-111.

Bertolino, S. Wauters, L. Pizzul, A., Molinari, A., Lurz, P. & Tosi G. (2009) A general approach of using hour-tubes to monitor the European red squirrel: A method applicable at regional and national scales. *Mammalian Biology* 74: 210-219.

Cavia, R., Andrade, A., Martin, E.Z., Fernandez, M.E, Cueto G.R. & Suarez O.V. (2008) Hair structure of small rodents from central Argentina: A tool for species identification. *Mammalia* 72:35-43.

De Marinis, A.M. & Asprea, A. (2006) Hair identification key of wild and domestic ungulates from Southern Europe. *Wildlife Biology* 12: 305-320.

Dick, J.T.A., Freeman, M., Provan, J. & Reid, N. (2010) First record of free-living Reeves' muntjac deer (*Muntiacus reevesi* (Ogilby 1839)) in Northern Ireland. *Irish Naturalists Journal* 31:151

Flaherty, M. (2016) Developing optimal strategies for limiting the spread of grey squirrels *Sciurus carolinensis* and conservation of reds *Sciurus vulgaris* in Ireland. PhD Thesis, NUI Galway.

Gannon, W.L. & Sikes, R.S. (1998) Guidelines for the capture, handling and care of mammals as approved by the American Society of Mammalogists. *Journal of Mammalogy* 79(4): 1416-1431.

Goldstein, E.A., Lawton, C., Sheehy, E. & Butler, F. (2014) Locating species range frontiers: a cost and efficiency comparison of citizen science and hair-tube survey methods for use in tracking an invasive squirrel. *Wildlife Research* 41: 64-65.

Harris, S. & Yalden, D.W. (2008) *Mammals of the British Isles: handbook (4th edition)*. The Mammal Society, Southampton.

Hayden, T. & Harrington, R. (2000). *Exploring Irish mammals*. Town House and Country House Ltd., Dublin.

Lawton, C., Flaherty, M., Goldstein, E.A, Sheehy, E. & Carey, M. (2015) Irish Squirrel Survey 2012. *Irish Wildlife Manuals*, No. 89. National Parks and Wildlife Service, Department of Arts, Heritage and the Gaeltacht, Ireland.

Lobert, B., Lumsden, L., Brunner, H. & Triggs, B. (2002) An assessment of the accuracy and reliability of hair identification of south-east Australian mammals. *Wildlife Research* 28(6): 637-641.

Lyra-Jorge, M.C., Ciocheti, G., Pivello, V.R., & Meirelles, S.T. (2008) Comparing methods for sampling large-and medium- sized mammals: camera traps and track plots. *European Journal of Wildlife Reservation* 34: 739-744.

Marnell, F., Donoher, D., Sheehy, E. & Lawton, C. (2015) First confirmed record of Hazel dormouse (*Muscardinus avellanarius*) in the wild in Ireland. *Irish Naturalists Journal* 33: 78-79.

McAney, K. (2011) *A pilot study to test the use of hair tubes to detect the Irish stoar along hedgerows in County Galway*. Vincent Wildlife Trust, http://www.hedgelaying.ie/images/1348511603.pdf

McDevitt, A.D. (2013) Invasions and expansions: the greater white toothed shrew in Ireland. *Genetics Society News* 68: 51-52.

Menike, U. Manawadu, D. & Meegaskumbura, S. (2012) Identification of Sri Lankan shrew using hair anatomy. *Ceylon Journal of Science (Bio Sci)* 41(1): 45-66.

Mullins, J., Statham, M.J., Roche, T., Turner, P.D., O'Reilly, C. (2010) Remotely plucked hair genotyping: a reliable and non-invasive method for censusing pine marten (*Martes martes*, L. 1758) populations. *European Journal of Wildlife Research* 56: 443-453.

Pocock, M.J.O. & Bell, S.C. (2011) Hair tubes for estimating site occupancy and activity- density of Sorex minutus. *Mammalian Biology* 76: 445-450.

Quinn, N. (2005). *The hair tube analysis and supplementary feeding of red squirrels (Sciurus vulgaris L.) in Portumna Forest Park, Co. Galway*. BSc Thesis. National University of Ireland, Galway.

Roche, N., Aughney, T., Marnell, F. & Lundy, M. (2014) *Irish Bats in the 21st Century.* Bat Conservation Ireland, Virginia, Ireland.

Ruell, W.E., Riley, S.P.D., Douglas, M.R, Pollinger, J.P. & Crooks, K.R. (2009) Estimating bobcat population sizes and densities in a fragmented urban landscape using non-invasive capture-recapture sampling. *Journal of Mammalogy* 90(1): 129-135.

Sheehy, E., O'Meara, D. B., O'Reilly, C., Smart, A. & Lawton, C. (2013) A non-invasive approach to determining pine marten abundance and predation. *European Journal of Wildlife Research* 60 (2): 223-236.

Sheehy, E. & Lawton, C. (2014) Population crash in an invasive species following the recovery of a native predator: the case of the American grey squirrel and the European pine marten in Ireland. *Biodiversity and Conservation*, 23 (3): 753-774.

Silveira, L., Jacomo, T. A., Jose Alexandra, J. & Filho, D. (2003) Camera trap, line transect census and track surveys: a comparative evaluation. *Biological Conservation* 114: 351-355.

Teerink, B.J. (1991) *Hair of Western European mammals: atlas and identification key.* Cambridge University Press, Cambridge.

Tosh, D.G., Lusby, J., Montgomery, W.I. & O'Halloran, J. (2008). First record of greater white-toothed shrew *Crocidura russula* in Ireland. *Mammal Review* 38 (4): 321-326.

Walsh, P.M. (1988). Black rat *Rattus rattus* (L.) as prey of short-eared owls *Asio flammeus* (Pontopiddan) on Lambay Island, Co Dublin. *The Irish Naturalists' Journal*. 22(12): 536.

Waters, C. & Lawton, C. (2011) Red Squirrel Translocation in Ireland. *Irish Wildlife Manuals* No. 51. National Parks and Wildlife Service, Department of the Environment, Heritage and Local Government, Dublin, Ireland.

Zapponi, L., Bianco, M. D., Luiselli, L., Catorci, A. & Bologna, M. A. (2013) Assessing environmental requirements effects on forest fragmentation sensitivity in two arboreal rodents. *Mammalian Biology* 78: 157-163.