Comparative analysis of structural and functional hair coat characteristics, including heat loss regulation, in the Lutrinae (Carnivora: Mustelidae)

Dissertation

Zur Erlangung des Doktorgrades des Departments Biologie

der Universität Hamburg

Fakultät für Mathematik, Informatik und Naturwissenschaften

vorgelegt von

Rachel Anne KUHN

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July 29, 2008

From: Dr. Nicole Duplaix, Instructor, Affiliate Faculty, Fisheries and Wildlife Department

RE: **English Language Evaluation of the Ph.D. Thesis of Ms. Rachel Anne Kuhn**

As an English native speaker, I have reviewed the excellent thesis of Ms. Rachel Anne Kuhn entitled "Comparative analysis of structural and functional hair coat characteristics, including heat loss regulation in the Lutrinae (Carnivora: Mustelidae)." I hereby confirm that the English employed in this thesis is correct and clear in both grammar and content.

Sincerely,

Dr Nicole Duplaix,
Instructor

SUMMARY

In this thesis, different characteristics of the hair coat of the Lutrinae (Mustelidae: Carnivora) and the regulation of heat loss through the surface of the body were investigated.

Chapter 1 was aimed at verifying how important the variability of the primary hair (PH) structure is within a single species, here the Eurasian otter (*Lutra lutra*). The morphology, colour, cross-section, length, width, cuticle and medulla of the PHs were examined. PH structure was not or only slightly influenced by sex, season, age, captivity and treatment of the samples. The structure of the PHs also tended to remain constant from one body region to the next, except for the head, the PHs of which diverged from those of the trunk for a few characteristics, principally by being shorter and thinner. A lesser divergence was also observed in the PHs from the abdomen. However, the observed variability was quite moderate, and the general structure of the PHs remained constant. The greatest differences were observed between the PHs of otters coming from temperate Europe and those from tropical South Asia, which were shorter and thinner with stockier cuticle scales.

Chapter 2 consists of an analysis of the hair structure of the 13 otter species known. The study focussed on the PHs, because the secondary (wool) hairs (SHs) show less species-specific characteristics, but the length and width of the SHs were also measured. The PHs of the different species showed many similar characteristics, but also some significant divergences. The most specific PHs were found in the Sea otter (*Enhydra lutris*), which morphologically and ecologically differs the most from the other species, and the Giant otter (*Pteronura brasiliensis*), which can also easily be recognized and is genetically the most divergent otter. Apparently, the macroscopic and microscopic hair structure in the Lutrinae has been influenced by both phylogenetic relationships and adaptive pressures. An influence of the climate and of the association to water could be recognized. For example, the species living in a colder climate had longer PHs and SHs and also longer lanceolate cuticle scales.

Chapter 3 focussed on the hair density of the Eurasian otter (*Lutra lutra*) and the Sea otter (*Enhydra lutris*). A mean hair density of about 70,000 hairs/cm² (whole body, n=6 individuals) was found in the Eurasian otter. The mean individual density ranged from about 60,000 to 80,000 hairs/cm². The majority were SHs (hair coat with only 1.26% of primary hairs). The SH density remained constant over the body (appendages excepted), whereas a few variations in PH density were observed. Neither an influence of the sex, nor a seasonal variation of the hair coat was found. The Sea otter appeared to have a density between 120,000 and 140,000 hairs/cm², the PHs representing less than 1% of the hairs. Hair density remained quite constant over the regions of the body trunk but was lower at the head (about 60,000 hairs/cm² on the cheek).

In chapter 4, the secondary hair cuticle structure of six otter species, chosen so that the different genera, climatic regions and degrees of association to water of the Lutrinae were represented, were examined by scanning electron microscopy (SEM). The cuticle of every examined hair, exhibited a specific shape and arrangement of the scales that allows a flexible interlocking of adjacent SHs, and thus facilitates the trapping of an insulating air layer.

In chapter 5, the body surface temperature in the Eurasian otter (*Lutra lutra*) and, to a lesser extent, in the tropical Giant otter (*Pteronura brasiliensis*) and Small-clawed otter (*Amblonyx cinereus*) was studied using an infrared thermographic camera. The Eurasian otters used mainly their feet to dissipate excess heat, whereas the two tropical species, particularly the Giant otter, lost more heat through the whole body and also through the tail. In all three species, the temperature at the surface of the feet tended to decrease to a temperature similar to that of the water when the animal was submerged. The same was observed for the tail in the tropical species, and also in the Eurasian otter during winter. In the Eurasian otter, temperatures of the air layer trapped within the fur being more than 10°C above the temperature of the ambient air or water were measured. A selective heating of the sensory organs at the head could be observed.

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INTRODUCTION

The 13 otter species known form the subfamily Lutrinae. They have a worldwide distribution and live in very different environments, from tropical to arctic climates. The group is morphologically rather uniform, with only a few species that can be easily identified. Otters are all semi-aquatic carnivores, but they show different degrees of adaptation to the aquatic habitat. Like other semi-aquatic and aquatic endotherms, otters have to cope with the high cooling power of water, and they achieve maintaining their body temperature mostly thanks to the insulative capacity of their hair coat. Otters are renowned for the exceptional quality of their fur, a reputation that unfortunately contributed to their regression. Another disadvantage of this hair coat is that it is highly sensitive to disturbance, which induces a decrease in the insulating capacity and thus an increase in heat loss, and also this “thermal wrap” can cause overheating.

In this study, we examined the role of the hair coat in the adaptation of the different otter species to their environment. The hair coat of mammals is characterised by typical macroscopic and microscopic features of the hairs and by hair density. It is commonly admitted that species with the highest insulating needs, i.e. those living in cold climates and/or partly in the water, have the densest furs, but the role of the structure of the single hairs, especially the role of microscopic hair structure is less known. The ecological diversity encountered within the Lutrinae, especially regarding climate and relation to water, makes the study of the different aspects of the hair coat of these semi-aquatic mammals particularly interesting. Moreover, within the subfamily, the anatomical adaptations do not always correlate with the taxonomic position of the different species, so species that are morphologically and ecologically close to each others are not necessarily the closest relatives from the phylogenetical point of view.

This brings us to the question: Do the hairs of the different otter species distinguish from each other, and do they reflect the ecological diversity or the phylogenetical relationships within the subfamily? Can even microscopic features of the hairs have an adaptive function? Moreover, the characteristics of the hairs, more precisely of the primary hairs, could be used in field studies to identify the different otter species, depending on how species-specific these primary hair features are. However, in order to better evaluate the importance of the difference in primary hair structure between species, it is helpful to get information on the intra-specific variability.

Otters are famous for their high numbers of hairs per cm^2, but actually the information available about hair density in the Lutrinae is quite incomplete, whereby only a few species are concerned. There are no data available on hair density in tropical otter species, for

example. Furthermore, there is a certain discrepancy in the published values for a single species, and it is unclear how hair density varies over the body or between sexes.

An important aspect of the hair coat of several mammal species is its seasonal variability. Indeed, many species grow a better insulating hair coat for the coldest period of the year. For semi-aquatic mammals, the challenge is different, because they need a good protection against heat loss all the year round. However, an increase of the insulating capacity of the fur by colder temperatures, involving possible changes in hair density and/or in length and width of the hairs, cannot be excluded. Thus, it is interesting to clarify if seasonal variability of hair coat characteristics occurs in the otter species exposed to seasonal climatic variations.

Actually, how effective is the fur of the different otter species in retaining heat, and how are the characteristics of the hair coat reflected by heat permeability? An increase of the insulative property of the fur implies a decrease of the possibility to lose excess heat, and thus exposes the animal to higher risks of overheating. Otters must use the less-furred parts of their body to lose excess heat, but how extended and how effective are these parts in regulating heat loss?

The objective of our study was to bring answers or more detailed information regarding these questions. Many parts of this work focussed on the Eurasian otter (*Lutra lutra*), because this was the species for which most material was available. Chapter 1 is aimed at the possible variability of the structure of primary hairs in the Eurasian otters according to different potential influence factors, for example body region or sex. Chapter 2 looked at the hair structure of each of the 13 otter species, including an exhaustive analysis of the macroscopic (colour, morphology, length, width) and microscopic (cuticle, medulla) characteristics of the primary hairs, and measurements of the length and width of the secondary hairs. The results were compared with the ecological and taxonomic position of the different otter species, and also with published information on the hair structure of semi-aquatic mammals and of the closest relatives and ancestor branch of the Lutrinae, in order to determine if the hair characteristics have an adaptive value or are rather influenced by the phylogeny of the group. Chapter 3 consists of a study of hair density at different body regions in the Eurasian otter (*Lutra lutra*) and in the Sea otter (*Enhydra lutris)*. Possible variations of the hair coat during the year were also analysed. In chapter 4, hair cuticle structure of secondary hairs of 6 otter species, chosen so that the different genera, climatic regions and degrees of association to water were represented, was examined by SEM. In chapter 5, the body surface temperature in the Eurasian otter (*Lutra lutra*) was studied using infrared thermography, in order to document which parts of the body are important thermoregulatory surfaces, and to demonstrate in this way mechanisms for heat conservation or dissipation. Measurements were also made in two species originating from tropical regions, the Giant otter (*Pteronura brasiliensis*) and the Small-clawed otter (*Amblonyx cinereus*).

CHAPTER 1:
PRIMARY HAIR STRUCTURE IN THE EURASIAN OTTER *LUTRA LUTRA*

1.1 Introduction

Hairs are typical skin products for mammals. They are filamentous outgrowths of the skin, produced by hair follicles. The hair shaft, which is the outer part of the hair and extends beyond the skin, has to fulfil several functions. The most important are: insulation, protection against UV radiation and mechanical hazards, but hairs also have functions in sense of touch as mechanical receptor, in the dispersion of glandular secretion and also in social communication and camouflage.

The hair coat consists of strong primary hairs (PH) or guard hairs and thin secondary hairs (SH) or wool hairs, which are often curled. The hair shaft is formed by the central medulla, surrounded by the cortex and the outer hair cuticle, which consists of a layer of flat, strongly cornified cells, the scales (Fig. 1). Along the hair shaft, three important regions can be distinguished: the pars apicalis (apical part), that in several mammalian groups has a spindle-shaped to strongly flattened thickening called the shield, then the pars intermedia (medial part), that takes about one quarter of the hair shaft length and shows a more or less constant hair cuticle pattern and finally comes the pars basalis (basal part), that takes about one third to one half of the hair shaft and often has a varying hair cuticle pattern (Fig. 2). The structure of primary hairs (PH) varies species or group related, and thus, it is possible to identify a group or even a species by analysing the PH structure. The first reference to hair cuticle structure is known from 1842 by QUEKETT, but the use of the hair cuticle pattern for species identification really began with the work of HAUSMAN (1920, 1924, 1930, 1932, 1944). Many publications about species identification by analysing the hair shaft have followed (DAY 1966, BRUNNER & COMAN 1974, KELLER 1978-1986, TEERINK 1991, MEYER et al. 2002). The hairs analysed are usually taken from the dorsal body region of the mammals studied. However, some work concentrated on the difference in hair structure between hairs taken from different body regions, and also between hairs of animals from different sexes, ages and seasons (WILLIAMSON 1950, MAYER 1952, DREYER 1966, KENNEDY 1982, USHAKOVA & TSELIKOVA 1998, SOKOLOV et al. 1999).

Several publications studied the hair structure of the semi-aquatic Eurasian otter, *Lutra lutra* (HAUSMAN 1920, 1930, JULIEN 1930, APPLEYARD 1960, FALIU et al. 1979, 1980, KELLER 1981, TEERINK 1991, COWELL & THOMAS 1999, MEYER et al. 2002, TÖTH 2002), but information on the variability of hair structure according to different possible influence factors is not available. Thus, it was the aim of the present study to analyse the PH structure (length, width, morphology, cross-section, medulla, cuticle pattern) of the Eurasian otter, re-

ferring to influences of the body region, sex, season, age, climatic region, differences between museum and fresh samples, and differences between wild and captive otters.

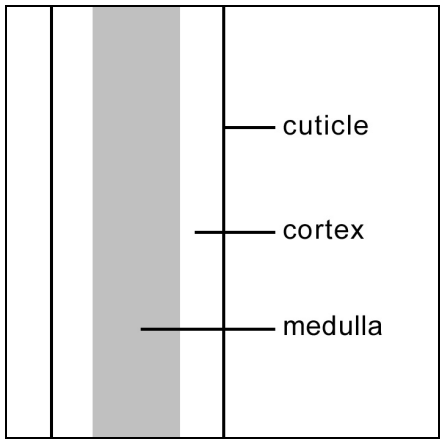


Fig. 1: Longitudinal section of the hair shaft showing the three principal structural layers

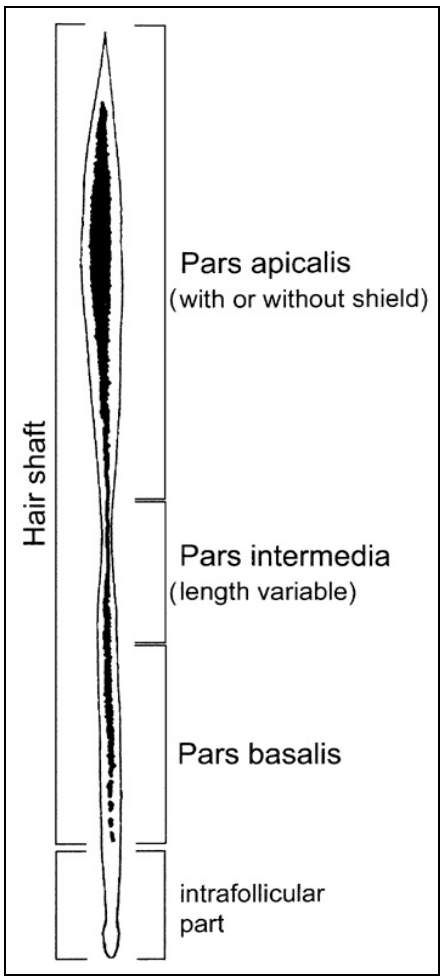


Fig. 2: Three subdivisions along the hair shaft
(from MEYER et al. 2002)

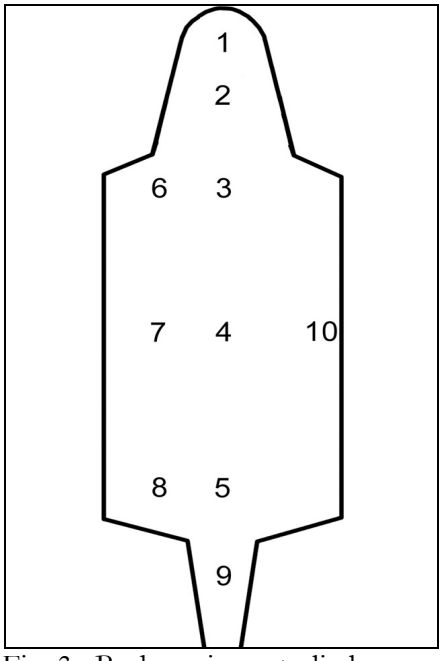
1.2 Material and methods

1.2.1 Samples

PH samples were collected from 30 Eurasian otters (*Lutra lutra*), which were regrouped in different categories, depending on the factor studied, each group consisting of 5 individuals. All samples were taken from dead animals (dried or tanned pelts or fresh carcasses) and from the dorsal body region (except for the factor “body region”). All animals were adults, (except for the “juvenile group”), wild (except for the “captivity” group) and came from Germany (except for the “climatic region” group). The individuals of the juvenile, fresh samples and captivity groups were not chosen according to their sex and to the season during which they died, because for these categories it was not always possible to obtain enough samples for all purposes. The sex and date of death of individuals coming from other climatic regions were unknown. The following possible influence factors were studied.

1.2.1.1 Body region

In order to study the possible influence of body regions on hair structure, PH samples were taken from 10 different parts of the pelts of 5 otters (2 males and 3 females), which had died accidentally at different times of the year (3 in winter und 2 in summer). The studied body regions were (Fig. 3):



1. Forehead
2. Neck
3. Dorsum (anterior, withers)
4. Dorsum (medium, back)
5. Dorsum (posterior)
6. Shoulder
7. Flank
8. Buttock
9. Tail
10. Abdomen (central)

Fig. 3 : Body regions studied

1.2.1.2 Sex and season

PH samples were taken from the dorsal body region of 5 males and 5 females, which had died during winter (November to February), and 5 males and 5 females, which had died during summer (Mai to August).

1.2.1.3 Age

PH samples were also taken from a group of juveniles (2 females and 3 males), aged between 4 and 6 months, which had died at different times of the year.

1.2.1.4 Tanning or drying process

All these PH samples were taken from dried or tanned pelts. In order to study a possible influence of the drying or tanning process on the hair structure, samples were also taken from fresh carcasses (2 females and 3 males, which had died at different times of the year).

1.2.1.5 Captivity

PH samples were taken from a group of animals born, raised and died in captivity (1 female and 4 males, which had died at different times of the year).

1.2.1.6 Climatic region

In order to study the possible influence of different climatic regions, PH samples were taken from animals coming from the following geographic areas:

- Lake Baikal (1 individual)
- Tunisia (1 individual)
- India (1 individual)
- Sri Lanka (2 individuals)

} South Asia group

The PH characteristics of the juvenile group, the fresh samples group, the captivity group, and of the individuals coming from different geographic areas were compared to the PH characteristics of a “standard group”. This standard group was made of 5 wild adult otters (2 males and 3 females), which had died accidentally in North-Eastern Germany (Saxony, Saxony-Anhalt and Brandenburg) at different times of the year (3 in winter und 2 in summer).

1.2.2 Documentation of hair shaft structures

First, the general appearance of the PHs (morphology and colour) was described. The length (total length and length of the shield) was measured with graph paper (5 hairs for each individual). Then the structure of the cross-section, medulla and cuticle of PHs were studied by light microscopy (magnification 400x) and documented with a digital camera (FUJI FinePix

S602). The structure of the cross-section, medulla and cuticle was described using the nomenclature of MEYER et al. (2002). The analysis of hair cuticle structure focused on three important hair shaft regions: the tip (pars apicalis), particularly the upper part, the medial part, directly beneath the shield (pars intermedia), and the lower part of the pars basalis (part directly above the epidermis). The form and arrangement pattern of the cuticle scales, and the shapes and distances of the free edges of the scales were described and classified.

1.2.2.1 Preparation of hair cross-sections

For cross-section analysis, PHs (3-5/ind.) were glued between two pieces of scotch tape, so that the upper half of the shield was freely overhanging, forming a right angle with the upper edge of the scotch tape. The hairs were cut along the upper (outer) edge of the double layer of scotch tape with a razor blade (so the hairs were cut at the middle of the shield, which is the thickest part of the hair shaft). Then it is possible by upright holding of this double scotch tape to look at the sectional planes of the hairs under light microscope (magnification 100x or 400x) (MEYER et al. 2002). This method does not allow obtaining "good pictures" of the cross sections, but it is a very easy and rapid way to recognize the shape of the sectional planes.

1.2.2.2 Preparation of the hair medulla

In order to obtain a view of the medulla, PHs (about 10/ind.) were glued to a non-greasy microscopic slide, covered with paraffin oil and then with a cover slip (MEYER et al. 2002). The oil penetrates into the hair and replaces the gas in the medullar spaces. After 30 minutes of treatment, the hair medulla (and the cortex) can be observed light microscopically (magnification: 400 X).

1.2.2.3 Preparation of casts of the hair cuticle

For the analysis of scale morphology, hair cuticle casts were prepared according to the following protocol (MEYER et al. 2002): The PHs had eventually to be cleaned by immersion in a mixture of acetone-ethanol (50/50) for 60 minutes or, when necessary, overnight, and afterwards dried on a sheet of absorbent paper. The cleaning procedure had to be done particularly for hair samples from old furs or from carcasses. A microscopic slide (26 x 76 mm) was covered with a very thin layer of a 5% solution of polyvinyl acetate (PVA) in distilled water, using a fine flat brush. 5-8 hairs were placed on the slide, some with the tip overhanging and others with the basal (proximal) part overhanging. For each individual, 15-20 hairs were used (the one half with the tip on the slide, the other half with the base on the slide). After 30 minutes drying at room temperature, the hairs were removed from the casting medium, be-

ginning with the overhanging parts. Now the pattern of the hair cuticle scales could be observed under light microscope (magnification: 400 X).

1.2.2.4 Computer assisted measuring of parameters

The medulla and cuticle scales were photographed with a digital camera (FUJI FinePix S602) fixed on the light microscope. The photographs were then transferred to a personal computer. The width of the medulla and hair (Fig. 4) and the size of the cuticle scales (area, perimeter, length and width) (Fig. 5) were measured on the digital pictures, using the software AxioVision (Zeiss Inc.). These measurements were made manually, which means that the scale borders were delineated digitally with the mouse to get the area and perimeter of each scale. Lines were traced to get measures of length (X) and width (Y). The length X is always the height of the scale, independently of the shape of the scale. The cuticle scales were always measured at the pars intermedia. The width of the medulla and hair was measured at the middle of the shield (largest part of the hair shaft).

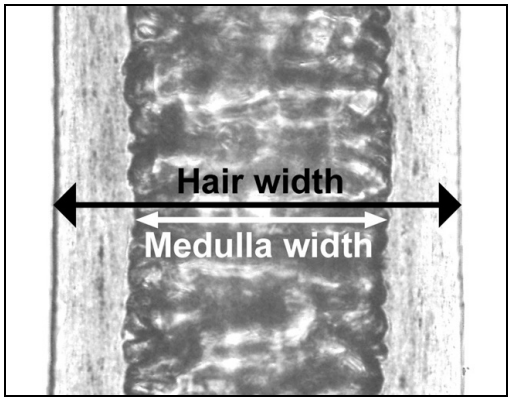


Fig. 4: Processing of hair and medulla width

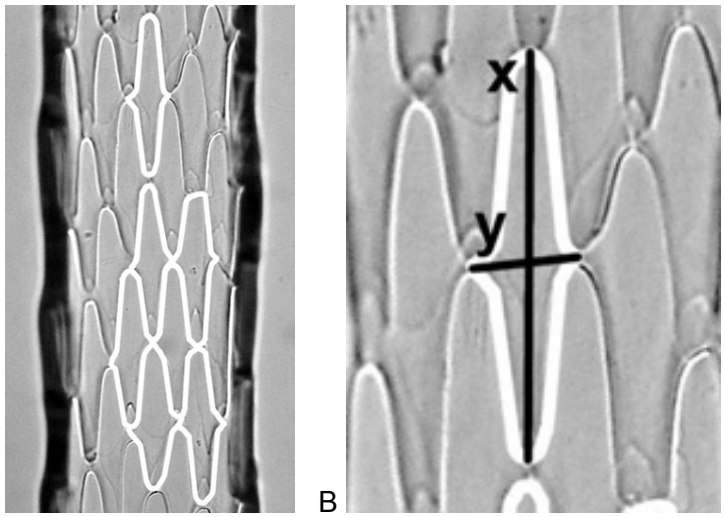


Fig. 5: Processing of the scale area and perimeter (A), and of the length X and width Y (B). The pictures were analysed using AxioVision. The white lines and the black lines on picture B were traced manually.

Hair and medulla width were obtained from 5 hairs/individual. For each individual, 100 cuticle scales were measured (e.g. 20 scales/hair drawn from 5 different hairs). The number of scales measured on each guard hair depended on the quality of the hairs and of the casts, but it was never necessary to measure scales from more than 10 hairs. The processed data were used to calculate the medullary index (ratio of medulla width to hair width) and the Y-/X-Feret (ratio of scale width to length).

1.2.3 Statistical analyses

Statistical analyses (ANOVA, Student's t-test) are based on mean values per individual. Differences between body regions and between groups are based on subsequent Post-hoc tests (Tukey).

1.3 Results

1.3.1 Description of hair characteristics

1.3.1.1 General characteristics

The hair characteristics observed (morphology, colour, shape of the cross section, structure of the medulla, arrangement pattern, shapes and distances of the free edges of the hair cuticle scales) were shared by all the individuals studied (Tab. 1). Only slight differences could be observed on the PHs from some body regions and on the PHs of the otters coming from South Asia (see 1.3.1.2).

Tab 1: General hair characteristics observed

Morphology (Fig. 6)	Fusiform (straight hair with typical shield)
Colour	Whitish/beige at the pars basalis and pars intermedia, shield (pars apicalis) brown to dark brown
Cross-section (Fig. 7)	Oblong-oval
Medulla (Fig. 8)	Wide lattice (A, B), tip (C) and base often fragmented (D) Cortex brown at the shield, whitish/beige at the pars intermedia and at the pars basalis
Hair cuticle pattern (Fig. 9)	Pars apicalis (A): irregular wave, rippled scale margins, distance between the scale margins: intermediate to close Pars intermedia (B): narrow diamond petal pattern, smooth scale margins, distance between the scale margins: intermediate Pars basalis (C): irregular wave, smooth scale margins, distance between the scale margins: intermediate

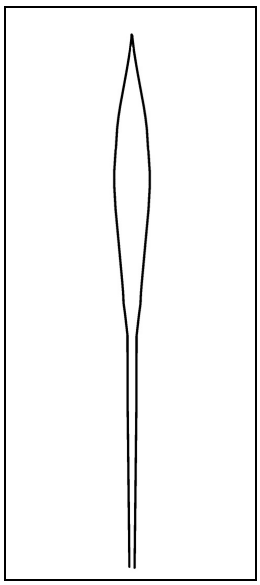


Fig. 6: Fusiform hair

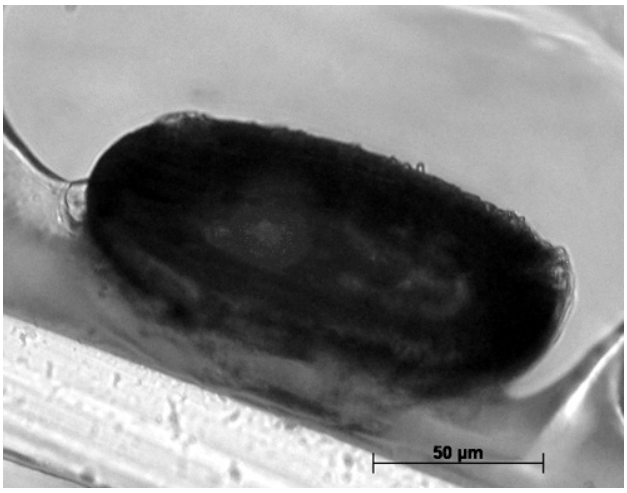


Fig. 7: Sectional plane at the middle of the shield of a hair glued between two pieces of scotch tape

Magnification scales: in the following pictures, the scales represent 10 µm. If not indicated otherwise, the scale in the picture on the right applies also to the photos beside.

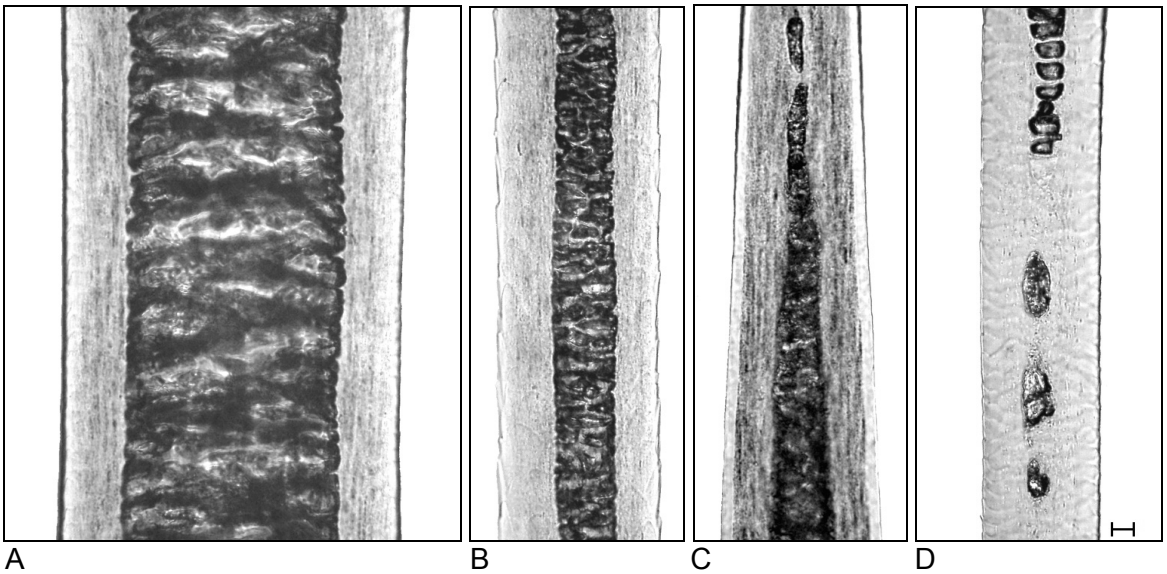


Fig. 8: Wide lattice medulla structure of a dorsal guard hair of the Eurasian otter *Lutra lutra* (A: view of the shield, B: view of the pars intermedia). The tip (C) and the base (D) are often fragmented (magnification bar: 10 µm)

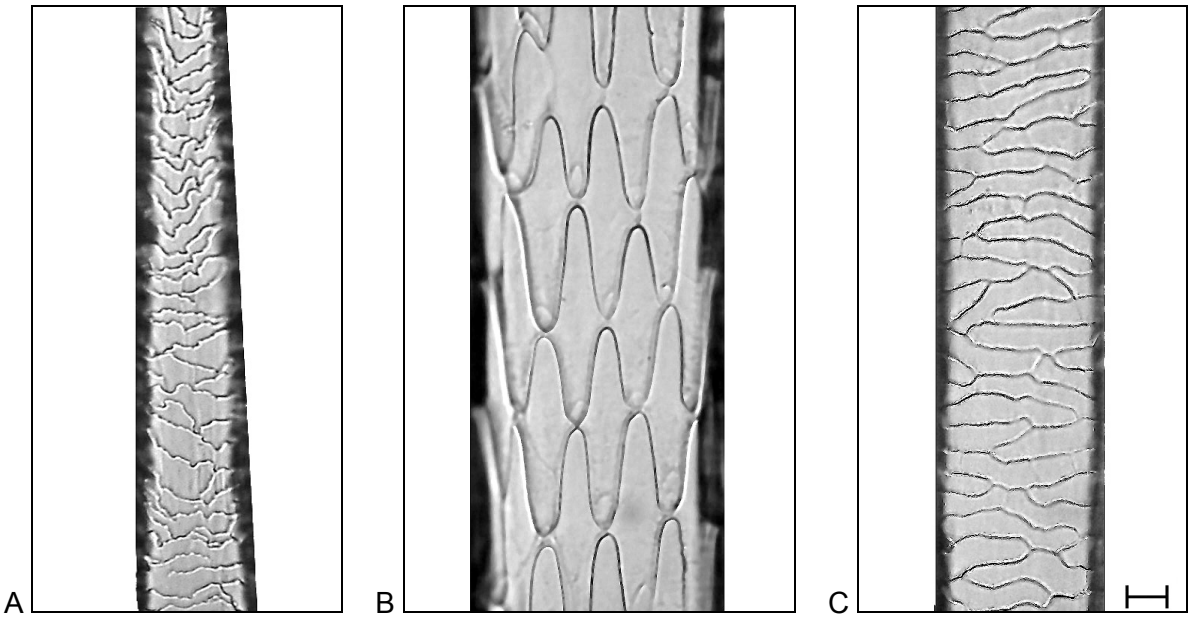


Fig. 9: Hair cuticle at the pars apicalis (A), pars intermedia (B) and pars basalis (C) of a dorsal guard hair of the Eurasian otter, *Lutra lutra* (magnification bar: 10 µm)

Concerning the hair cuticle structure, some comments can be made to complete the information given in Tab. 1. The irregular wave pattern seen at the tip was observed along the whole shield, with the scale margins becoming closer at the middle of the shield (Fig. 10A). At the lower part of the shield, the pattern changed into a mosaic pattern over a very short distance (Fig. 10B), before becoming a broad diamond petal pattern (with short scales, which were often as wide as long), at the part where the edges of the hair shaft became parallel (Fig. 10C). Beneath this narrowing of the hair shaft, the scales became longer and the cuticle pattern changed into the narrow diamond petal shown in Fig. 9B, with long, lanceolate and mostly symmetrical scales. This pattern was found along the rest of the hair shaft, which means also along the parallel edged part of the hair shaft, until the "final" pattern of the pars basalis (shown in Fig. 9C) was observed. The pars intermedia was quite short and it was difficult to define where this part exactly began and where it ended. Moreover, the shield became thinner very gradually at the PHs of *Lutra lutra*, without an obvious stricture at the middle of the hair shaft, like the one seen in Fig. 2. This can make it a little bit ambiguous to determine which pattern is the "pars intermedia pattern". As a rule, the pars intermedia pattern was the first "constant" pattern beneath the shield. Here the broad diamond petal pattern observed between the shield and the parallel-edged part of the hair shaft was seen only over a very short distance, and therefore was considered as a transition pattern. So the narrow diamond petal pattern was found to be the first constant pattern beneath the shield and the pars intermedia pattern proper. As already said, the pars basalis often has a varying hair cuticle pattern, and the structure described here was the lowest one observed, situated directly above the epidermis.

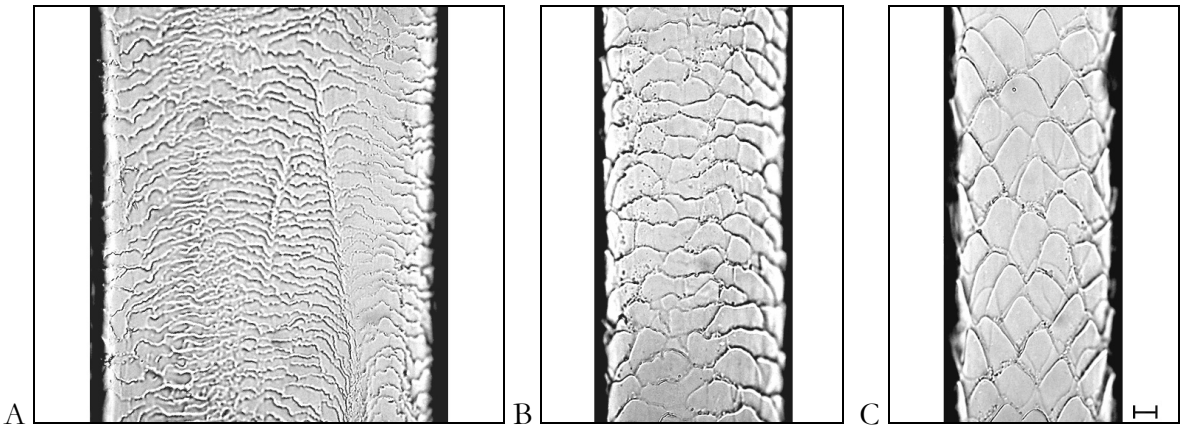


Fig. 10: Hair cuticle at the middle of the shield (A), at the base of the shield (B) and at the transition between the shield and the parallel edged part of the hair shaft (C)
(magnification bar: 10 μm)

1.3.1.2 Variability observed

1.3.1.2.1 Variability related to body region

Morphology: The morphology of the PHs from the abdomen (10) differed slightly from the rest of the body. The PHs were also fusiform but the shield was less distinctive.

Colour: The PHs from the abdomen (10) were whitish/beige all along the hair shaft, whereas the PHs from the other body regions were brown at the shield. The shield of the PHs from the shoulder (6), flank (7) and buttock (8) was light brown (brown to dark brown on the other body regions).

Medulla: In the PHs from the forehead (1) and abdomen (10), the medulla was clearly thinner. Moreover, in the hairs from the forehead and neck (2), the medulla was very thin and fragmented at the proximal part of the pars apicalis (base of the shield) and at the pars intermedia. Those interruptions were more important in the hairs from the forehead (Fig. 11A, B) but were not observed in the hairs from the abdomen.

Cuticle pattern: The cuticle pattern was similar in all the PHs studied, but in the hairs from the shoulder (6), flank (7) and buttock (8), the transition between the irregular wave pattern observed at the pars apicalis and the narrow diamond petal pattern observed at the pars intermedia was slightly longer. This transition was even longer in the PHs from the abdomen (10).

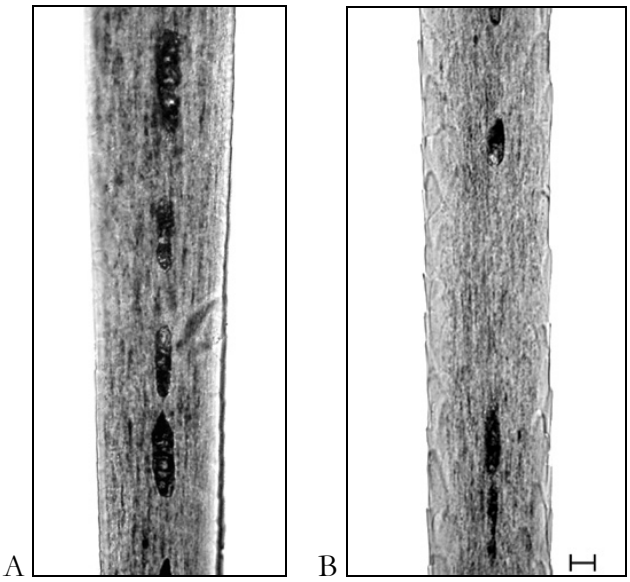


Fig. 11: Proximal part of the pars apicalis (A) and pars intermedia (B) of a PH from the forehead, with a fragmented medulla (magnification bar: 10 µm)

1.3.1.2.2 Variability related to climatic region

Medulla: The structure of the medulla of the otters coming from South Asia was the same as in the hairs of otters coming from Germany, but the medulla and the hair shaft were clearly thinner (Fig. 12).

Cuticle pattern: In the PHs of the otters coming from South Asia, the transition between the irregular wave pattern observed at the pars apicalis and the narrow diamond petal pattern observed at the pars intermedia was slightly longer. The scales were visibly smaller and stockier, particularly in the individuals from Sri Lanka (Fig. 13).

There were no visible difference between the PHs of the otters coming from Germany, the individual coming from the Lake Baikal, and the individual coming from Tunisia.

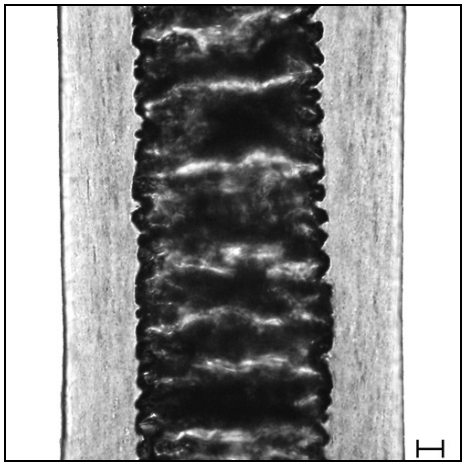


Fig. 12: Medulla at the middle of the shield of a PH of an otter coming from Sri Lanka. For comparison see Fig. 8A (the two pictures have the same magnification) (magnification bar: 10µm)

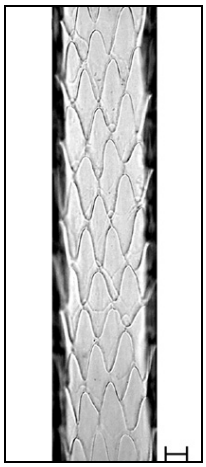


Fig. 13: Cuticle at the pars intermedia of a PH of an otter coming from Sri Lanka (magnification bar: 10 µm)

1.3.2 Metrical parameters

The shortest of all the measured guard hairs was 7 mm long and the longest 32 mm. The shield took up 35% to maximal 50% of the total hair shaft length (mostly between 40 and 50%). As it has already been remarked earlier (see 1.3.1.1), there was a smooth transition between the shield, which became thinner very gradually toward its base, and the parallel-edged part of the hair shaft. So the measured shield length can be influenced subjectively by the observer (estimated error:+/-1 mm). The analysed PH had a width between 86 µm and 182 µm. The medulla width extended from 39 to 122 µm, and the medullary index from 0.54 to 0.72. The cuticle scales had an area between 172 and 561 µm² and a perimeter between 65 and 146 µm. The scale length X ranged from 27 to 70 µm and the Y-/X-Feret from 0.18 to 0.62.

1.3.2.1 Possible influence of the body region

1.3.2.1.1 Hair length

Like in most mammals, the Eurasian otter had distinctly shorter hairs on the head than on the rest of the body. Indeed, the PHs measured in this study had a mean length ranging from 11 mm on the forehead (1, see Fig. 3) to 23 mm on the dorsum (medium and posterior) and flank (4,5,7). The graph in Fig. 14 shows the PH length measured on each body region (mean, minimal and maximal length). The shortest PHs, situated on the forehead, differed significantly from the PHs from every other body region (ANOVA, $F=17.67$, $p<0.001$, $n=50$, $R^2=0.80$). The longest PHs, situated on the mid- and posterior dorsum and on the flank, differed significantly, from the PHs on the forehead, and also from the PHs situated on the neck (2) and shoulder (6). The PHs that had an intermediate length were situated on the anterior dorsum, buttock, tail and abdomen (3,8,9,10), and differed significantly only from the PHs on the forehead.

Table 2 indicates the means and standard deviations calculated from the mean hair length of each individual on the different body regions.

1. Forehead
2. Neck
3. Dorsum (anterior, withers)
4. Dorsum (medium, back)
5. Dorsum (posterior)
6. Shoulder
7. Flank
8. Buttock
9. Tail
10. Abdomen (central)

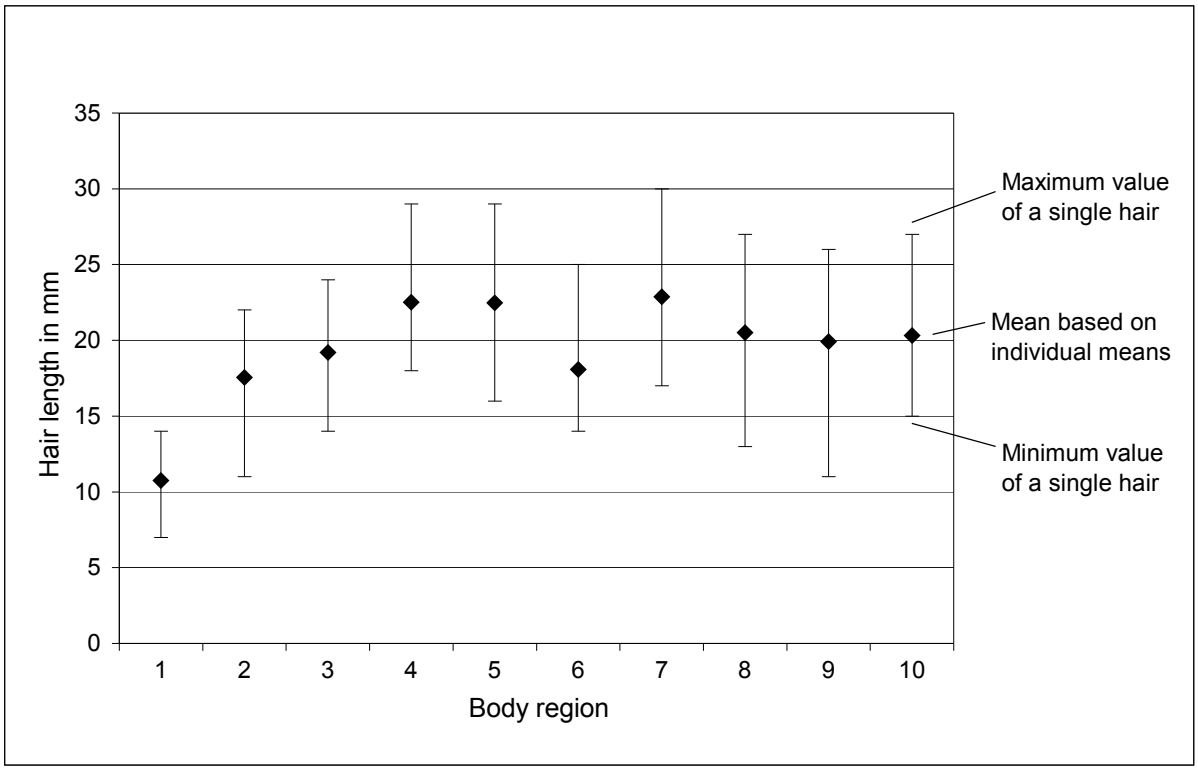


Fig. 14: Hair length on different body regions (mean, minimum and maximum values) (n=5 individuals)

Tab. 2: Means and standard deviations calculated from the mean hair length of each individual on the different body regions

	1	2	3	4	5	6	7	8	9	10
Hair length in mm	11 ± 1.4	18 ± 1.9	19 ± 1.2	23 ± 1.8	22 ± 2.1	18 ± 2.1	23 ± 1.4	21 ± 2.9	20 ± 1.9	20 ± 1.7

1.3.2.1.2 Hair width, medulla width and medullary index

The graphs in Figs. 15 and 16 show the hair width, medulla width and the medullary index (ratio of medulla width to hair width) for each body region (mean, minimum and maximum values).

Hair width: the mean hair width ranged between 132 µm on the forehead (1) and 165 µm on the tail (9). The posterior dorsum, shoulder, flank and buttock (5,6,7,8) had a mean hair width

of 160 µm. The PH width on the forehead differed significantly from every other body region, except for the neck (ANOVA, $F=8.68$, $p<0.001$, $n=50$, $R^2=0.66$). There was no significant difference between all the regions situated apart from the head (regions 3 to 10). The hair width at the neck differed significantly from the other regions except, of course from the forehead, and also from the anterior- and mid dorsum and the abdomen (1,3,4,10).

Medulla width: the mean medulla width ranged between 77 µm on the forehead and 105 µm on the tail. On the posterior dorsum and on the flank, the medulla width was 103 and 104 µm respectively. The medulla width on the forehead differed significantly from every other region, except from the neck and from the abdomen (ANOVA, $F=16.96$, $p<0.001$, $n=50$, $R^2=0.79$). These three regions (1,2,10) differed significantly from the mid- and posterior dorsum, from the flank, the buttock and the tail (4,5,7,8,9).

Medullary index: the lowest values for the mean medullary index were found on the forehead (0.58) and the abdomen (0.57), meaning that in these body regions the medulla was not only thinner, but also took up a smaller part of the hair diameter, and that the cortex was relatively more important. The maximum values (0.64-0.66) were found on the mid- and posterior dorsum, on the flank and on the tail (4,5,7,9). Only these two groups (1,10 vs. 4,5,7,9) differed significantly from each others (ANOVA, $F=6.62$, $p<0.001$, $n=50$, $R^2=0.60$).

Table 3 indicates the means and standard deviations calculated from mean hair width, medulla width and medullary index of each individual related to the different body regions.

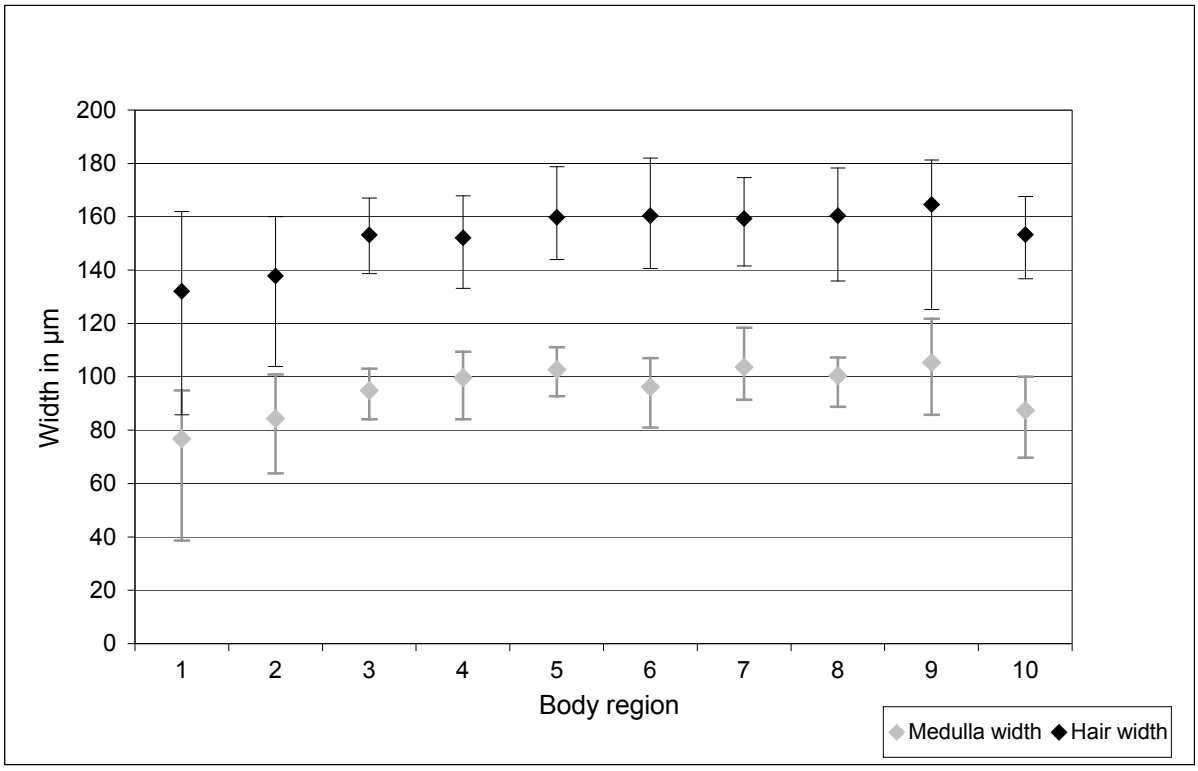


Fig. 15: Hair and medulla width on different body regions (mean, minimum and maximum values) (n=5 individuals)

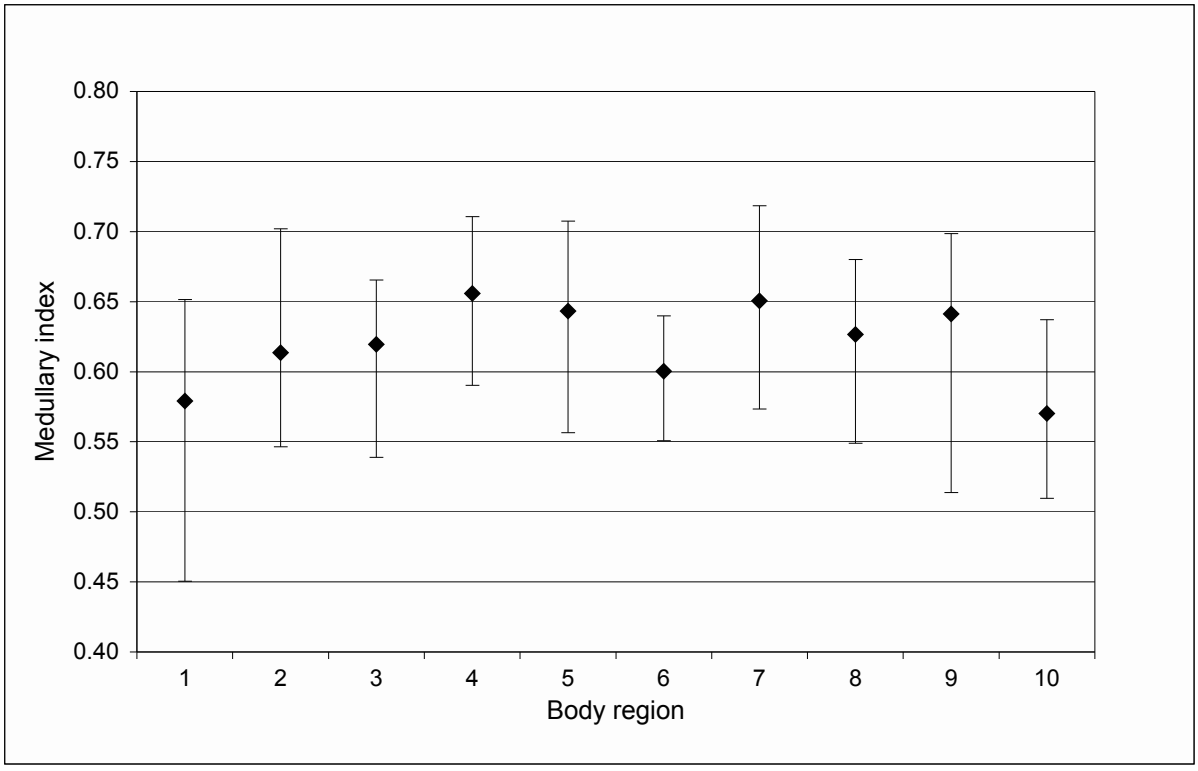


Fig. 16: Medullary index on different body regions (mean, minimum and maximum values) (n=5 individuals)

Tab. 3: Means and standard deviations calculated from the mean hair width, medulla width and medullary index of each individual on the different body regions

	1	2	3	4	5	6	7	8	9	10
Hair width in µm	132 ± 10.3	138 ± 3.9	153 ± 4.3	152 ± 3.3	160 ± 2.6	160 ± 1.5	159 ± 6.4	160 ± 2.3	165 ± 6.0	153 ± 4.2
Medulla width in µm	77 ± 12.7	84 ± 10.9	95 ± 6.9	100 ± 5.1	103 ± 6.5	96 ± 3.9	104 ± 7.3	100 ± 3.9	105 ± 10.8	87 ± 6.6
Medullary index	0.58 ± 0.04	0.61 ± 0.03	0.62 ± 0.02	0.66 ± 0.03	0.64 ± 0.02	0.60 ± 0.01	0.65 ± 0.02	0.63 ± 0.01	0.64 ± 0.04	0.57 ± 0.01

1.3.2.1.3 Cuticle scale parameters (area, perimeter, length and Y-/X-Feret)

The graphs in Figs. 17, 18, 19 and 20 show the cuticle scale area, perimeter, length and the Y-/X-Feret (ratio of scale width to scale length) for each body region (mean, minimum and maximum values). The width of the cuticle scales (Y) was used to calculate the Y-/X-Feret, but is not shown graphically and was not analysed statistically.

Scale area: the mean area of the cuticle scales ranged between 304 µm on the forehead (1) and 391 µm on the mid dorsum (4). The area on the forehead differed significantly from the area on the body regions 4,5,7 and 9 (ANOVA, $F=7.90$, $p<0.001$, $n=50$, $R^2=0.64$). The area on the mid dorsum differed significantly from the area on the body regions 1,2,3,6 and 10. All

the 3 regions with the smallest area (1,2,10) differed significantly from the 3 regions with the largest area (4,5,9).

Scale perimeter: the mean perimeter of the cuticle scales ranged between 97 µm on the forehead and 112 µm on the mid dorsum. Significant differences were found only between the 3 regions with the smallest perimeter (1,2,10) and the region with the largest perimeter, the mid dorsum. The region 1 also differed significantly from the region 5 (ANOVA, F=4.25, p=0.001, n=50, R^2=0.49).

Scale length X: the mean scale length ranged between 44 µm on the forehead and abdomen (1,10) and 50 µm on the anterior dorsum (5) and 51 µm on the mid dorsum (4). Significant differences were found only between the 3 regions with the shortest scales (1,2,10) and the region with the longest scales, the mid dorsum (ANOVA, F=3.65, p=0.002, n=50, R^2=0.45).

Y-/X-Feret: the mean values for the Y-/X-Feret varied only slightly from one body region to the other (values between 0.29 and 0.33). There were no significant differences between the different regions (ANOVA, F=0.77, p=0.65, n=50, R^2=0.15).

Table 4 indicates the means and standard deviations calculated from the mean scale area, scale perimeter, scale length and Y-/X-Feret of each individual on the different body regions.

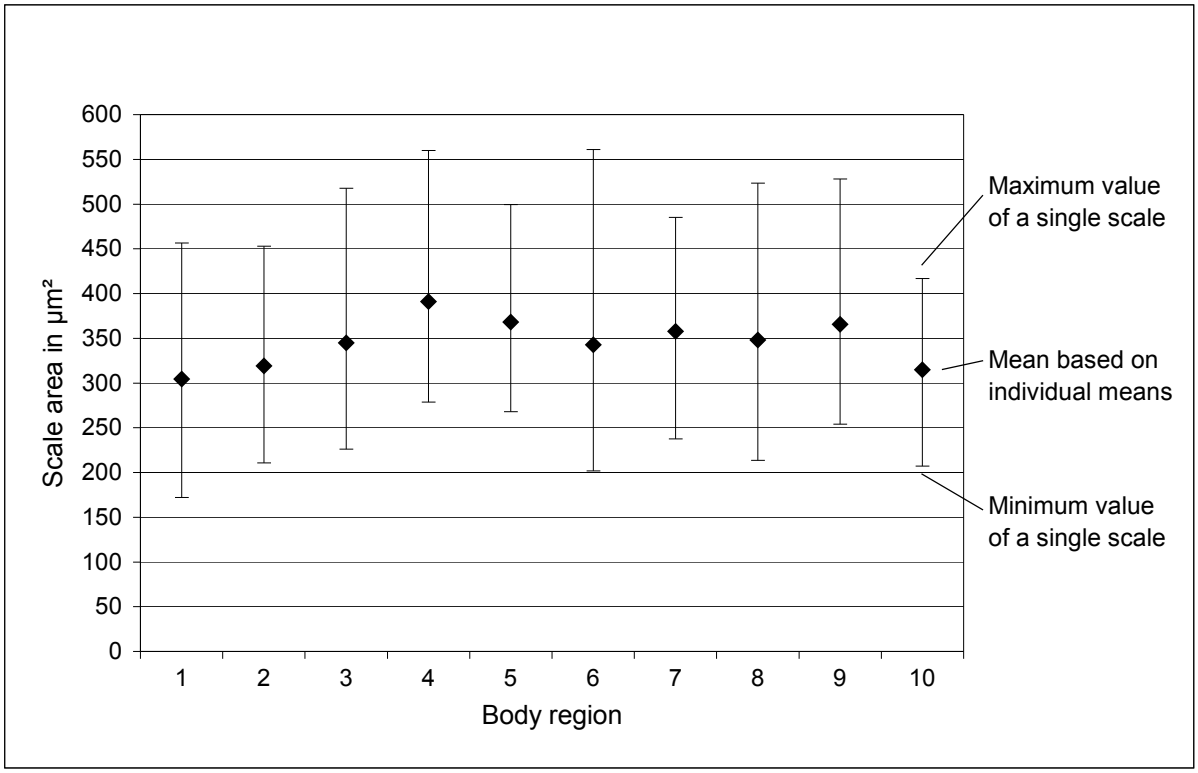


Fig. 17: Cuticle scale area on different body regions (mean, minimum and maximum values) (n=5 individuals)

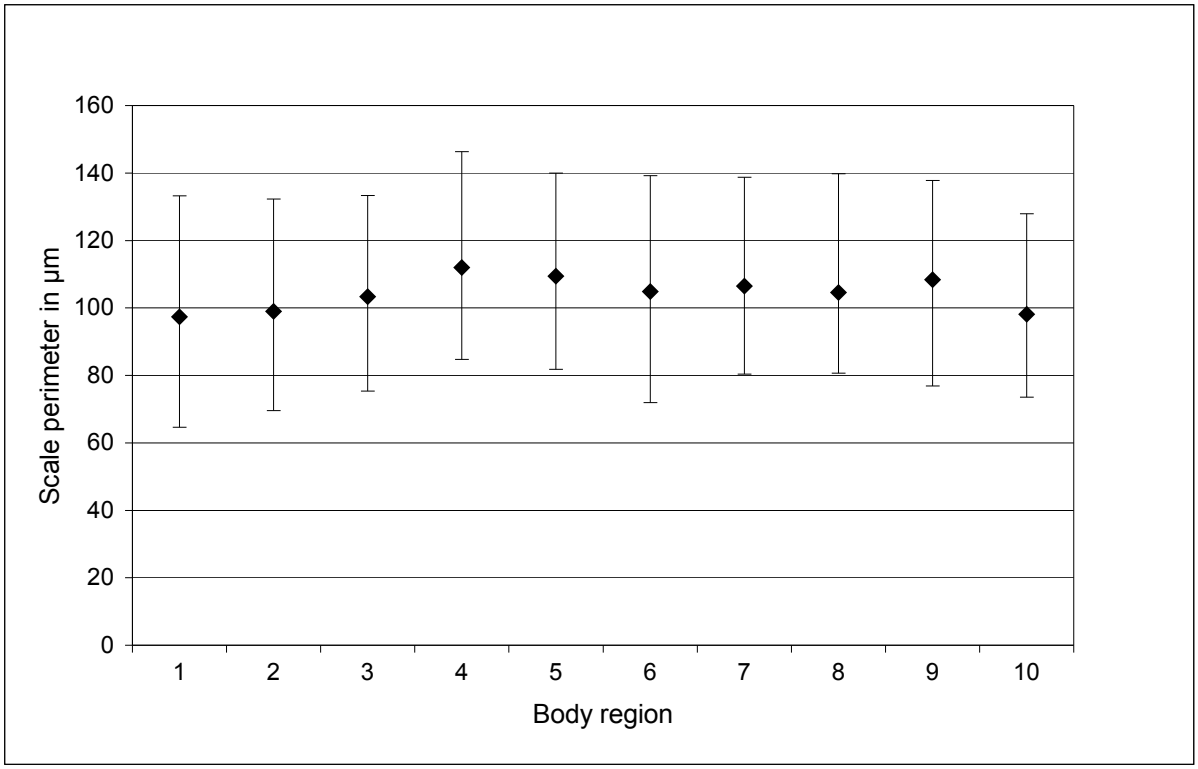


Fig. 18: Cuticle scale perimeter on different body regions (mean, minimum and maximum values) (n=5 individuals)

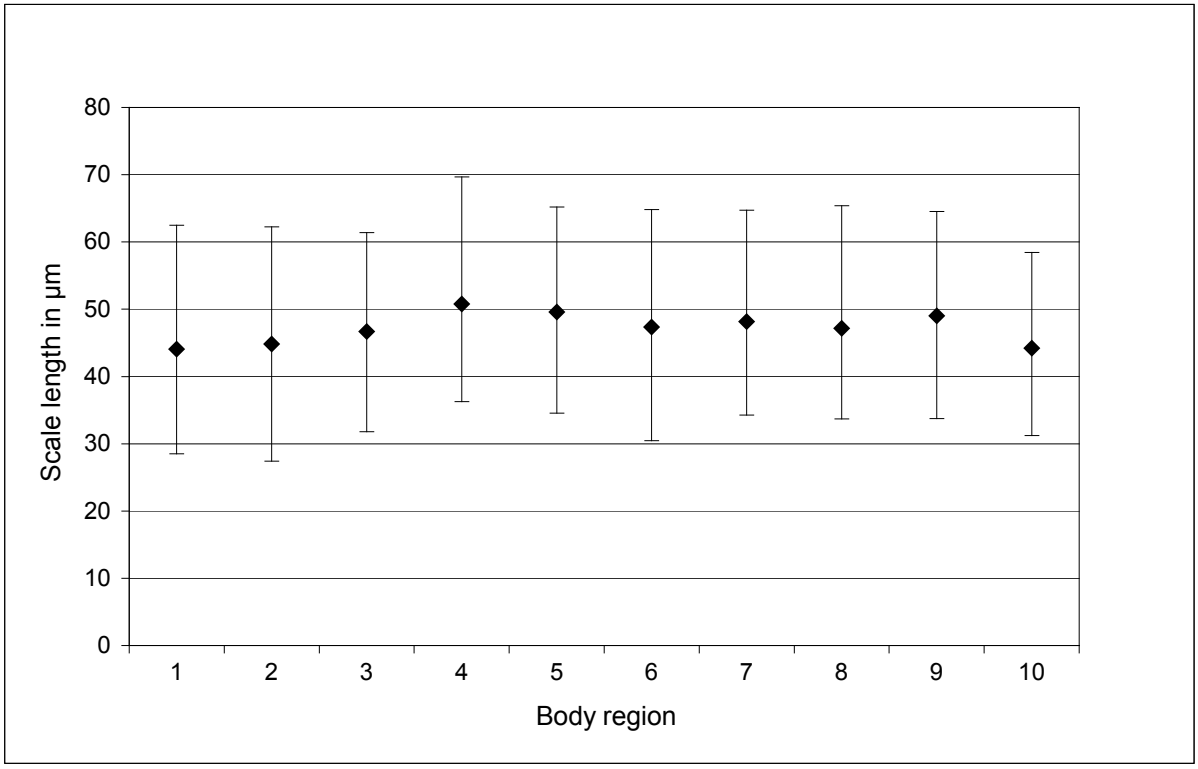


Fig. 19: Cuticle scale length X on different body regions (mean, minimum and maximum values) (n=5 individuals)

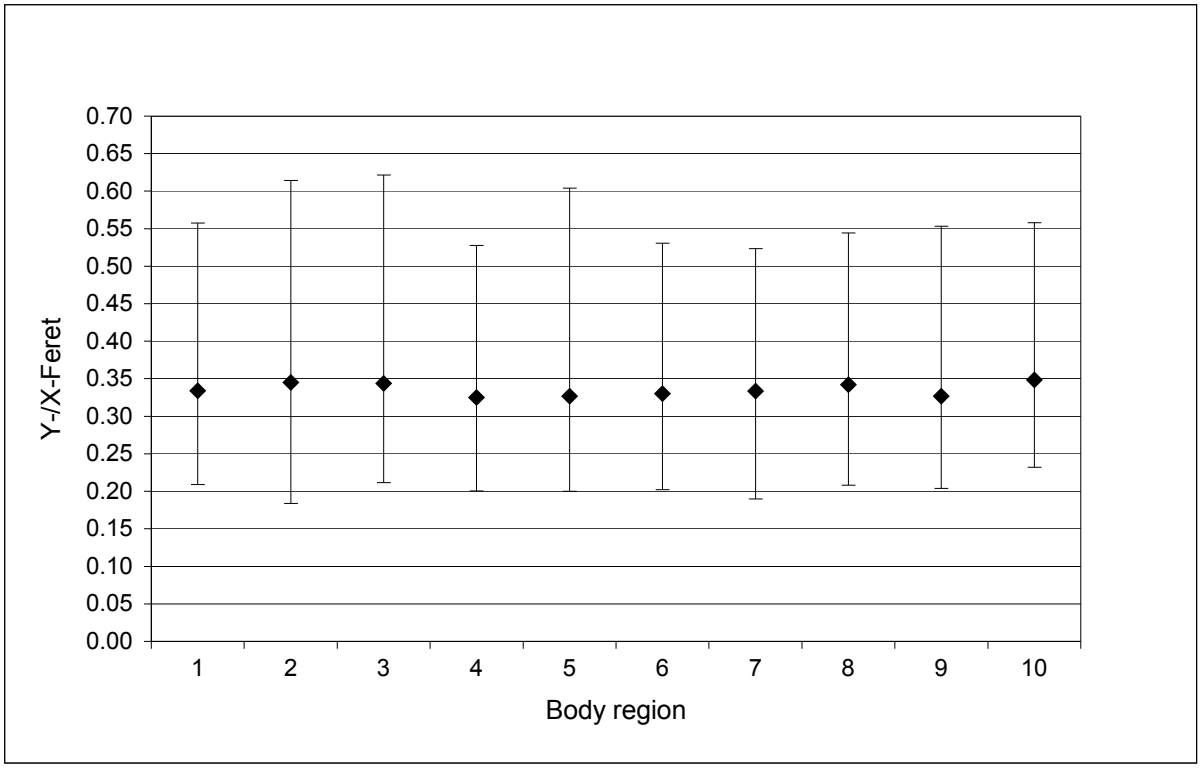


Fig. 20: Y-/X-Feret on different body regions (mean, minimum and maximum values) (n=5 individuals)

Tab. 4: Means and standard deviations calculated from the mean scale area, scale perimeter, scale length and Y-/X-Feret of each individual on the different body regions

	1	2	3	4	5	6	7	8	9	10
Area in µm²	304 ± 30.8	319 ± 20.8	345 ± 11.3	391 ± 14.7	368 ± 12.7	343 ± 25.4	358 ± 26.2	348 ± 26.6	366 ± 16.9	315 ± 18.7
Perim. in µm	97 ± 8.4	99 ± 7.6	103 ± 4.5	112 ± 2.5	109 ± 2.1	105 ± 6.0	107 ± 5.1	105 ± 5.0	108 ± 3.0	98 ± 6.1
Length X in µm	44 ± 4.0	45 ± 3.9	47 ± 2.5	51 ± 1.2	50 ± 1.0	47 ± 2.9	48 ± 2.5	47 ± 2.4	49 ± 1.5	44 ± 3.0
Y-/X-Feret	0.33 ± 0.03	0.35 ± 0.04	0.34 ± 0.02	0.33 ± 0.01	0.33 ± 0.01	0.33 ± 0.02	0.33 ± 0.02	0.34 ± 0.02	0.33 ± 0.01	0.35 ± 0.03

1.3.2.2 Possible influence of the sex

1.3.2.2.1 Hair length

The PH length on the dorsal body region of the females and the males is shown in Fig. 21 (mean, minimum and maximum values). The individual mean hair length of the females studied ranged from 20 mm to 23 mm, whereas the individual mean hair length of the males studied ranged between 22 and 25 mm. The hair length of the males differed significantly from the hair length of the females (Student's t-test, two-tailed, n=20, $p<0.05$).

Table 5 indicates the means and standard deviations calculated from the mean hair length of each individual from both sexes.

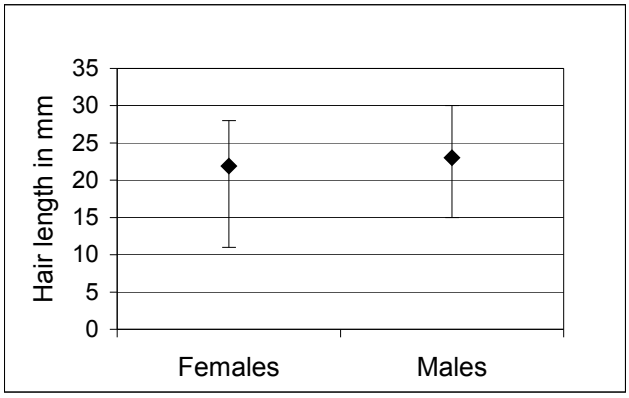


Fig. 21: Hair length of each sex (mean, minimum and maximum values) (n=10 individuals/sex)

Tab. 5: Means and standard deviations calculated from the mean hair length of each individual

	Females	Males
Hair length in mm	22 ± 0.8	23 ± 1.3

1.3.2.2.2 Hair width, medulla width and medullary index

The graphs in Figs. 22 and 23 show the hair width, medulla width and the medullary index (ratio of medulla width to hair width) of the females and the males (mean, minimum and maximum values).

Hair width and medulla width:

There was no significant difference between the sexes for the hair width and the medulla width (Student's t-test, two-tailed, n=20, p>0.05).

Medullary index: The medullary index for the females (0.66) was slightly higher than the medullary index for the males (0.64), this difference was significant (Student's t-test, two-tailed, n=20, p=0.049).

Table 6 indicates the means and standard deviations calculated from the mean hair width, medulla width and medullary index of each individual.

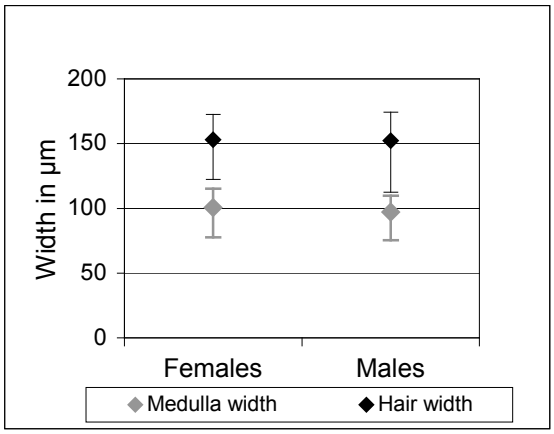


Fig. 22: Hair width and medulla width of each sex (mean, minimum and maximum values) (n=10 individuals/sex)

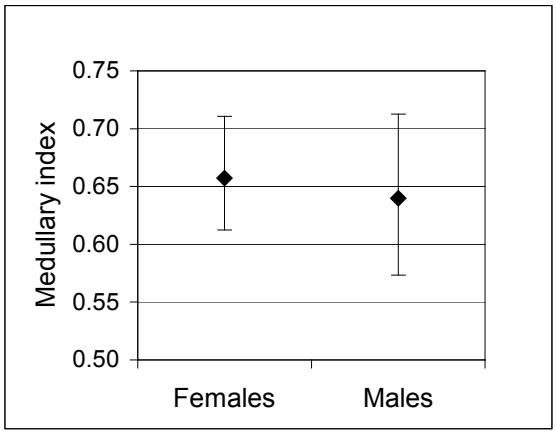


Fig. 23: Medullary index of each sex (mean, minimum and maximum values) (n=10 individuals/sex)

Tab. 6: Means and standard deviations calculated from the mean hair width, medulla width and medullary index of each individual

	Females	Males
Hair width in µm	153 ± 3.9	152 ± 5.4
Medulla width in µm	101 ± 7.0	97 ± 10.3
Medullary index	0.7 ± 0.02	0.6 ± 0.02

1.3.2.2.3 Cuticle scale parameters (area, perimeter, length X and Y-/X-Feret)

The cuticle scale area, perimeter, length X and Y-/X-Feret of the females and males are shown in Figs. 24, 25, 26 and 27 (mean, minimum and maximum values). There were no statistically significant differences between the sexes for any of the parameters analysed (Student's t-test, two-tailed, n=20, p>0.05).

Table 7 indicates the means and standard deviations calculated from the mean scale area, perimeter, length and Y-/X-Feret of each individual.

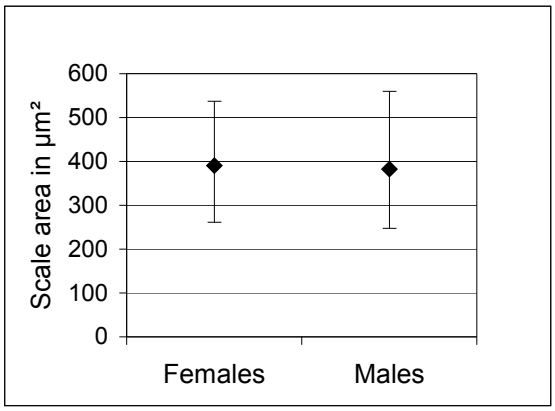


Fig. 24: Cuticle scale area of each sex (mean, minimum and maximum values) (n=10 individuals/sex)

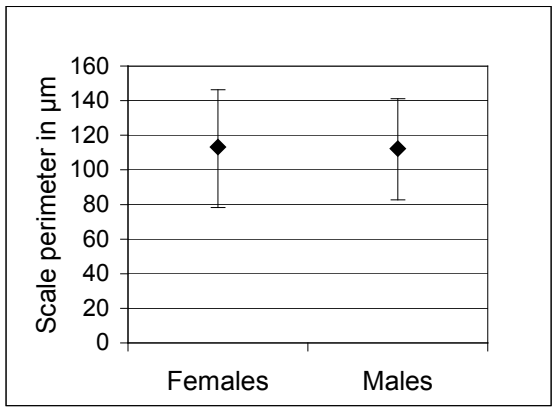


Fig. 25: Cuticle scale perimeter of each sex (mean, minimum and maximum values) (n=10 individuals/sex)

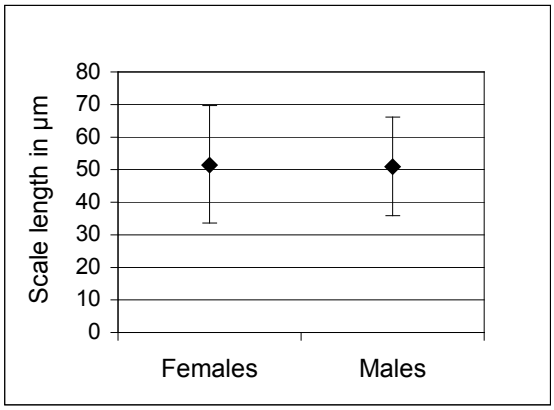


Fig. 26: Cuticle scale length X of each sex (mean, minimum and maximum values) (n=10 individuals/sex)

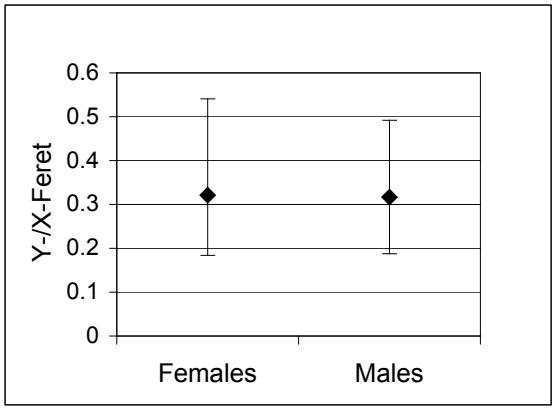


Fig. 27: Y-/X-Feret of each sex (mean, minimum and maximum values) (n=10 individuals/sex)

Tab. 7: Means and standard deviations calculated from the mean scale area, perimeter, length and Y-/X-Feret of each individual

	Females	Males
Area in µm²	390 ± 15.4	382 ± 21.3
Perimeter in µm	113 ± 3.8	112 ± 3.5
Length X in µm	51 ± 1.9	51 ± 1.7
Y-/X-Feret	0.32 ± 0.01	0.32 ± 0.01

1.3.2.3 Possible influence of the season

1.3.2.3.1 Hair length

The PH length of the individuals, which had died in winter and of those, which had died in summer is shown in Fig. 30 (mean, minimum and maximum values). There was no significant difference between the two seasons (Student's t-test, two-tailed, n=20, p>0.05).

Table 8 indicates the means and standard deviations calculated from the mean hair length of each individual.

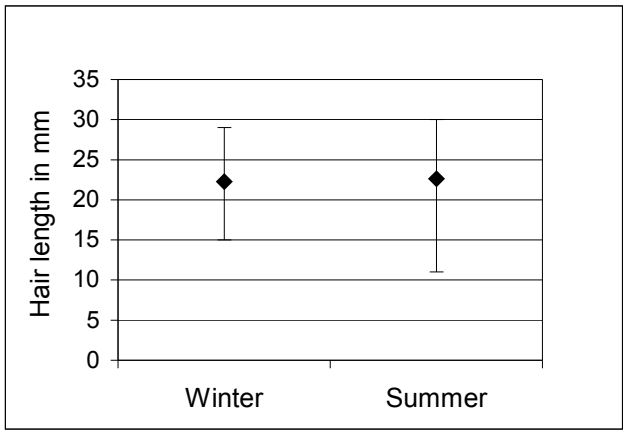


Fig. 28: Hair length in winter and summer (mean, minimum and maximum values)
(n=10 individuals/season)

Tab. 8: Means and standard deviations calculated from the mean hair length of each individual

	Winter	Summer
Hair length in mm	22 ± 1.0	23 ± 1.4

1.3.2.3.2 Hair width, medulla width and medullary index

The hair width, medulla width and medullary index of the individuals, which had died in winter and those, which had died in summer are shown in Figs. 29 and 30 (mean, minimum and maximum values). There were no significant differences found between the seasons for any of these parameters (Student's t-test, two-tailed, n=20, p>0.05).

Table 9 indicates the means and standard deviations calculated from the mean hair width, medulla width and medullary index of each individual.

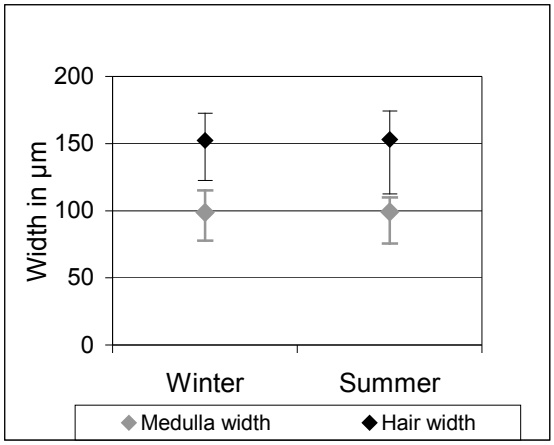


Fig. 29: Hair width and medulla width in winter and in summer (mean, minimum and maximum values) (n=10 individuals/season)

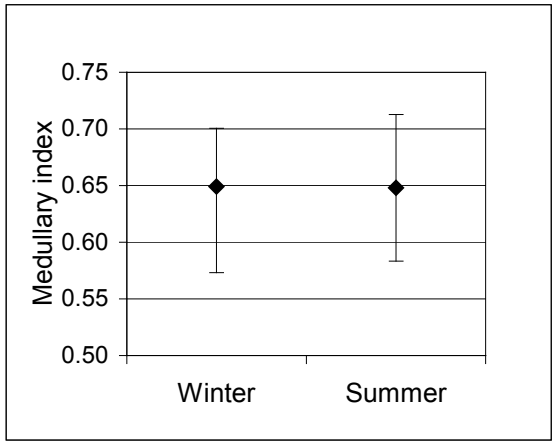


Fig. 30: Medullary index in winter and in summer (mean, minimum and maximum values) (n=10 individuals/season)

Tab. 9: Means and standard deviations calculated from the mean hair width, medulla width and medullary index of each individual

	Winter	Summer
Hair width in µm	152 ± 5.5	153 ± 11.2
Medulla width in µm	99 ± 3.4	99 ± 6.2
Medullary index	0.6 ± 0.02	0.6 ± 0.02

1.3.2.3.3 Cuticle scales parameters (area, perimeter, length X and Y-/X-Feret)

The cuticle scale area, perimeter, length X and Y-/X-Feret of the individuals, which had died in winter and those, which had died in summer are shown in Figs. 31, 32, 33 and 34 (mean, minimum and maximum values). There were no statistically significant differences between the seasons for any of the parameters analysed (Student's t-test, two-tailed, n=20, p>0.05). Table 10 indicates the means and standard deviations calculated from the mean scale area, perimeter, length and Y-/X-Feret of each individual.

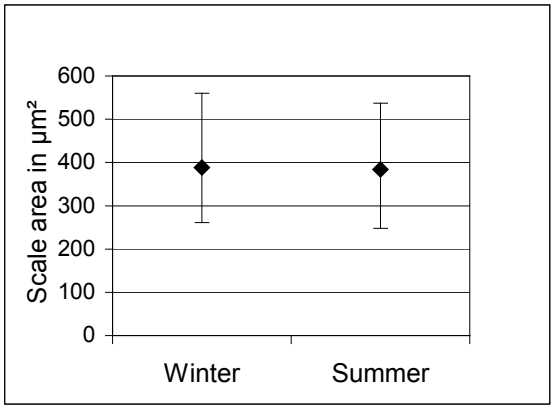


Fig. 31: Cuticle scale area in winter and in summer (mean, minimum and maximum values) (n=10 individuals/season)

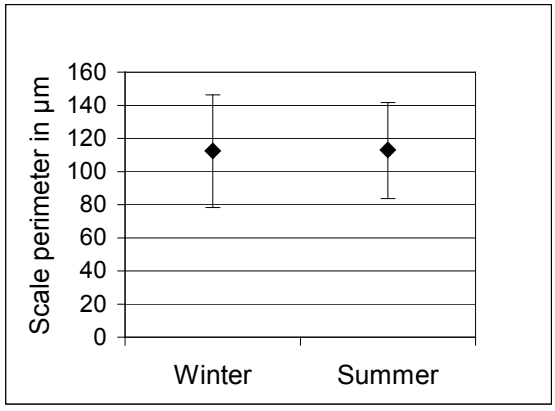


Fig. 32: Cuticle scale perimeter in winter and in summer (mean, minimum and maximum values) (n=10 individuals/season)

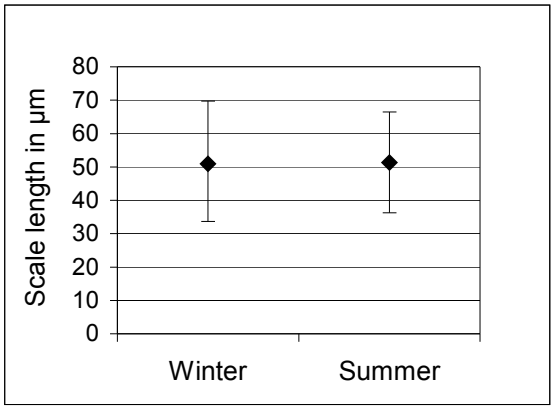


Fig. 33: Cuticle scale length X in winter and in summer (mean, minimum and maximum values) (n=10 individuals/season)

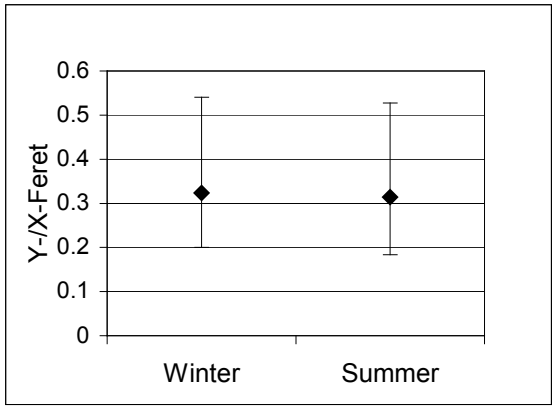


Fig. 34: Y-/X-Feret in winter and in summer (mean, minimum and maximum values) (n=10 individuals/season)

Tab. 10: Means and standard deviations calculated from the mean scale area, perimeter, length and Y-/X-Feret of each individual

	Winter	Summer
Area in µm²	389 ± 15.2	384 ± 22.0
Perimeter in µm	112 ± 3.3	113 ± 4.0
Length X in µm	51 ± 1.6	51 ± 2.0
Y-/X-Feret	0.32 ± 0.01	0.31 ± 0.01

1.3.2.4 Possible influences of the sample treatment, age and captivity

1.3.2.4.1 Hair length

The PH length of the standard group, the fresh samples group, the juveniles and the captivity group (mean, minimal and maximal values measured) is shown in Fig. 35. To remember, the standard group was made up by wild adult otters (2 males and 3 females), which had died accidentally in Germany at different times of the year (3 in winter und 2 in summer).

Standard and fresh samples groups had both a mean hair length of 23 mm; the minimum and maximum hair length was also the same for both groups. The juveniles had a higher mean hair length (25 mm) and also a higher maximum hair length (32 mm) than the standard group. Each individual in this group had a mean hair length between 24 and 26 mm. The captive otters showed a smaller mean hair length than the standard group (20 mm), and their PHs were not longer than 25 mm. Those differences were not statistically significant (ANOVA, $F=15.32$, $p<0.001$, $n=23$, $R^2=0.77$).

Table 11 indicates the means and standard deviations calculated from the mean hair length of each individual.

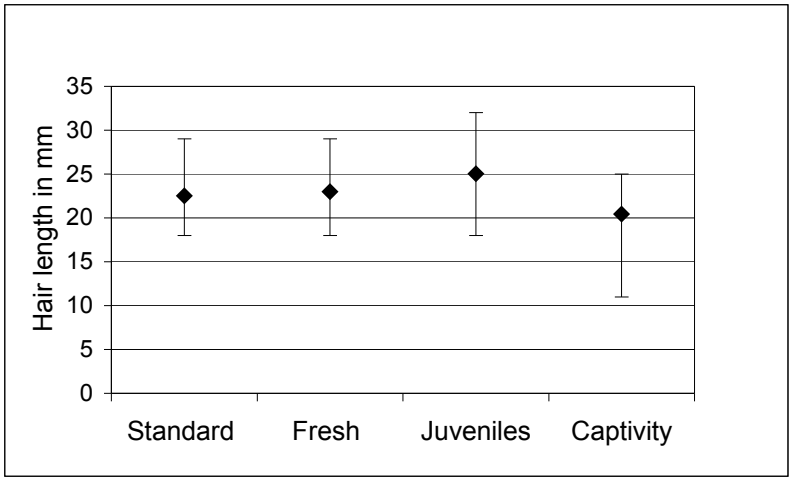


Fig. 35: Hair length of the standard, fresh samples, juveniles and captivity group (mean, minimum and maximum values) (n=5 ind./category)

Tab. 11: Means and standard deviations calculated from the mean hair length of each individual

	Standard	Fresh	Juveniles	Captivity
Hair length in mm	23 ± 1.8	23 ± 0.9	25 ± 0.9	20 ± 1.8

1.3.2.4.2 Hair width, medulla width and medullary index

The hair width, medulla width and medullary index of the standard group, the fresh samples group, the juveniles and the captivity group are shown in Figs. 35 and 36 (mean, minimum and maximum values). The PHs and the medulla were thinner in the captivity group but none of the differences between the different groups were significant for any of the analysed parameter (ANOVA, n=23, $p=0.017$, $F=3.99$ and $R^2=0.47$ for the hair width; $p<0.001$, $F=11.79$ and $R^2=0.72$ for the medulla width; $p<0.001$, $F=9.75$ and $R^2=0.68$ for the medullary index.) Table 12 indicates the means and standard deviations calculated from the mean hair width, medulla width and medullary index of each individual.

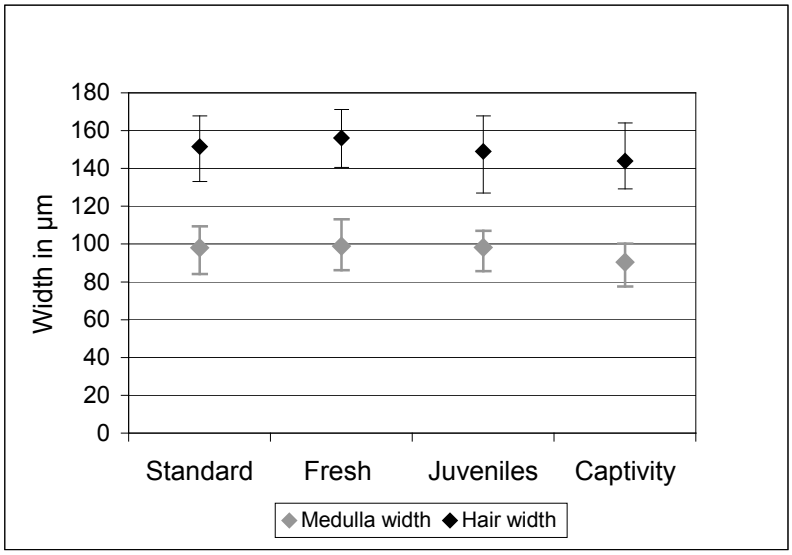


Fig. 36: Hair width and medulla width of the standard, fresh samples, juveniles and captivity group (mean, minimum and maximum values) (n=5 ind./category)

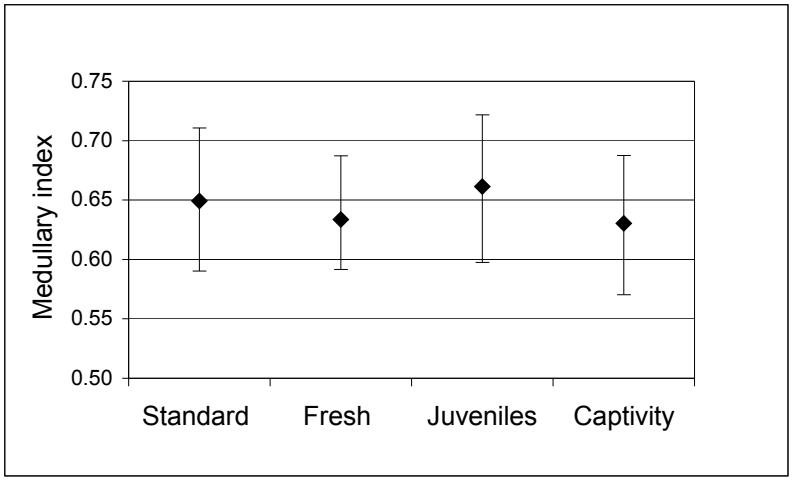


Fig. 37: Medullary index of the standard, fresh samples, juveniles and captivity group (mean, minimum and maximum values) (n=5 ind./category)

Tab. 12: Means and standard deviations calculated from the mean hair width, medulla width and medullary index of each individual

	Standard	Fresh	Juveniles	Captivity
Hair width in µm	151 ± 5.1	156 ± 3.8	149 ± 6.3	144 ± 5.6
Medulla width in µm	98 ± 3.3	99 ± 4.6	98 ± 5.5	90 ± 3.6
Medullary index in µm	0.65 ± 0.03	0.63 ± 0.02	0.66 ± 0.02	0.63 ± 0.02

1.3.2.4.3 Cuticle scale parameters (area, perimeter, length X and Y-/X-Feret)

The cuticle scale area, perimeter, length X and Y-/X-Feret of the standard group, the fresh samples group, the juveniles and the captivity group are shown in Figs. 38, 39, 40 and 41 (mean, minimal and maximal values measured). Only the captivity group differed significantly from the standard group, for every parameter (ANOVA, $p<0.001$, $n=23$, $F=21.80$ and $R^2=0.83$ for the area; $F=27.71$ and $R^2=0.86$ for the perimeter; $F=29.08$ and $R^2=0.87$ for the scale length; $F=35.10$ and $R^2=0.89$ for the Y-/X-Feret).

Table 13 indicates the means and standard deviations calculated from the mean scale area, perimeter, length and Y-/X-Feret of each individual.

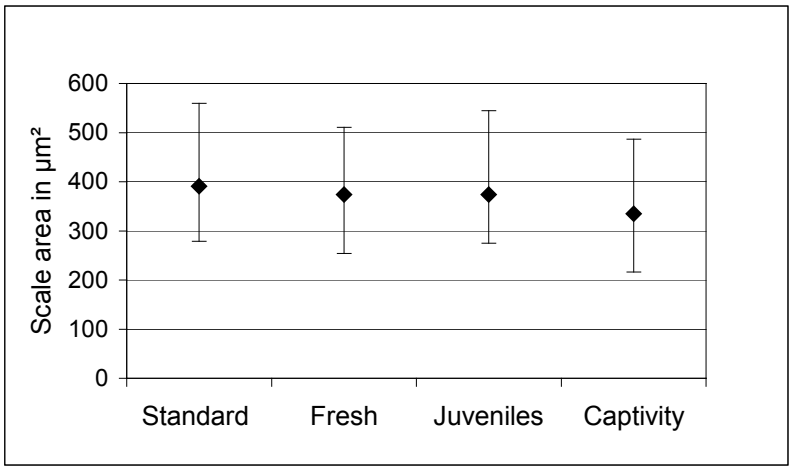


Fig. 38: Cuticle scale area of the standard, fresh samples, juveniles and captivity group (mean, minimum and maximum values) (n=5 ind./category)

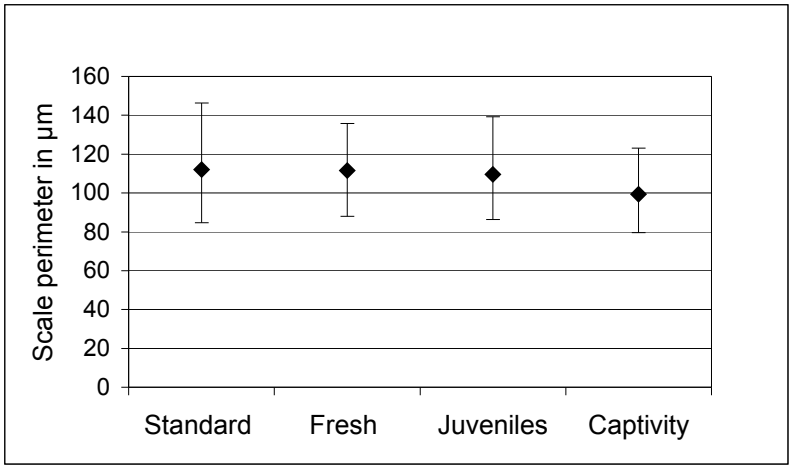


Fig. 39: Cuticle scale perimeter of the standard, fresh samples, juveniles and captivity group (mean, minimum and maximum values) (n=5 ind./category)

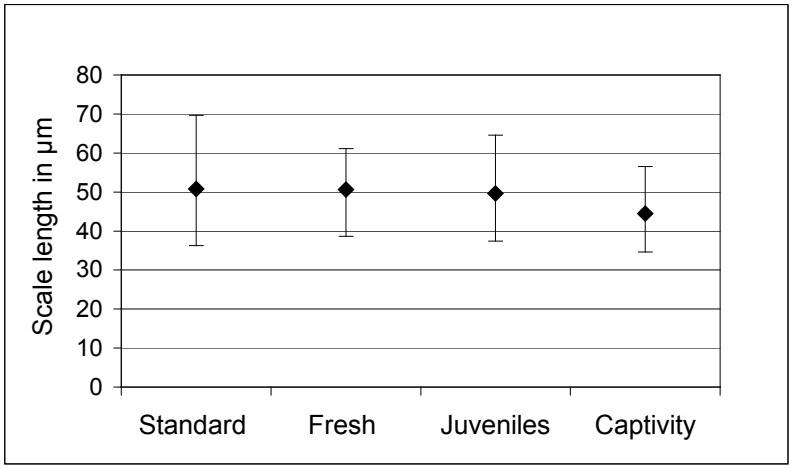


Fig. 40: Cuticle scale length X of the standard, fresh samples, juveniles and captivity group (mean, minimum and maximum values) (n=5 ind./category)

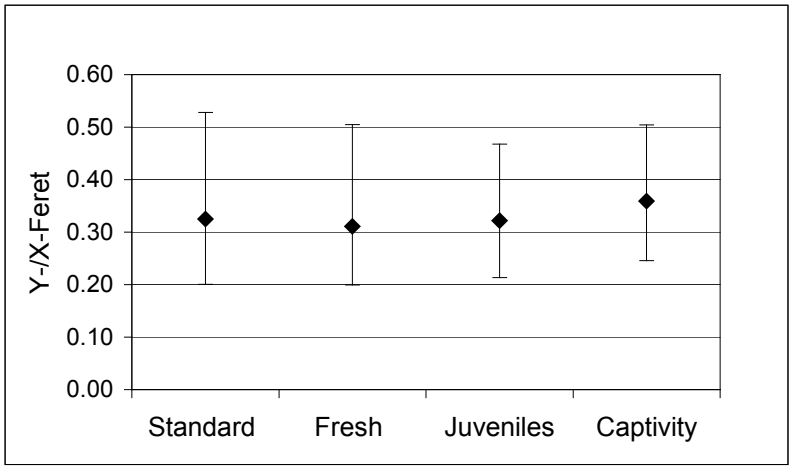


Fig. 41: Y-/X-Feret of the standard, fresh samples, juveniles and captivity group (mean, minimum and maximum values) (n=5 ind./category)

Tab. 13: Means and standard deviations calculated from the mean scale area, perimeter, length and Y-/X-Feret of each individual

	Standard	Fresh	Juveniles	Captivity
Area in µm²	391 ± 14.7	374 ± 22.2	374 ± 12.8	335 ± 18.3
Perimeter in µm	112 ± 2.5	111 ± 4.6	110 ± 3.8	99 ± 3.3
Length X in µm	51 ± 1.2	51 ± 2.1	50 ± 1.9	44 ± 1.5
Y-/X-Feret	0.33 ± 0.01	0.31 ± 0.01	0.32 ± 0.01	0.36 ± 0.01

1.3.2.5 Possible influence of the factor climatic region

1.3.2.5.1 Hair length

The PH length of the standard group from Germany and the individuals coming from Lake Baikal, Tunisia, India and Sri Lanka is shown in Fig. 42 (mean, minimum and maximum values). The standard group had a mean hair length of 23 mm, but the individuals in this group exhibited a mean hair length between 20 and 25 mm. So the mean hair length of the Lake Baikal (25 mm) and the Tunisia (24 mm) otters were within the range of the values of the standard group, whereas the mean hair length of the individuals coming from India (18 mm) and from Sri Lanka (17 mm and 19 mm), were below this range. The individual coming from India and the two individuals coming from Sri Lanka were put together in a “South Asia group”, which had a mean hair length of 18 mm. The PHs of the South Asia group were significantly shorter than the PHs of the standard group (ANOVA, $F=15.32$, $p<0.001$, $n=23$, $R^2=0.77$).

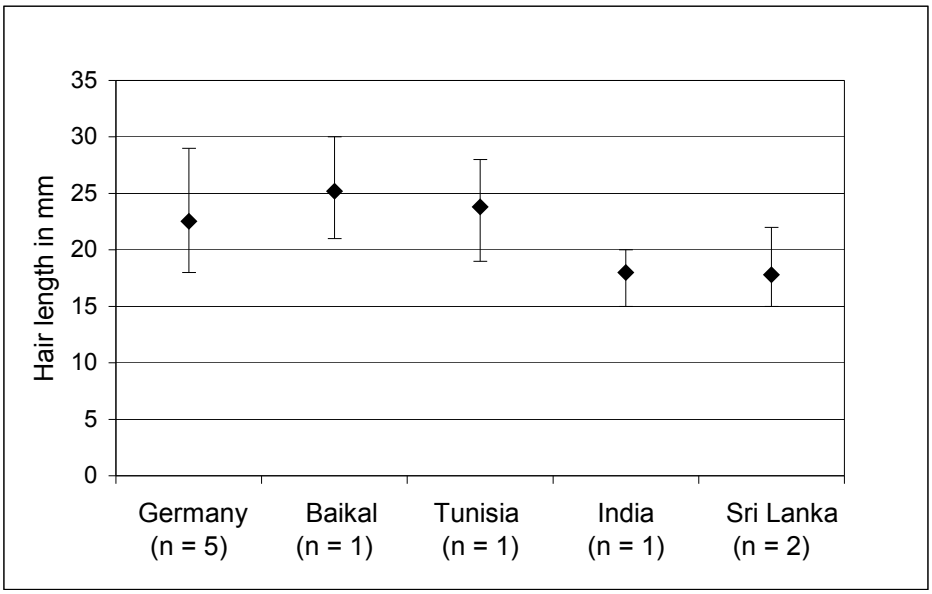


Fig. 42: Hair length of the individuals coming from Germany, Lake Baikal, Tunisia, India and Sri Lanka (mean, minimum and maximum values)

1.3.2.5.2 Hair width, medulla width and medullary index

The hair width, medulla width and medullary index of the standard group coming from Germany and the individuals coming from Lake Baikal, Tunisia, India and Sri Lanka are shown in Figs. 43 and 44 (mean, minimum and maximum values).

Hair width: The mean hair width of the Lake Baikal and of the India otters were within the range of the individual values of the Germany group (146 to 158 µm). The mean hair width of the Tunisia individual (127 µm) and of the two Sri Lanka individuals (130 and 134 µm) were below this range. The mean hair width of the South Asia group (139 µm) was lower than the mean hair width of the Germany group (151 µm), this difference was not significant (ANOVA, $F=3.99$, $p=0.017$, $n=23$, $R^2=0.47$).

Medulla width: The individuals of the Germany group had a mean medulla width ranging from 96 µm to 105 µm. The mean medulla width of the Baikal otter (95 µm) was within this range, whereas the values of the Tunisia (84 µm), India (86 µm) and Sri Lanka individuals (73 and 78 µm) were lower. The medulla of the South Asia group (mean medulla width: 79 µm) was significantly thinner than the medulla of the Germany group (ANOVA, $F=11.79$, $p<0.001$, $n=23$, $R^2=0.72$).

Medullary index: The otters from Germany had a mean medullary index going from 0.62 to 0.69. The mean medullary index of the Baikal individual (0.62) and of the Tunisia individual (0.66) were within this range, whereas the medullary index of the India (0.56) and the two Sri Lanka otters (0.56 and 0.58) were lower. The medullary index of the South Asia group (mean medullary index: 0.57) was significantly lower than the medullary index of the Germany group (ANOVA, $F=9.75$, $p<0.001$, $n=23$, $R^2=0.68$).

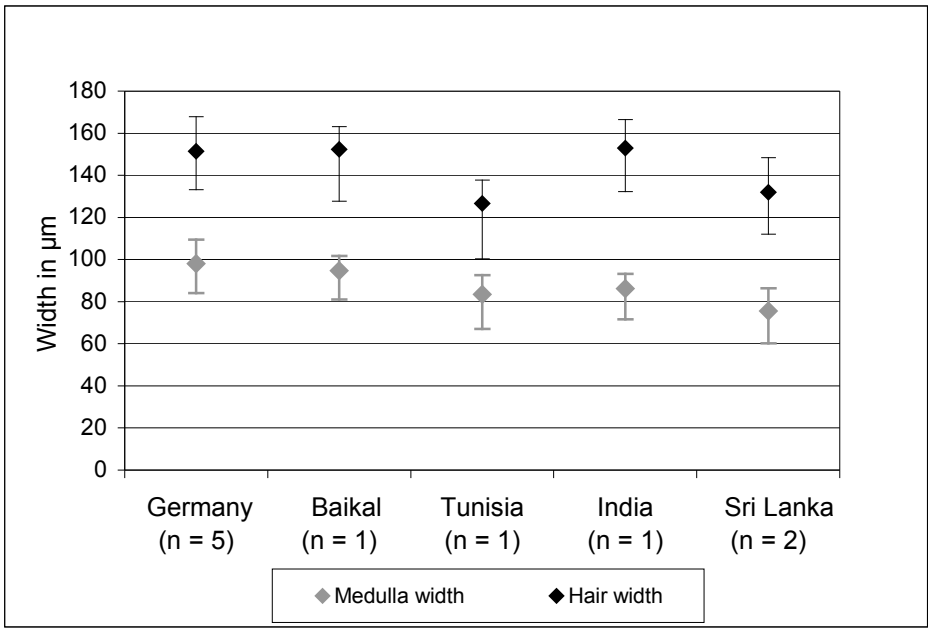


Fig. 43: Hair width and medulla width of the individuals coming from Germany, Lake Baikal, Tunisia, India and Sri Lanka (mean, minimum and maximum values)

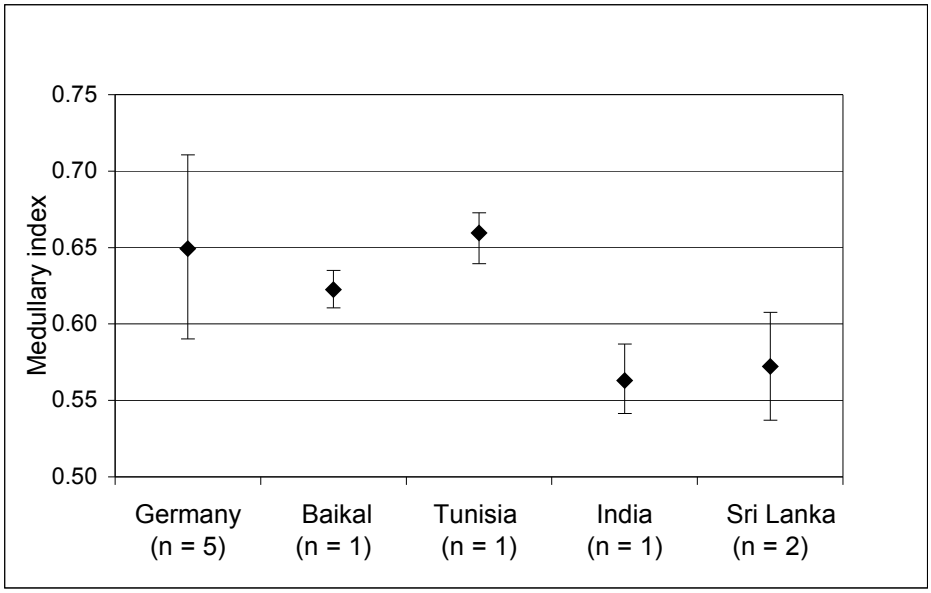


Fig. 44: Medullary index of the individuals coming from Germany, Lake Baikal, Tunisia, India and Sri Lanka (mean, minimum and maximum values)

1.3.2.5.3 Cuticle scales parameters (area, perimeter, length X and Y-/X-Feret)

The cuticle scale area, perimeter, length X and Y-/X-Feret of the standard group coming from Germany and the individuals coming from Lake Baikal, Tunisia, India and Sri Lanka are shown in Figs. 45, 46, 47 and 48.

Scale area: The otters coming from Germany had a mean cuticle scale area ranging from 379 to 417 µm². The mean scale area of the Lake Baikal individual (366 µm²), the Tunisia individual (318 µm²), the India individual (305 µm²) and the two Sri Lanka individuals (259 and 279 µm²) were below this range. The scale area of the South Asian otters (mean scale area: 281 µm²) was significantly smaller than the scale area of the German otters (ANOVA, $F=21.80$, $p<0.001$, $n=23$, $R^2=0.83$).

Scale perimeter: The otters coming from Germany had a mean cuticle scale perimeter ranging from 109 to 116 µm. The mean scale perimeter of the Lake Baikal individual (104 µm), the Tunisia individual (98 µm), the India individual (93 µm) and the two Sri Lanka individuals (81 and 85 µm) were below this range. The scale perimeter of the South Asia group (mean scale perimeter: 86 µm) was significantly smaller than the scale perimeter of the Germany group (ANOVA, $F=27.71$, $p<0.001$, $n=23$, $R^2=0.86$).

Scale length X: The otters coming from Germany had a mean cuticle scale length ranging from 49 to 53 µm. The mean scale length of the Lake Baikal individual (47 µm), the Tunisia individual (44 µm), the India individual (42 µm) and the two Sri Lanka individuals (36 and 37 µm) were below this range. The scale length of the otters coming from South Asia (mean scale length: 38 µm) was significantly smaller than the scale length of the German otters (ANOVA, $F=29.08$, $p<0.001$, $n=23$, $R^2=0.87$).

Y-/X-Feret: The otters coming from Germany had a mean Y-/X-Feret ranging from 0.32 to 0.34. The mean Y-/X-Feret of the Lake Baikal individual (0.35), the Tunisia individual (0.35), the India individual (0.38) and the two Sri Lanka individuals (0.43) were above this range. The Y-/X-Feret of the South Asia group (mean Y-/X-Feret: 0.42) was significantly higher than the Y-/X-Feret of the Germany group (ANOVA, $F=35.10$, $p<0.001$, $n=23$, $R^2=0.89$).

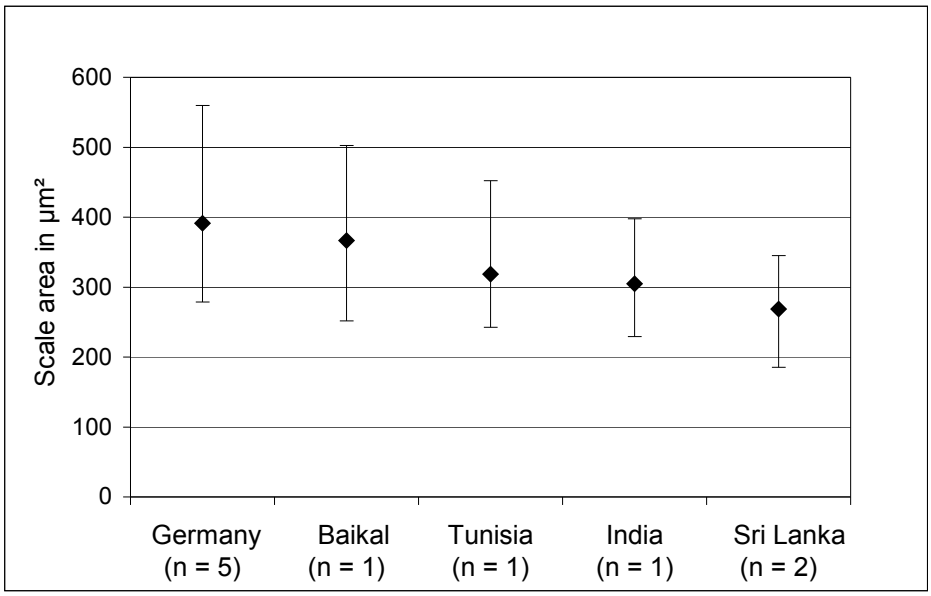


Fig. 45: Cuticle scale area of the individuals coming from Germany, Lake Baikal, Tunisia, India and Sri Lanka (mean, minimum and maximum values)

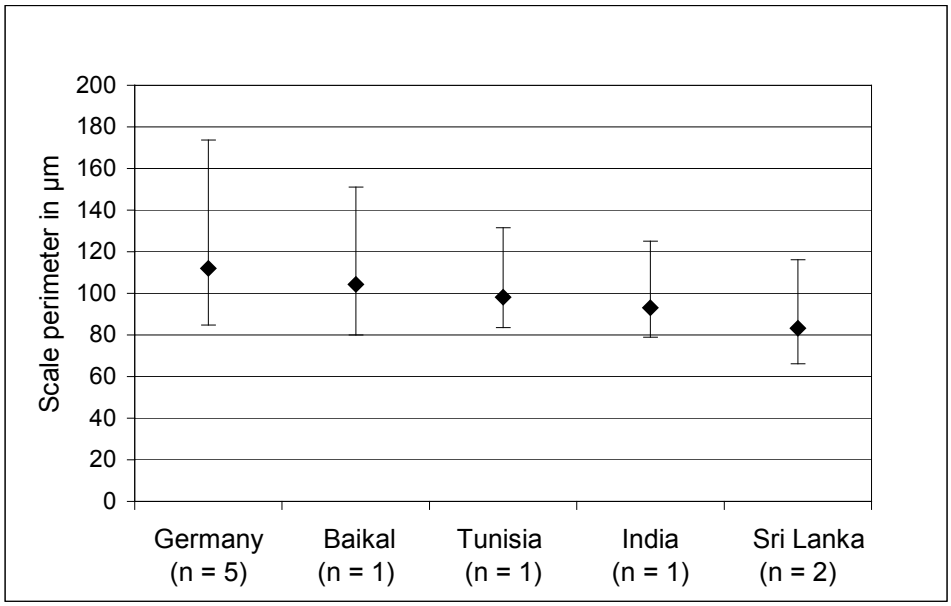


Fig. 46: Cuticle scale perimeter of the individuals coming from Germany, Lake Baikal, Tunisia, India and Sri Lanka (mean, minimum and maximum values)

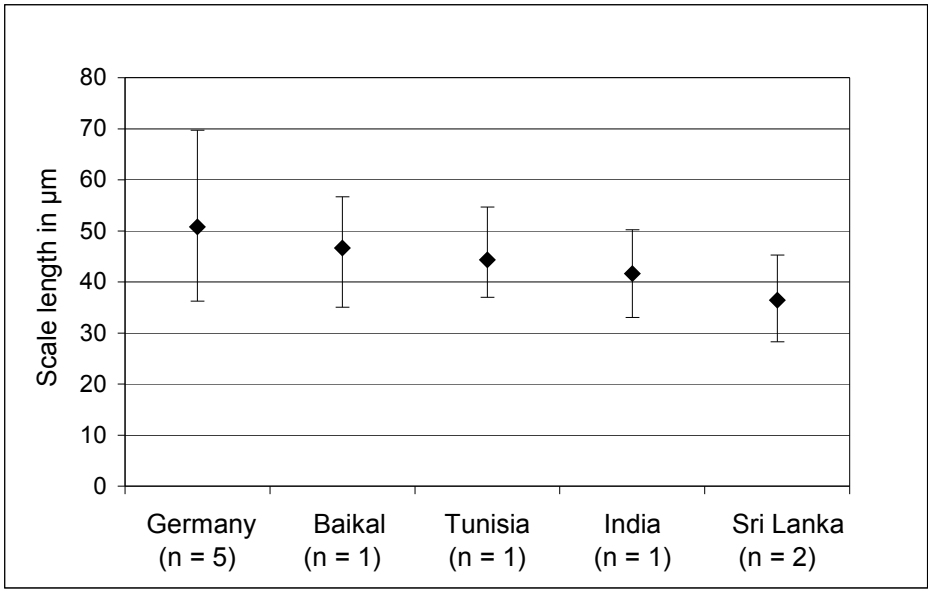


Fig. 47: Cuticle scale length X of the individuals coming from Germany, Lake Baikal, Tunisia, India and Sri Lanka (mean, minimum and maximum values)

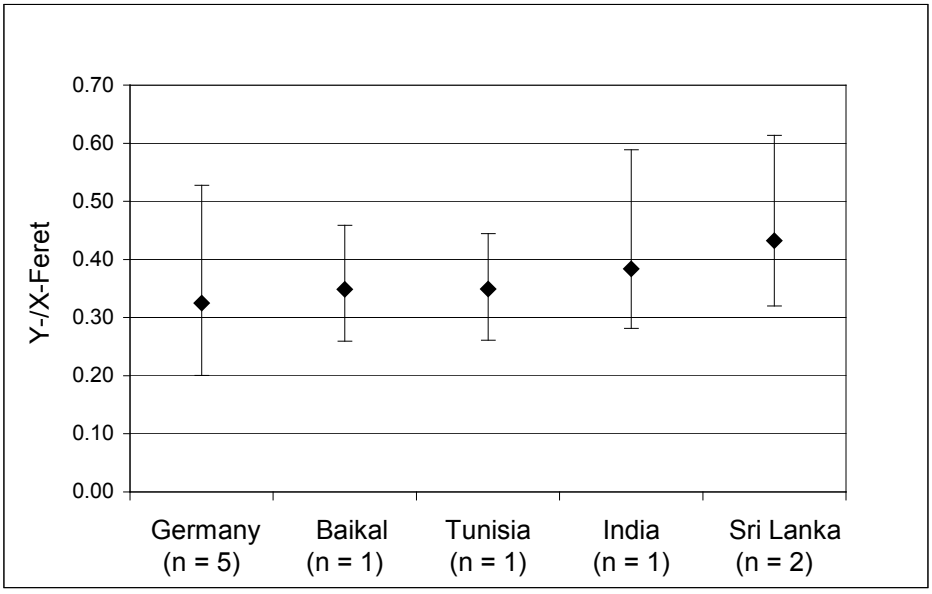


Fig. 48: Y-/X-Feret of the individuals coming from Germany, Lake Baikal, Tunisia, India and Sri Lanka (mean, minimum and maximum values)

1.3.2.6 Summary

The factors body region and climatic region had the most intensive influence on the PH parameters measured in the court of this study, confirming the observations made in 1.3.1.2.

The factor body region had an influence on every parameter, except for the Y-/X-Feret. The most distinct differences between the body regions were observed for the hair length. For each parameter, the minimum values were observed on the forehead, except for the me-

dullary index, which was slightly smaller on the abdomen. Forehead and neck were the regions with the thinnest hairs. Forehead, neck and abdomen constituted the group with the thinnest medulla and the smallest cuticle scales (area, perimeter and length). The maximum values were not always found on the same body regions. However, the dorsal body regions (mid- and/or posterior dorsum) always belonged to the group with the highest values. High values were also measured on the flank, buttock and tail, but not for every parameter. The PHs on the shoulder were relatively short (only the PHs on the forehead and neck were shorter) but rather thick. The medulla was comparatively thin, and therefore the medullary index was low (only the forehead and abdomen had a lower medullary index).

The factor sex had an influence only on hair length, i.e. the females had shorter PHs. The factor season had no influence on the PH structure. The PHs taken from fresh carcasses did not differ from the PHs of the standard group, taken on tanned or dried pelts. The juveniles had longer hairs than the standard group, but this difference was not statistically significant. They did not differ from the standard group for the other parameters. The captive otters differed significantly from the standard group for the cuticle scale parameters (area, perimeter and length had lower values, Y-/X-Feret was higher).

The values measured in the South Asia group were significantly lower than the values measured in the Germany (standard) group, for every parameter, except for the Y-/X-Feret, which was significantly higher in the South Asia group, which means that the scales were wider relatively to their length. The differences between the South Asian otters and the otters coming from the other regions were particularly obvious for the hair length and the medullary index (see Figs. 42 and 44). These differences were less obvious for the hair width, because the PHs from India and the PHs from Germany had a similar width, and the Sri Lanka otters had hairs, which were thinner than those of the Germany otters, but thicker than those of the Tunisia otter. The Sri Lanka otters had the thinnest medulla, and the smallest cuticle scales with the highest Y-/X-Feret. The Tunisia otter showed thinner hairs, a thinner medulla and smaller scales with a higher Y-/X-Feret than the Germany otters. The values of the hair length and medullary index were within the range of the values measured in the Germany otters. The values measured on the PHs of the Baikal otter were within the range of the values measured in the Germany otters, except for the cuticle scale parameters, which were lower (area, perimeter and length) or higher (Y-/X-Feret), but remained close to the values of the Germany otters.

1.4 Discussion

The general characteristics of the cross-section, medulla structure and hair cuticle pattern corresponded to former descriptions (HAUSMAN 1920, 1930, APPLEYARD 1960, FALIU et al. 1979, 1980, KELLER 1981, TEERINK 1991, MEYER et al. 2002, TÖTH 2002). The values that we found for PH length, PH width, medulla width and medullary index also fitted in with the values published by some of the previous authors (FALIU et al. 1979, 1980, TEERINK 1991, MEYER et al. 2002, TÖTH 2002). Whereas metrical analyses of the hair shaft are usually limited to get values of length, width and medulla width, some studies included metrical data on the cuticle of mammalian hairs, mostly length and width of the scales (HAUSMAN 1944, LYNE & MCMAHON 1951, TRAPP 1980, CHAKRABORTY & DE 1995, DE et al. 1998). MEYER et al. (1997, 2000, 2001, 2002), USHAKOVA & TSELIKOVA (1998) and SOKOLOV et al. (1999) also measured parameters like area and perimeter of the scales. As far as we know, the only available metrical data on the cuticle scales of the Eurasian otter have been published by MEYER et al. (1997, 2002), and are quite close to our findings (area: 349±77 µm², perimeter: 101±7 µm, Y-/X-Feret: 0.32±0.06).

The general descriptive features of the Eurasian otter PHs remained unchanged. Previous authors who looked at the variability of hair structure, also reported that the general characteristics remain unchanged, except for DAGNALL et al. (1995) who observed some differences in the shape of the cross-section of hairs taken from different body regions of the Red squirrel (*Sciurus vulgaris*). VOGEL & KÖPCHEN (1978) observed that even the very characteristic H-shaped profile of hairs from insectivores belonging to the subfamily Soricinae, particularly developed in the aquatic forms (HUTTERER & HÜRTER 1980), is constant at different body regions and at different seasons. The shape of the cross-section of the Eurasian otter also remains constant.

1.4.1 Variation according to body region

Fur colour was quite uniform, which actually is a common characteristic of semi-aquatic mammals, with only the ventrum being paler.

According to the results shown in Fig. 14, the length of the PHs of the Eurasian otter increases from the forehead to the central part of the body (mid dorsum and flank), remains constant until the posterior part of the trunk (posterior dorsum), and decreases slightly on the buttock and on the tail. The hair length on the abdomen equals the hair length on the anterior dorsum, the buttock and the tail (+/-1mm). Despite of the significant differences between the hair length on the forehead, neck and shoulder (1,2,6) and the hair length on the mid- and posterior dorsum and on the flank (4,5,7), the variability between the regions was moderate, also due to the generally rather moderate length of the *Lutra lutra* hairs (max 3 cm). The difference between the means did not exceed 5 mm, if the forehead was excluded, and the

ranges of the values measured on the different body regions were overlapping. Only the PH length on the forehead was really distinct from the other body regions.

The variations of the hair width between the different body regions did not exactly follow the same pattern as the variations of the hair length. The anterior part of the body (1 and 2) still showed the minimum values, but the other body regions did not differ significantly from each other. The largest differences between the two patterns were observed on the shoulder, whose PHs were relatively short (only the hairs on the forehead and neck were shorter), but belonged to the thickest ones.

TSEREVITINOV (1958) found a PH length of 24.2 mm on the back of *Lutra lutra* and 21.0 on the belly. ERMILOV & MARVIN (1971) found a similar PH length on the midback and belly (22.5 mm) but higher values on the withers (24.5 mm), sacrum and side (26 mm), and thicker PHs on the dorsum than on the ventrum, the flank showing an intermediate value (in SOKOLOV 1982). However, the differences between the values were small and those results had not been analysed statistically. In our study, the PHs on the midback were 2.2 mm longer than those from the abdomen, but this difference was not significant, and the PHs from the trunk all had a similar width.

More studies on the variations of hair length and hair width over the body are available for the Sea otter (*Enhydra lutris*). BARABASCH-NIKIFOROW (1947) measured slightly longer PHs on the dorsal side (ant: 24.3 mm, mid: 27.7 mm, post: 24.5 mm) than on the belly (23.4 mm), with the hairs from the flank having an intermediate length (25.9 mm) close to the length on the chest, and the hairs from the root of the tail being shorter (19.0 mm). He found thicker PHs on the abdomen (147.6 µm) than on the dorsum (ant: 98.7 µm, mid: 123.9 µm, post: 125.0 µm). The PHs from the flank again had an intermediate value (136.6 µm). The thickest PHs were on the chest (169.5 µm), and the root of the tail was in second position (153.2 µm).

WILLIAMS (1992) measured the longest PHs on the back and on the flank (mean length: 26.9 and 25.7 mm). The PHs were shorter on the rump (23.5 mm), abdomen (22.7 mm) and chest (17.3 mm), and also on the limbs. The thickest PHs were found on the abdomen (106 µm), followed by the rump (76 µm), the side (70 µm), the chest (47 µm) and the back (44 µm). The PHs from the limbs were thicker than on some parts of the trunk.

TARASOFF (1974) observed that the PHs of both, North American River otter (*Lontra canadensis*) and Sea otter (*Enhydra lutris*) had the same length on the midback than on the tail (20 mm in *L. canadensis*, 30 mm in *E. lutris*), the PHs on the limbs being shorter. In *L. canadensis*, the PHs were thicker on the tail (220 µm) than on the midback (198 µm). In *E. lutris* the PHs from the tail and midback had quite a similar width (tail: 105 µm, midback: 97 µm). The PHs from the limbs were thinner than those on the tail and back in *L. canadensis*, whereas they were thicker in *E. lutris*.

Mammals often have longer hairs on the back (MEYER et al. 1982, KORHONEN et al. 1984), however in semi-aquatics, this difference is not relevant (see DAWSON & FANNING 1981 and this study). A body region-related variability of the hair coat of semi-aquatic mammals will be further discussed in chapter 3.

The medulla of the PHs from the neck and, particularly, from the forehead of *Lutra lutra* was interrupted at the pars intermedia. According to our knowledge, variations in the continuity of the medulla according to body region or other possible influence factors have not been reported previously. The adaptive function of the medulla will be discussed in chapter 2. The variations of the medulla width between body regions have more similarities with the variations observed for the hair length than for the hair width, except that the medulla on the tail was not thinner than on the dorsum (mid and posterior), and that the medulla on the abdomen was thinner than on every other regions, except the forehead and neck. As a result, the abdomen had the lowest medullary index. Indeed, the width of the medulla was not proportional to the width of the hair. The PHs from the forehead were not only shorter and thinner, with a thinner medulla, their medulla also took up a smaller part of the hair diameter. The PHs from the mid- and posterior dorsum, from the flank and from the tail had a medulla, which took up a larger part of the hair diameter, and therefore a relatively thin cortex was present. The medullary index of the PHs from the shoulder occupied the same rank as the hair length (third rank on a scale from the minimal to the maximal value). The fact that the medulla on the forehead and on the abdomen was thinner than on the other regions could already be recognised from the light microscope photographs. However, the medulla on the neck had a width in between the values measured on the forehead and on the abdomen, and did not look thinner in the pictures. Actually, this was due to differences in the medullary index. The medulla on the forehead and on the abdomen appeared clearly thinner because the cortex was relatively thicker. Despite of the significant differences between some of the regions, the variability of hair and medulla width and of the medullary index between the regions remained moderate, even more moderate than for the hair length.

DAY (1966) observed the cuticle scale pattern, medulla and cross-section of PHs taken from the head, neck, back and belly of several species of insectivores, chiropters, small rodents and lagomorphs and found no differences. Hairs taken from extremities, like feet, lower leg or snout had features, showing reduced or simplified versions of those found on the rest of the body. No differences were found between the PH structure of the flank, ventrum and dorsum in *Talpa caeca*, *Sorex coronatus* and *Crocidura russula* (KELLER 1978). PERRIN & CAMPBELL (1980) observed slight variations in the cuticle of PHs taken from different body regions of the Vlei rat (*Otomys irroratus*). However, the pattern remained the same with only the size and to some extent the shape of the scales changing. Canids showed very little intraspecific variability in the microstructure of either dorsal, lateral or ventral guard hairs (KENNEDY

1982). The cuticular scales from PHs taken from different body regions in several species of the Cervidae also remained similar in general characters (WILLIAMSON 1950).

The difference that we observed in the cuticle of the PHs from the shoulder (6), flank (7) and buttock (8) was very subtle, and it was necessary to analyse the PHs very carefully to notice that the transition between the irregular wave pattern of the pars apicalis and the narrow diamond petal pattern observed on the pars intermedia was slightly longer in the PHs of these three regions. This difference was more visible in the PHs from the abdomen but was not striking. So we come to the same conclusion as MAYER (1952), which is that hairs of the venter and other areas usually resemble the dorsal guard hairs closely enough to be identified.

The variability observed between the body regions for the cuticle scale metrical parameters follows approximately the same pattern as seen previously. The smallest scales were on the shortest and thinnest hairs (except for the abdomen) and the PHs with the largest scales belonged to the longest and thickest ones. The regions where a slightly longer transition between the pars apicalis and the pars intermedia pattern could be observed (shoulder, flank and buttock), and which were all situated at the lateral part of the body, had scale sizes that were intermediate between the maximum and minimum values, but closer to the maximum values. The ratio of scale width to scale length remained constant from one body region to the other.

The same comment can be made as for the other parameters, stating that the variability between the regions was not very important, particularly when we look at the overlapping of the ranges between the minimum and maximum values. The fact that the PHs on the forehead and on the neck had a thinner medulla and smaller cuticle scales was not surprising, because they were shorter and thinner than the PHs from the other body regions. The medulla at the shield of the PHs from the forehead was even thinner than expected, as proven by the low medullary index. The thin medulla and the small cuticle scales of the PHs from the abdomen were more unexpected, because these hairs were not significantly shorter or thinner than the PHs from the dorsal region.

1.4.2 Variation according to sex

The male otters analysed had longer PHs than the females. Moreover, in the group of individuals in which the hair length was measured on 10 body regions, the three females had a lower mean hair length, or equal mean hair length, than the males, at every body region. Despite of being significant, the difference between the sexes was small. The longest PHs of the males were only 2 mm longer than the longest PHs of the females, the difference between the means was only 1.1 mm, and the ranges between minimum and maximum values were overlapping. Male otters tend to be bigger than females. However, this dimorphism is

low and females can be taller and heavier than some males (MOORS 1979 and pers. observation). The difference between the hair lengths is more important in species where sexual dimorphism is more pronounced, like in seals or deers (SCHEFFER 1964b, RYDER 1977).
The difference between the medulla indices of each sex was not considered as being relevant, because this difference was very small, the statistical test was not really conclusive ($p=0.049$), and also because of the fact that hair width and medulla width did not differ significantly between the sexes. No differences were found between the sexes for the cuticle scale parameters. DREYER (1966) also found that sex had no influence on the cuticle structure of bovids, and KEOGH (1975 in KEOGH 1983) came to the same conclusion for rodents. However, USHAKOVA & TSELIKOVA (1998) observed an influence of sex on some metrical morphometric parameters of the hair in the Saiga antelope (*Saiga tatarica*), again a species, which displays a higher sexual dimorphism than the otter.

1.4.3 Variation according to season

Curiously the studies that deal with the variability of hair structure between seasons, are principally related to Cervidae and Bovidae. WILLIAMSON (1950) observed that the cuticular scales from PHs taken from different seasons in several species of cervids remained similar in general characters, whereas DE MARINIS & ASPREA (2006) found a variability in the cuticular features of the winter and summer coats of deer (*Dama dama, Cervus elaphus, Capreolus capreolus*), and considered that these 3 species can be separated from each other only when comparing winter hairs. USHAKOVA & TSELIKOVA (1998) and SOKOLOV et al. (1999) observed that the summer and winter cuticle of the Saiga antelope (*Saiga tatarica*) differ morphologically.
We did not observe any influence of the season on the PH structure of Eurasian otters, neither on the length and width nor on the microscopic features. The seasonal variation of the hair coat of otters and mammals in general, particularly semi-aquatics, will be discussed in chapter 3.

1.4.4 Variation according to sample treatment

The otter hairs taken from fresh carcasses did not differ from the hairs of the standard group, taken from tanned pelts. It has already been observed in previous studies that digestion, putrefaction and taxidermy processing do not cause severe damage to the medullar and scale pattern (SHORT 1978, QUADROS & MONTEIRO-FILHO 1998). The effect of museum storage also appeared to be negligible and even mammoth hairs show no apparent deterioration (MAYER 1952, KEOGH 1983). However, in old museum specimens, the hairs can be discoloured and the cuticle scale pattern is sometimes destroyed through physical abrasion (PERRIN & CAMPBELL 1980, pers. observation).

1.4.5 Variation according to age

The juveniles aged between 4 and 6 months had slightly longer hairs than the adults. This result could not be influenced by the sex of the individuals because both groups had almost the same sex ratio (3 females and 2 males in the standard group, and 2 females and 3 males in the juveniles group) and, as previously seen, the difference between the mean hair lengths of both sexes was significant but moderate. The difference between the PH length of the juveniles and the standard group was also not significant, but it was interesting to observe that every studied juvenile had a maximal PH length between 30 and 32 mm, which was not the case for the adult animals (not every studied adult had a PH length up to 30 mm and none had PHs longer than 30 mm). The young otters look more “woolly” then the adults, even when they are close to adult size. This seams to be due to the fact that their hairs emerge somewhat steeper, which is common in young mammals (MEYER 1982), and may also be caused by slightly longer hairs. Young Sea otters (*Enhydra lutris*) of about 1 month had longer hairs than the adults, but juveniles at an age between 3 and 5 months showed already similar lengths than the adults (BARABASH-NIKIFOROV et al. 1968, in SOKOLOV 1982).

The other analysed PH features were identical in adult and juvenile otters. DAY (1966) observed the cuticle scale pattern, medulla and cross-section of PHs taken from several species of insectivores, chiropters, small rodents and lagomorphs, and found that the juveniles exhibited the characteristics described in the adults, albeit in some cases to a lesser degree. MUKHERJEE et al. (1994a) compared the medullary index of the hairs of Chitals (*Axis axis*) belonging to different age classes and found little variation. DREYER (1966) observed that the cuticle structure of young Kudu differs slightly from the adults. Young of deer and wild bovids exhibit a cuticle scale pattern from birth to 3-4 months old that distinguishes them from adults, but the hairs get similar to adults after the first molt (about 4 months), whereas young of domesticed ungulates show the same microscopic hair features as adults (DE MARINIS & ASPREA 2006). In rodents, the cuticular scale pattern remained unchanged from the age of 6 months through life (KEOGH 1975, in KEOGH 1983). In our study, not even slight differences were found between adults and juveniles aged between 4 and 6 months, nor in the medulla width and medullary index, neither in the metrical parameters of the cuticle scales. Changes in the metrical characters would probably appear in the hairs of younger specimens, but we expect the general pattern to remain the same. Indeed, one month Sea otter pups exhibit the same cuticular scale pattern as 7-8 years old adults (ZAGREBELNY 1998).

1.4.6 Variation according to health, diet and keeping in captivity

The captive otters had slightly shorter hairs than the otters of the standard group (this difference was not significant, albeit clearly visible on Fig. 35) and also showed smaller cuticle scales with a higher Y-/X-Feret. This result could not have been biased by the sex ratio within the captivity group because this group was made of 4 males and 1 female, and so an influence of the sex could only have resulted in a higher mean hair length and not a lower. The result could have been influenced by the fact that the animals, which had died in captivity, died because of illness and/or age, and were not in good shape. That was not necessarily the case for the wild animals, which had died accidentally. So the health condition probably was the important influency factor on hair structure, and not the fact that the animals lived in captivity. Hairs taken using hair traps on 4 alive captive adult otters were up to 3 cm long, like the hairs of the wild otters, which would confirm this hypothesis. Few studies looked at the influence of health, diet and captivity on hair structure, however it is known that diet has no effect on the cuticular structure of rodents (KEOGH 1975, in KEOGH 1983, SOKOLOV et al. 1999).

1.4.7 Inter-individual variability between otters coming from the same population

As we can see from the figures, the difference between the minimum and maximum values was important, and this for every PH parameter and for every influence factor studied. However, as we can see in the Tabs. 2-13, the mean value of each individual within a group was always close to the mean value of the group (see standard deviations). So we can conclude that within a group (for example, females or captivity group), the inter-individual variability was low. This also shows the importance of analysing several hairs from an individual to obtain a reliable mean value.

1.4.8 Variation according to climatic region

The PHs of the otter coming from the Lake Baikal were comparable to the PHs of the individuals from Germany. Only the mean cuticle scale area, perimeter, length and Y-/X-Feret were not within the respective range of mean values of the Germany group, but still close to these values. The lake is situated in the south of Siberia, where the climate is continental subarctique with an average annual temperature of 0°C, an average temperature of -15°C in January and +20°C in July. The lake is frozen from January to May. The Lake Baikal is approximately at the same latitude as North-Eastern Germany (53.5°N). The climate in North-Eastern Germany is continental and warmer than in Siberia, but the temperature during winter can also be clearly below 0°C. Our observations showed that the season had no influence on PH structure, and the differences between the climate of southern Siberia and Germany was probably not large enough to affect hair structure. However, it was surprising to see that

the cuticle scales of the Baikal otter were smaller but, as already said, this difference was only subtle, and it would be necessary to analyse more individuals to make some relevant conclusions.

The PH length of the otter coming from Tunisia was comparable to the hair length of the otters coming from Germany. The hairs were thinner, with a thinner medulla but the medullary index was comparable to the medullary index of the Germany group, and the cuticle scales were smaller. Tunisia is situated between the latitudes 30° and 37°N and has a warm Mediterranean climate without frost in winter. This would indicate that the PHs tend to become thinner, with a thinner medulla and smaller cuticle scales in warmer climate but further analyses would be necessary to confirm this.

The 3 individuals coming from India and Sri Lanka showed shorter PHs than the otters from Germany, Baikal and Tunisia. Former data indicate that the Eurasian otters living in South and South-East Asia have shorter hairs than the individuals living in Europe (HARRIS 1968). The individual from India had no thinner PHs than the German otters, which is quite surprising, but a thinner medulla. The individuals from Sri Lanka had thinner PHs than the individuals from Germany and the Lake Baikal, but not than the Tunisia otter. However, they had the thinnest medulla, and all the 3 South Asian otters exhibited a lower medullary index. This is probably the most obvious difference between the South Asian otters and the other individuals analysed, and it was clearly visible on the photographs of the shield (see Fig. 12). The South Asian otters also had significantly smaller cuticle scales, but here an almost gradual decrease of cuticle scale size was observable from Germany to Sri Lanka. Moreover, the individuals from South Asia had not only smaller scales but also wider scales relatively to their length, as proven by the value of the Y-/X-Feret. The Baikal and Tunisia otters showed values of the Y-/X-Feret, close to the Y-/X-Feret of the Germany group. The fact that the South Asia otters, and particularly the Sri Lanka otters had smaller and stockier scales is obvious for example, on the photographs of the cuticle at the pars intermedia (see Fig. 13). Sri Lanka is near the equator and has a tropical climate. Unfortunately, the exact origin of the Indian otter is unknown, but the biggest part of India also has a tropical climate and only the Himalayan region in the extreme north has temperatures below 20°C. The results of the analysis of the hairs of the South Asian otters confirm the observation made on the hairs of the Tunisian otter, that the values of the hair parameters tend to decrease with increasing temperatures. The larger differences between the Germany otters and the South Asia otters could be explained by climatic differences, but also by the large geographic distance. The geographic isolation due to insularity could also have an influence on the PH structure of the Sri Lanka individuals and moreover, these otters belong to the subspecies *Lutra lutra nair,* whereas the Germany otters belong to the subspecies *Lutra lutra lutra*. *Lutra lutra nair* also occurs in the South of India (HARRIS 1968). Further differences in the PH characteristics

could maybe be observed in the Eurasian otters living in South-East Asia, due to geographic isolation and to the presence of different subspecies. Siberian and Tunisian otters belong to the same subspecies as German otters.

It is known that within a species having a large distribution range over different climates, the populations living in the colder areas show longer hairs (TÄNZER 1932). In our study, only the Asiatic otters confirm this principle. However, climate may have less influence on the hair coat of semi-aquatic animals. We observed that the otter from Tunisia had not shorter but thinner hairs than those from Germany, whereas the otter from India had shorter but not thinner hairs than the German. This could be a coincidence and analyses of further individuals from those regions would be needed to clear that point. The two otters from Sri Lanka were the only ones which differed from the German otters for every parameter, having shorter and thinner PHs, a thinner medulla, a lower medullary index, smaller and stockier scales. However, this could be due not only to the influence of climate, but also to larger geographical distance, geographical isolation and to the fact that the Sri Lanka otters belong to a different subspecies. The adaptive possibilities of hair structure and the influence of climate on hair coat will be further discussed in the following chapters.

1.5 Conclusion

Despite a few significant differences for some of the parameters, the PHs from the dorsal body region of Eurasian otters coming from Germany were very similar to each others, and even if the hair characteristics are influenced by some of the factors studied, this is not really relevant for the identification criteria of the species hairs. The influence of the factor body regions on the hair characteristics was moderate, the hairs of the trunk showing a great similarity. The hairs from every body region, even from the head can be identified as being a hair from *Lutra lutra* with great reliability. The general morphology of the hair, the cross-section, the structure of the medulla and the cuticle are the same in all the PHs examined. For the purpose of species identification, one should keep in mind the existing range of values for the length, width, medulla width and cuticle scales parameters. If metrical characters of microscopic features are used, we recommend the medullary index and the Y-/X-Feret. Particularly the Y-/X-Feret is an interesting parameter, because it is quite constant, independently of the variation of cuticle area and perimeter. It not even differs between body regions, and only a slight influence of captivity/health was observed. Moreover, it is much less time consuming to just measure the length and width of the scales than to measure the area and perimeter and can be done without any particular software.

However, the differences that we found in all metrical parameters were limited, even the differences in length and width, probably due to the small size of the otters and also to the aquatic nature of their environment. The climate has apparently a moderate influence on the

PH structure. The hairs of otters from South Asia, and particularly from Sri Lanka, are distinguishable best from all the other hairs examined, which may be due to climate and perhaps also other factors like geographical and genetic distance. They can still be identified as belonging to an otter, but the identification of the exact species may be ambiguous, as we will see in the next chapter.

CHAPTER 2:
COMPARATIVE HAIR STRUCTURE IN THE LUTRINAE

2.1 Introduction

The 13 otter species known have worldwide distribution and live in very different environments, from tropical to arctic climates. In some regions of Africa, Asia and South America, two to four species occur sympatrically. Otters are all considered as semi-aquatic carnivores, but they have different degrees of adaptation to the aquatic environment. Indeed, the Sea otter (*Enhydra lutris*) only rarely leaves the ocean waters, and is often considered as being a marine mammal, whereas the Congo otter (*Aonyx congicus*) is a swamp inhabitant, which searches the mud for worms and other invertebrates.

Otters are well known for the quality of their fur, but the characteristics of their pelage and, particularly, the structure of their hairs has been studied in only few species, principally the Eurasian otter (*Lutra lutra*), North American River otter (*Lontra canadensis*) and Sea otter (*Enhydra lutris*), which are actually the best known otter species (HAUSMAN 1920ab, 1930, BROWN 1942, BARABASCH-NIKIFOROW 1947, APPLEYARD 1960, TARASOFF 1974, MOORE et al. 1974, FALIU et al. 1979, 1980, KELLER 1981, TEERINK 1991, WALLIS 1993, WILLIAMS et al. 1992, MEYER et al. 2002, TÖTH 2002). Some information is also available on the hair structure of the African Cape clawless otter (*Aonyx capensis)* (PERRIN & CAMPBELL 1980), and the South American Neotropical (*Lontra longicaudis*) and Southern River otter (*Lontra provocax*) (CHEHÉBAR & MARTIN 1989, VÁZQUEZ et al. 2000).

The hair coat consists of strong primary hairs (PHs) or guard hairs and of thin, often curled, secondary hairs (SHs) or wool hairs, which form the insulating wool hair coat (underhair). The structure of guard or primary hair varies species- or group-related, and this feature initiated the development of identification methods for wild mammals based on hair structure (HAUSMAN 1920-1944, DAY 1966, BRUNNER & COMAN 1974, KELLER 1978-1986, TEERINK 1991, MEYER et al. 2002). Recent studies have shown, however, that structural hair characteristics include some adaptive functions (HOWELL & HODGKIN 1976, HUTTERER & HÜRTER 1981, MEYER et al. 1995). Moreover, PH morphology can give information about the evolution of zoosystematical subgroups within one family (MEYER et al. 2001).

The hair shaft is formed by the central medulla, the cortex, and the hair cuticle, which consists of a layer of flat cornified cells, the scales. The medulla is usually absent in wool hairs. Along the hair shaft, three important regions can be distinguished: the pars apicalis (apical part), that in several mammalian groups has a spindle-shaped to strongly flattened thickening called the shield; then the pars intermedia (medial part), that takes about one quarter of the hair shaft length and shows a more or less constant hair cuticle pattern; finally comes the

pars basalis (basal part), that takes about one third to one half of the hair shaft and often has a varying hair cuticle pattern (see Figs. 1 and 2 in chap.1)

In this study, several structural PH features (length, width, morphology, cross-section, medulla, cuticle structure) of the 13 otter species were analysed, in order to find out whether they can be identified using hair morphology, and to see how hair characteristics correlate with geographic distribution, foraging behaviour and taxonomic position. Additionally, the length and width of the secondary hairs (wool hairs) were measured to give some complementary information on the pelage characteristics of the Lutrinae.

2.1.1 The Lutrinae subfamily

Otters belong to the Mustelidae, the largest family of the Carnivora, which also comprises the Mustelinae (weasels, martens and minks), Melinae (badgers), Mellivorinae (honey-badgers), Taxidiinae (American badgers) and Mephitinae (skunks). The Mustelinae are the closest to otters and are their ancestor branch (MASUDA & YOSHIDA 1994, KOEPFLI & WAYNE 1998). They are small carnivores. The males tend to be bigger than the females; however, the importance of dimorphism differs from one species to the other, but is never pronounced. The otter lineage is quite old; the oldest lutrine fossil known is *Mionictis* from the Miocene from France (WILLEMSEN 1992). The Lutrinae subfamily is a rather uniform and also monophyletic group (KOEPFLI & WAYNE 2003), clearly distinct from the other mustelids, and each species can immediately be recognized as being an otter. The otters comprise 13 species, which belong to 7 different genera: *Lutra, Lontra, Lutrogale, Pteronura, Enhydra, Amblonyx* and *Aonyx*. The classification of this group is subject to many controversies and is continuously changing. Indeed, 9 to 19 species have been described, mostly because of the fact that the different subspecies of the Neotropical otter (*Lontra longicaudis)* used to be considered as different species (HARRIS 1968, DAVIS 1978). On the other hand, DAVIS discussed the validity of classifying *Lontra longicaudis* and *Lontra canadensis* as distinct species. The *Lontra* species and also sometimes *Lutrogale*, used to belong to the *Lutra* genus, whereas *Lutra maculicollis* was considered to belong to the separate genus *Hydrictis*. *Lutra sumatrana* used to be considered as being a subspecies of *Lutra lutra*. The Small-clawed otter *Amblonyx cinereus* is always switching between the genera *Amblonyx* and *Aonyx*, and some consider that *Aonyx congicus* and *Aonyx capensis* belong to the same species. Mating between different otter species has been observed, for example, *L. canadensis* with *L. longicaudis* or *L. lutra* with *L. sumatrana* (DAVIS 1978, DUPLAIX 1982), and even between species belonging to different genera, *Lutrogale perspicillata* with *Amblonyx cinereus* (MELISCH & FOSTER-TURLEY 1996), but data on the fecundity of these hybrids are not available.

The Lutrinae can be divided in two groups (DUPLAIX 1982):
The "*Lutra* group" comprises the genera *Lutra*, *Lontra*, *Lutrogale* and *Pteronura* (River otters, Smooth and Giant otter), which are very well adapted to aquatic life, have all webbed fore and hind feet, are rather piscivorous and have a long, slim and flexible body. The *Lutra* and *Lontra* also have a penis that is entirely sheathed within the abdominal skin, to reduce drag in aquatic locomotion.
The "*Aonyx* group" comprises the genera *Aonyx*, *Amblonyx* and *Enhydra* (Clawless, Small-clawed and Sea otter), which are stockier and less flexible than the members of the *Lutra* group, have shorter skulls, a different dentition, and feed rather on crabs and other invertebrates. The fore and hind feet are not or only rudimentary webbed, except the Sea otter (*Enhydra lutris*), whose fore feet are not webbed but whose hind feet are real flippers. The Sea otter is the most aquatic otter, whereas the other members of this group are less aquatic than the members of the *Lutra* group, and are better adapted to terrestrial life, particularly regarding locomotion on land.

However, the position of *Lutrogale perspicillata* (Smooth otter) and *Pteronura brasiliensis* (Giant otter) is controversial. They share the slim body and webbed feet with the members of the *Lutra* group (their webbings are even larger than in *Lutra* and *Lontra*), but some characteristics like the morphology of the baculum and genitalia, dentition and shorter skull, relate them to the *Aonyx* group. DUPLAIX (1982) suggested an alternative division of the Lutrinae in 4 groups, based on morphological, ecological and ethological characters: Lutrini (with the current genera *Lutra* and *Lontra*), Aonychini (with *Aonyx, Amblonyx, Lutrogale* and *Pteronura*), Hydrictini (with the current *Lutra maculicollis*) and Enhydrini (with *Enhydra lutris*). DAVIS (1978) adopted the same grouping, except that he put *Enhydra* in the *Aonychini* group. VAN ZYLL DE JONG (1987) considered that the New World river otters (*Lontra*) are closer to *Aonyx* and *Amblonyx* as to the Old World river otters (*Lutra*), according to some morphological data, and that *Enhydra* and *Pteronura* are the most divergent otters, *Pteronura* being close to *Lutrogale*, which in turn is close to the *Lutra* genus. Actually, he recognized three branches on the otter tree: one resulting in *Enhydra lutris*, the second in *Lontra*, *Aonyx* and *Amblonyx*, and the third in *Lutra, Lutrogale* and *Pteronura*.
The shape of the baculum (DAVIS 1978) separates the *Lutra* and *Lontra* genera from the other species. Both have a baculum, which looks like a "hockey stick", but the sharp distal bend of the stick is ventral in Old World species (*Lutra*) and dorsal in New World species (*Lontra*). The suggestion to put Old and New World river otters in two different genera is quite ancient (VAN ZYLL DE JONG 1972), but has been accepted only after the results of recent genetic analyses (KOEPFLI & WAYNE 1998, 2003, MARMI et al. 2004). So molecular biology helped to provide a clearer picture of the relationship between otter species, but

some points are still unclear. KOEPFLI & WAYNE (1998, 2003) suggested that otters are divided in three primary clades: the first with the *Lontra* genus, the second with *Enhydra, Lutra, Aonyx* and *Amblonyx*, and the third with *Pteronura*. *Lutrogale* has not been analysed in this study. *Lutra* is not a monophyletic genus because *L. lutra* is genetically closer to *Aonyx* and *Amblonyx* than to *L. maculicollis*. Moreover, *L. maculicollis* has a very similar external morphology than the two other *Lutra* but differs by other characters like the morphology of the baculum (DAVIS 1978). That is why it used to be classified as separate genus. MARMI et al. (2004) also observed a branch with *Lontra* and a branch with *Lutra*, *Aonyx*, *Amblonyx*, and *Enhydra*, the position of *Pteronura* being unclear. BININDA-EDMONDS et al. (1999) proposed the following tree based on both fossil and molecular data:

First branch: → *Enhydra lutris*

Second branch: → *Lutra maculicollis, Lutrogale perspicillata, Pteronura brasiliensis*

Third branch: → A*mblonyx cinereus*

→ *Aonyx capensis, Aonyx congicus*

Fourth branch: → *Lutra lutra, Lutra sumatrana*

→ *Lontra* → *Lontra felina, Lontra provocax*

→ *Lontra longicaudis*

→ *Lontra canadensis*

The *Lutra* and *Lontra* species probably have their origin in southern Asia, from where the different species branched out (KRUUK 2006). The earliest fossils ascribed to the genus *Lutra* are found in the Pliocene of Europe and *Lontra* apparently descended from the fossil *Lutra licenti,* which lived in China in the early Pleistocene, and migrated from there into North America during the Pleistocene (VAN ZYLL DE JONG 1972, WILLEMSEN 1992). *Lontra* colonised the Americas, resulting in *Lontra canadensis, L. longicaudis, L. provocax* and *L. felina,* and also many extinct fossil species. However, morphological data suggest an earlier date for the separation between the two lineages, so the modern *Lontra* species could be descendants of the earliest genus *Mionictis,* found with several species in early Miocene deposits of both Europe and North America, and present in North America until the Pliocene (WILLEMSEN 1992, KOEPFLI & WAYNE 1998). Whatever, the *Lontra* genus forms a well-supported monophyletic group and the South American river otters diverged from *Lontra canadensis* about 1.7 M years ago, which corresponds to the dispersal of river otters into South America after the formation of the Panamanian landbridge.

In Eurasia, the *Lutra* branch produced *Lutra lutra*, *Lutra sumatrana* and the African *Lutra maculicollis* (KRUUK 2006). *L. lutra* and *L. sumatrana* are sister species, whereas the position of *L. maculicollis* is still somewhat unclear. Molecular biology strongly suggests that

Aonyx and *Amblonyx* descend from the *Lutra* branch (KOEPFLI & WAYNE 1998). *Aonyx* and *Amblonyx* shared a common ancestor with *L. lutra* about 6.3-8 M years ago. The authors also suggest that the three species are so close that they can all be put in the genus *Aonyx.* However, in this study, we will keep the genus *Amblonyx.* The common ancestor of these three species probably evolved fingers and large crushing molars to adapt to the rich resources of crustaceans in Asia and in Africa (KRUUK 2006). The Sea otter *Enhydra lutris*, which morphologically and ecologically differs from the other otters the most, diverged from the *Lutra* lineage about 13 M years ago. The Giant otter (*P. brasiliensis*) is related only distantly to the American *Lontra* and actually has the highest percentage divergence from the other otters (KOEPFLI & WAYNE 1998). WILLEMSEN (1992) suggests that it derived from the genus *Satherium,* which appeared in Asia and emigrated to America during the Pliocene, *Pteronura* being the only living descendant of this genus. No DNA analyses of *Lutrogale perspicillata* are available, but morphological and ecological similarities let several previously mentioned authors suggest that the Giant otter is its closest living relative.

Many fossil findings and morphological features of the Lutrinae, like the fact that *L. maculicollis, L. sumatrana* and *L. lutra* have more primitive teeth than *L. canadensis, Lutrogale* and *Pteronura* (DUPLAIX 1982), corroborate the results of genetic analyses.

The thirteen otter species

General references: DUPLAIX 1982, KRUUK 2006

See also Figs. 49 and 50 for illustrations and distribution ranges

EUROPE, ASIA & NORTH AFRICA

Eurasian otter (*Lutra lutra*)

Colour: dark brown, somewhat lighter underneath, in some areas with light patches on the throat

Total length: 100 to 130 cm

Weight: 6 to 10 kg

Feet: webbed

Habitat: fresh water, also sea water (coastal area) but with access to fresh water for drinking and rinsing the fur

Diet: dominated by fish, also frogs, crayfish, crabs, reptiles, birds and mammals
fishes by swimming along the surface, then diving and searching for prey along the bottom; catches its preys with the mouth, and eats the small fishes on the surface, whereas larger preys are taken ashore

Distribution: has the widest distribution of all otters; occurs in whole Europe, North Africa and whole Asia, with the exception of desert regions and some islands in the southeast

AFRICA

Congo clawless otter (*Aonyx congicus*)

Colour: brown, the hairs on the top of the head, neck and shoulder are grey frosting, white throat and cheeks with a distinctive dark patch

Total length: 130 to 150 cm

Weight: 15 to 25 kg

Feet: fore feet with long fingers and not webbed, rudimentary webbing on the hind feet

Diet: earthworms, crabs, principally invertebrates, also fish, frogs, insects

Habitat: rainforest swamps

Distribution: Congo Basin (Cameroon, Central African Republic, Gabon, Congo, Democratic Republic of Congo, North of Angola)

Cape clawless otter (*Aonyx capensis*)

Colour: brown with a clear white throat and cheeks

Total length: 120 to 160 cm

Weight: 15 to 25 kg

Feet: fore feet with long fingers and not webbed, rudimentary webbing on the hind feet

Diet: mostly crabs, also fish, frogs and molluscs
often catches its preys in shallow water; catches its preys with the fingers

Habitat: fresh water, all kind of wetlands where there are crabs, also coastal water

Distribution: Africa south of the Sahara, except in the Congo Basin and in dry areas (part of Ethiopia, Somalia, Botswana and Namibia)

Spotted-necked otter (*Lutra maculicollis)*

Colour: dark chocolate brown with white patches on the underside

Total length: 90 to 100 cm

Weight: 3-6 kg

Feet: webbed

Diet: principally fish, also crabs, frogs and insects

Habitat: fresh water, common in the large East African lakes

Distribution: Africa south of the Sahara, absent from the eastern fringe, Namibia, Botswana, Zimbabwe and dry areas of South Africa

ASIA

Small-clawed otter (*Amblonyx cinereus*)

Colour: dark brown greyish above, whitish throat and sides of mouth

Total length: 60 to 90 cm

Weight: 3 to 5 kg

Feet: small with long fingers (short webbing only between the proximal part of the fingers)

Habitat: fresh water, small streams, marshes, rice paddies, mangroves and sea coasts

Diet: mostly crabs and other invertebrates, sometimes frogs and fish, probably the less piscivorous of all otters, searches for its food with the forelegs

Distribution: Southern India, Nepal, Bhutan, Bangladesh and Southeast Asia (Myanmar, Thailand, Vietnam, Laos, Cambodia, Malaysia, Indonesia)

Smooth-coated or Smooth otter (*Lutrogale perspicillata*)

Colour: dark brown, light underneath, often with a clear demarcation
Total length: 100 to 130 cm
Weight: 7 to 10 kg
Feet: webbed
Habitat: fresh water, mangroves, seashores, mostly larger water bodies
Diet: mostly fishes (principally large ones), also crabs, frogs and snakes
Distribution: Afghanistan, Pakistan, India, Nepal, Bhutan, Bangladesh, Southeast Asia, separate population in the Iraqi marshes

Hairy-nosed otter (*Lutra sumatrana*)

Colour: brown, throat and upper lips white
Total length: 100 to 130 cm
Weight: 5 to 8 kg
Feet: webbed
Habitat: swamps, rivers and streams situated in dense forests, possibly also in sea-water
Diet: small fishes, also snakes, frogs, small mammals, crabs and insects
Distribution: Cambodia, Vietnam (especially Mekong delta), south of Thailand, Malaysian Peninsula, Indonesia (Sumatra, Borneo, Java)

NORTH AMERICA

Sea otter (*Enhydra lutris*)

Colour: dark brown greyish
Total length: 110 to 140 cm
Weight: 20 to 40 kg
Feet: large webbed hind feet (flippers)
Habitat: coastal water, especially rocky costs
rarely leaves the water (in some areas even gives birth in the sea; spends most of its time floating belly-up on the sea surface)
Diet: sea urchins, crabs, molluscs, sometimes fish
searches its food on the ocean floor during dives of up to 60 m (max 100)
Distribution: along the coasts of the North Pacific, from the north of Japan to California

North American River otter (*Lontra canadensis*)

Colour: dark brown, somewhat lighter underneath
Total length: 120 to 140 cm
Weight: 7 to 10 kg
Feet: webbed
Habitat: fresh water and also sea water
Diet: principally fish, also frogs, crayfish, small mammals, birds and reptiles
Distribution: USA and Canada, as far north as there are trees

CENTRAL & SOUTH AMERICA

Neotropical otter (*Lontra longicaudis)*

Colour: dark brown, somewhat lighter underneath
Total length: 100 to 130 cm
Weight: 10 to 13 kg
Feet: webbed
Habitat: fresh water, also mangroves and rocky coasts
Diet: principally fish, also crustaceans, amphibians and mammals
Distribution: Central and South America (Mexico, Belize, Guatemala, El Salvador, Honduras, Nicaragua, Costa Rica, Panama, Colombia, Venezuela, Guyana, Suriname, French Guiana, Brazil, Peru, Ecuador, Bolivia, Paraguay, Uruguay, Argentina) absent from Chile and from the South of Argentina

Giant otter (*Pteronura brasiliensis*)

Colour**:** dark brown with white-yellow throat patches
Total length: 150 to 200 cm
Weight: 25 to 35 kg
Feet: webbed
Habitat: exclusively freshwaters situated in dense forests (slow rivers, oxbow lakes, flooded areas)
Diet: almost exclusively fish, often large specimens
Distribution: Amazon Basin (Brazil, French Guiana, Guyana, Suriname, Venezuela, Columbia, Peru, Bolivia, Paraguay), absent from coastal regions

Marine otter or sea cat (*Lontra felina*)

Colour: dark brown, lighter underneath
Total length: 90 to 100 cm
Weight: 3 to 6 kg
Feet: webbed, hairy underneath
Habitat: saltwater, mostly rocky coast, rest in holts or in caves, does not need freshwater
Diet: mostly crabs, also fish and sometimes molluscs
Distribution: along the Pacific coast, from Peru to Tierra del Fuego, also on islands

Southern River otter or huilin (*Lontra provocax*)

Colour: dark brown, lighter underneath, greyish throat
Total length: 100 to 110 cm
Weight: over 5 kg
Feet: webbed
Habitat: fresh water, also rocky coasts
Diet: mostly crabs, also fish and molluscs, in some areas small fish predominate
Distribution: southern half of Chile, narrow strip of Argentina, also Tierra del Fuego and other islands

Fig. 49: The 13 otter species

Eurasian otter
(Lutra lutra)
© Nicole Duplaix

Congo clawless otter
(*Aonyx congicus*)
© Nicole Duplaix

Cape clawless otter
(*Aonyx capensis*)
© Hélène Jacques

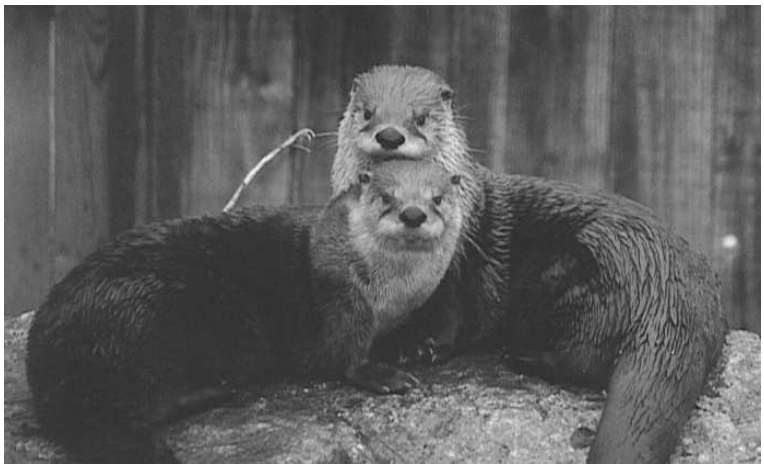
Spotted-necked otter
(*Lutra maculicollis*)
© Claus Reuther

Small-clawed otter
(*Amblonyx cinereus*)
© Nicole Duplaix

Smooth-coated otter
(*Lutrogale perspicillata*)
© Nicole Duplaix

Hairy-nosed otter
(*Lutra sumatrana*)
© Annette Olsson

Sea otter
(*Enhydra lutris*)
© Nicole Duplaix



North American
River otter
(*Lontra canadensis*)
© Rachel Kuhn

Neotropical otter
(*Lontra longicaudis*)
© Nicole Duplaix

Giant otter
(*Pteronura brasiliensis*)
© Nicole Duplaix

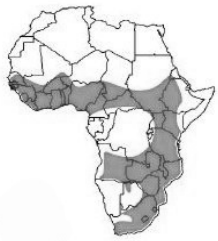
Marine otter
(*Lontra felina*)
© José Luis Bartheld

Southern River otter
(*Lontra provocax*)
© José Luis Bartheld

Fig. 50: Distribution range of the otter species

Eurasian otter
(*Lutra lutra*)

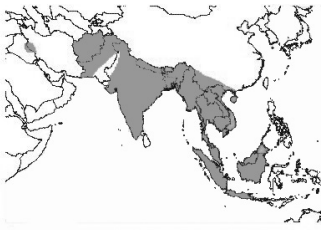
Congo clawless otter
(*Aonyx congicus*)



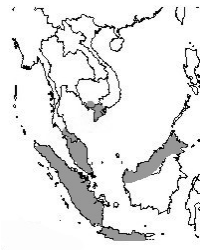
Cape clawless otter
(*Aonyx capensis*)

Spotted-necked otter
(*Lutra maculicollis*)

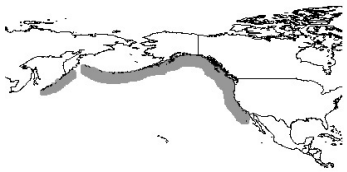
Small-clawed otter
(*Amblonyx cinereus*)



Smooth otter
(*Lutrogale perspicillata*)



Hairy-nosed otter
(*Lutra sumatrana*)



Sea otter
(*Enhydra lutris*)

North American River
otter (*Lontra canadensis*)

Neotropical otter
(*Lontra longicaudis*)

Giant otter
(*Pteronura brasiliensis*)

Marine otter
(*Lontra felina*)

Southern River otter
(*Lontra provocax)*

Source: IUCN/SSC Otter Specialist Group

2.2 Material and methods

2.2.1 Samples

Primary and secondary hairs were collected from the dorsal body region on pelts (museum specimens) of each of the otter species studied. The hairs were cut with small scissors as close as possible to the epidermis (the best way is to press slightly the point of the scissors against the skin). The hair structure was analysed in 5 individuals of each species, except 4 for the Marine otter (*Lontra felina)* and the Southern River otter (*Lontra provocax)*. The sex of the analysed individuals was generally unknown (except for *Lutra lutra*). The origin of the studied individuals (when known) is indicated in Table 14 (according to the information available in the museums the samples came from; many specimens were quite old or customs seizures, which explains the sometimes incomplete information about the origin of the animals). Because the hairs of the Eurasian otters from South Asia were significantly different from those of the Eurasian otters from Germany (for example shorter, see chap. 1), the individuals from South Asia were included in the present study.

Tab. 14: Origin of the individuals studied

Species	**Origin of the studied individuals**
Eurasian otter *Lutra lutra*	Germany (5 individuals) and South Asia (1 ind. from India and two from Sri Lanka)
Congo clawless otter *Aonyx congicus*	Gabon (2 individuals), Congo, Democratic Republic of Congo, 1 probably from Cameroon
Cape clawless otter *Aonyx capensis*	Chad, Gabon, South-Africa, Liberia, 1 unknown
Spotted-necked otter *Lutra maculicollis*	Gabon, Congo-Chad, Tanzania, 1 probably from South-Africa, 1 probably from Angola
Small clawed otter *Amblonyx cinereus*	Indonesia (1 from Java, 1 from Sumatra), 2 from the Philippines, Germany (captivity)
Smooth-coated otter *Lutrogale perspicillata*	India, Indonesia, 2 from Indochina (nowadays Cambodia, Laos and Vietnam) but died in captivity in France, 1 unknown
Hairy-nosed otter *Lutra sumatrana*	Malaysia (Borneo), Thailand, 2 from Indonesia (Sumatra), 1 unknown
Sea otter *Enhydra lutris*	2 from the Bering sea, 2 from Alaska, 1 from Kamtschatka (Russia)
North American River otter *Lontra canadensis*	Canada (Labrador), USA (South-East) 3 unknown
Neotropical otter *Lontra longicaudis*	French Guiana (3 individuals), 1 from Mexico 1 from Panama
Giant otter *Pteronura brasiliensis*	2 from French Guiana, 2 from the Upper Amazon (Peru, Ecuador, Venezuela, Colombia and Brazil), 1 unknown
Marine otter *Lontra felina*	Chile
Southern River otter *Lontra provocax*	Chile

2.2.2 Documentation of hair shaft structures

2.2.2.1 Primary hairs

First, the general appearance of the PHs (morphology and colour) was described and the length (total length and length of the shield) measured (10 hairs for each individual). Then, the structure of the cross-section, medulla and cuticle of PHs were studied by light microscopy (magnification 400x) and documented (FUJI FinePix S602). For cross-section analysis, PHs were glued between two pieces of scotch tape and cut with a razor blade at their thickest part, situated at the middle of the shield. In order to obtain a view of the medulla, PHs were glued to a microscopic slide and cleared up with paraffin oil. For the analysis of scale morphology, hair cuticle casts were prepared with a PVA solution. The three methods were used according to MEYER et al. (2002) and are described in detail in chap. 1. The structure of the cross-section, medulla and cuticle was described using the nomenclature of the same author. For hair cuticle structure analysis, attention was focused on three important regions: the tip (pars apicalis), particularly the upper part of the shield; the medial part, beneath the shield (pars intermedia); and the lower part of the pars basalis (part above the root). The form and arrangement pattern of the cuticle scales, and the shapes and distances of the free edges of the scales were described and classified. The width of the medulla and of the hair at the middle of the shield, and the size of the cuticle scales (area, perimeter, height, width) of the pars intermedia were measured using the software AxioVision (Zeiss Inc.) (see chap. 1). The hair and medulla width were measured for 10 hairs/ind. and the cuticle scale size for 100 scales/ind. The processed data were used to calculate the medullary index (ratio of medulla width to hair width) and the Y-/X-Feret (ratio of scale width to length).

2.2.2.2 Secondary hairs

The length and width of the secondary or wool hairs were also measured (10 hairs/ind.) To measure the width, the wool hairs were glued on a microscopic slide and photographed. Then, the width was measured on the pictures using the software Axiovision, as for the PH width. Unlike the PHs, the SHs have a constant width all along the hair shaft, so the measurements were made on an arbitrarily chosen part of the hair shaft. In otters, the secondary hairs have no medulla (TARASSOF 1974, SOKOLOV 1982, TEERINK 1991). The cuticle structure of the wool hairs of 6 otter species studied by scanning electron microscopy (SEM) will be described in chap. 4.

2.2.3 Statistical analyses

Statistical analyses (ANOVA) are based on mean values per individual. Differences between species were determined using subsequent Post-hoc tests (Tukey).

2.3 Results

2.3.1 Atlas on the primary hair structure of the Lutrinae

The results of hair structure analysis will be presented in an atlas for a better comparative information about the species. For the PH length and width and the medulla width, the mean, minimal and maximal values are indicated (exact values measured). For the medullary index and the cuticle scale parameters (area, perimeter, width, length, Y-/X-Feret) only the mean values are indicated.

The structure of the medulla was usually constant all along the hair shaft. The width of the medulla varied more or less proportionally to the hair shaft width. The tip and the base were often fragmented, but interruptions of the medulla could also be seen at other regions of the hair shaft. For each otter species, a view of the medulla at the central part of the shield and eventually at other regions is shown. The colour of the cortex seen on the preparations with paraffin oil is also described.

Structural features of the medulla encountered in the otter species

The medulla normally consists of pigmented and non-pigmented cell residues that are fused to form secondary wall units, thus separating medullar gas spaces of different sizes (MEYER et al. 2002). In the Lutrinae the medullar structure was usually wide lattice, which means the gas spaces between the cells are in a lattice arrangement. In the preparations, the paraffin oil has partially or fully replaced the air present in the medulla, and so the gas spaces have become more or less transparent, depending on how the paraffin had penetrated in the medulla.

The information pertaining to the cuticle of the PHs is shown according to the three regions: pars apicalis, pars intermedia and pars basalis.

The pictures of the pars apicalis are always from the very distal part. However, for every otter species, the described pattern was observed at the whole shield (eventually with slight variations in distance between the scale margins), except at the lower part where transition patterns can be formed.

It was a little bit ambiguous to determine which pattern was typical for the pars intermedia. Actually, the pars intermedia was quite short and it was sometimes difficult to say where exactly this part began and where it ended, particularly when the transition between the shield and the parallel-edged part of the hair shaft was very gradual, which was the case in the otter species studied (in some species more than in others). As a rule, the pars intermedia pattern was the first “constant” pattern beneath the shield.

With the pars basalis, it is the very proximal pattern, which is meant here (we already said that the pars basalis can take one half of the hair shaft and often has a varying structure).

However, comments will be made on the pattern (or patterns) situated distally. The pattern of the pars intermedia could be observed all along the lower half of the hair shaft until the base (like in the Eurasian otter), but this was not always the case (see the Congo and Cape clawless otter).

Structural features of the hair cuticle encountered in the otter species

The general shape and arrangement of hair cuticle scales observed in the otter species are the following (description of the arrangement pattern according to MEYER et al. 2002):

Petal types - the scales overlap like a series of overlapping flower petals. In the *diamond petal type*, the scales are relatively small, only slightly longer than wide. In the *broad diamond petal pattern*, the scales are longer than large, are of irregular size and have rounded distal edges. In the *narrow diamond petal pattern*, the scales are long, narrow and lanceolate.

Mosaic types – the patterns have an overall angular appearance (the adjacent scales have rather smooth margins). In the *regular mosaic* pattern, the width of the scales is similar to their length. In the *irregular mosaic pattern*, the scales are of dissimilar sizes and shapes. In the *flattened mosaic pattern*, the scales are always wider than long.

Waved types - this pattern appears like a parallel series of waves. The waves are not necessarily continuous. The wave form is *regular* when the waves have quite a constant amplitude and *irregular* when not.

The scale margins are *smooth* – smooth edge line without any indentations, or *rippled* – indentations on the edge line. The distance between the scale margins can be *close* or *intermediate.*

Magnification scale: The scale bars represent 10 µm. The scale bar in the photo on the right also applies to the other photos on the page. All the pictures of the atlas have the same magnification.

Eurasian otter *Lutra lutra*

Morphology: fusiform

Cross-section: oblong-oval

Colour: whitish/beige, shield brown to dark brown

Hair length: 22 mm (16-29) Shield: 35-50%

Hair width: 156 µm (133-179) Medulla width: 101 µm (84-111) Medullary index: 0.65

Cuticle scales parameters:

Scale area: 391 µm² Scale width Y: 16 µm

Scale perimeter: 112 µm Scale length X: 51 µm

Y-/X-Feret: 0.33

South-Asia

Hair length: 18 mm (15-22 mm) Shield: 35-50%

Hair width: 139 µm (112-167) Medulla width: 79 µm (60-93) Medullary index: 0.57

Cuticle scales parameters:

Scale area: 281 µm² Scale width Y: 16 µm

Scale perimeter: 86 µm Scale length X: 38 µm

Y-/X-Feret: 0.42

Medulla structure

Wide lattice, tip and base often fragmented

Cortex brown at the shield and whitish/beige at the parallel-edged part of the hair shaft; base whitish

Hair cuticle pattern

Pars apicalis: irregular wave, rippled scale margins, distance between the scale margins: intermediate to close

Pars intermedia: narrow diamond petal, smooth scale margins, distance between the scale margins: intermediate

Pars basalis: irregular wave, smooth scale margins, distance between the scale margins: intermediate

N.B. The narrow diamond petal pattern was seen all along the parallel-edged part of the hair shaft, until the irregular wave pattern near the root.

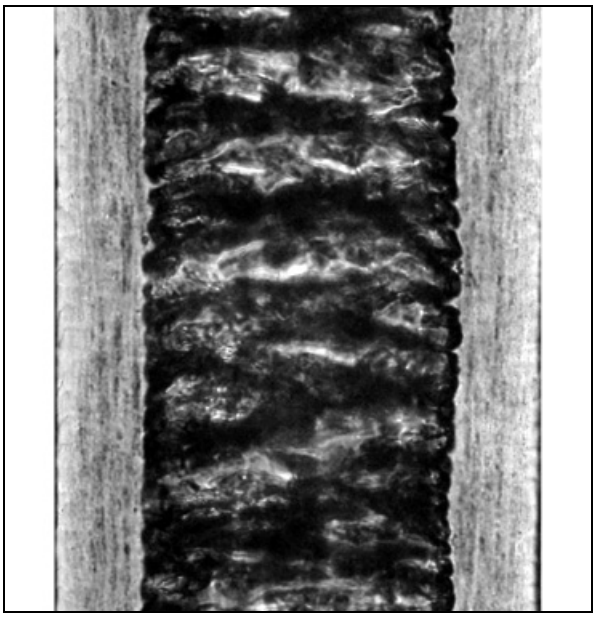


Fig. 51: Medulla at the shield of the Eurasian otter *Lutra lutra*

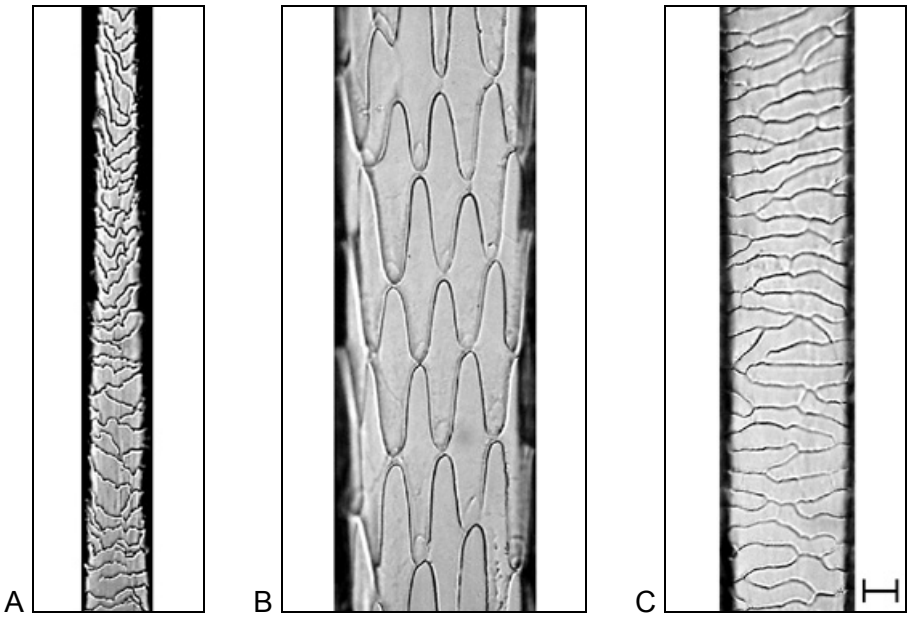


Fig. 52: Hair cuticle at the pars apicalis (A), pars intermedia (B) and pars basalis (C) of the Eurasian otter *Lutra lutra* (magnification bar: 10 µm)

Congo clawless otter *Aonyx congicus*

Morphology: fusiform

Cross-section: oblong-oval

Colour: beige/light brown, shield mostly dark brown with a white tip (sometimes half of the shield white)

Hair length: 16 mm (12-25) Shield: 40-60%

Hair width: 117 µm (86-142) Medulla width: 63 µm (43-78) Medullary index: 0.54

Cuticle scales parameters:

Scale area: 256 µm² Scale width Y: 27 µm

Scale perimeter: 70 µm Scale length X: 13 µm

Y-/X-Feret: 2.13

Medulla structure

Wide lattice, tip and base often fragmented. At the base of the shield and beneath, medulla very thin, sometimes interrupted (Fig. 53B)

Cortex whitish to dark brown at the shield. The tip of the shield was mostly whitish, sometimes light brown. Cortex of the parallel-edged part of the hair shaft beige to light brown; base whitish

Hair cuticle pattern

Pars apicalis: irregular wave pattern, rippled scale margins, distance between the scale margins: intermediate to close

Pars intermedia: irregular mosaic pattern, smooth scale margins, distance between the scale margins: intermediate

Pars basalis: irregular wave pattern, smooth scale margins, distance between the scale margins: intermediate

N.B. Beneath the pars intermedia, the scale pattern changed into a diamond petal (small scales about as wide as long) and then into a broad diamond petal pattern. A narrow diamond petal pattern was encountered over a very short distance just before the base.

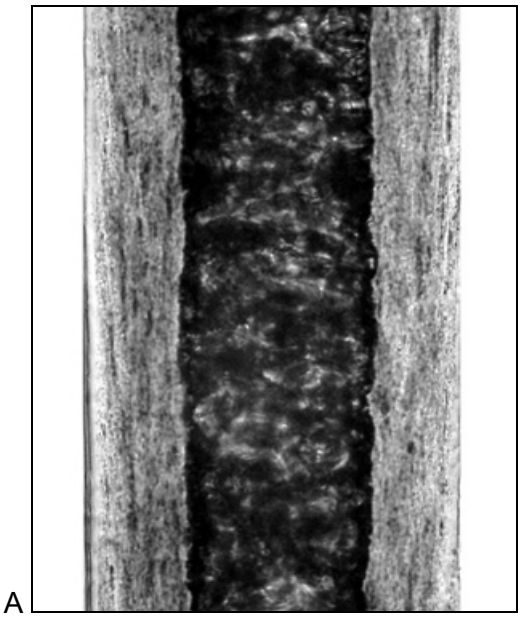


Fig. 53A: Medulla at the middle of the shield of the Congo clawless otter *Aonyx congicus*

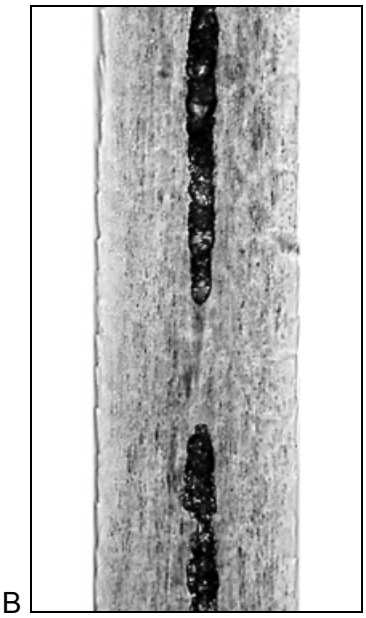


Fig. 53B: Fragmented medulla at the pars intermedia

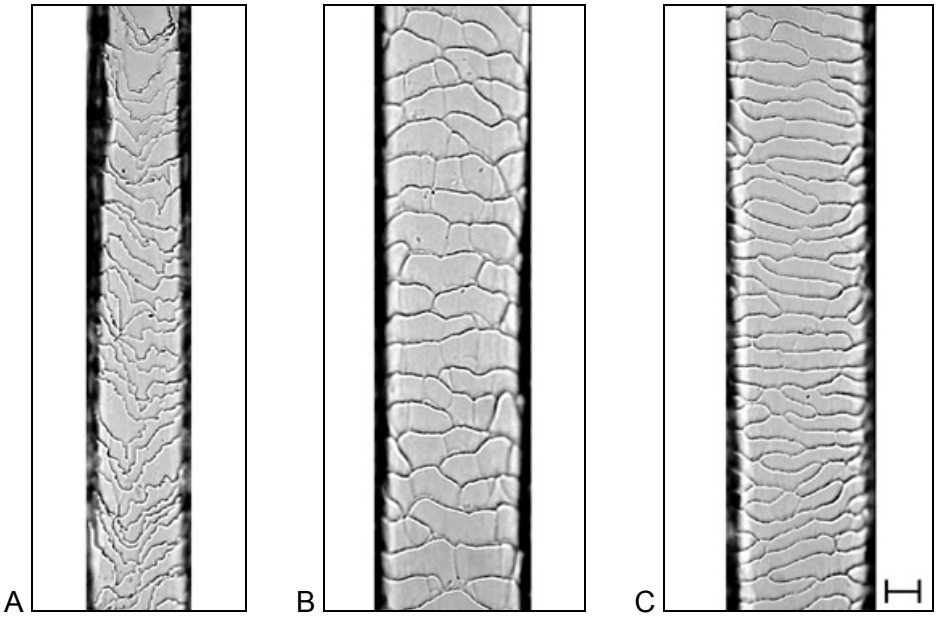


Fig. 54: Hair cuticle at the pars apicalis (A), pars intermedia (B) and pars basalis (C) of the Congo clawless otter *Aonyx congicus* (magnification bar: 10 μm)

Cape clawless otter *Aonyx capensis*

Morphology: fusiform
Cross-section: oblong-oval
Colour: beige/light brown, shield mostly dark brown, sometimes white tip (max 2 mm)

Hair length: 17 mm (11-22) Shield: 40-55%
Hair width: 110 µm (82-141) Medulla width: 53 µm (37-68) Medullary index: 0.49

Cuticle scales parameters:
Scale area: 237 µm² Scale width Y: 26 µm
Scale perimeter: 68 µm Scale length X: 12 µm
Y-/X-Feret: 2.23

Medulla structure

Wide lattice, tip and base often fragmented. At the base of the shield and beneath, medulla very thin, sometimes interrupted
Cortex brown to dark brown at the shield. The tip of the shield was whitish to dark brown. Cortex of the parallel-edged part of the hair shaft beige to light brown; base whitish

Hair cuticle pattern

Pars apicalis: irregular wave pattern, rippled scale margins, distance between the scale margins: intermediate to close

Pars intermedia: irregular mosaic pattern, smooth scale margins, distance between the scale margins: intermediate

Pars basalis: irregular wave pattern, smooth scale margins, distance between the scale margins: intermediate

N.B. Beneath the pars intermedia, the scale pattern changed into a diamond petal and then into a broad diamond petal pattern. A narrow diamond petal pattern was encountered over a very short distance just before the base.

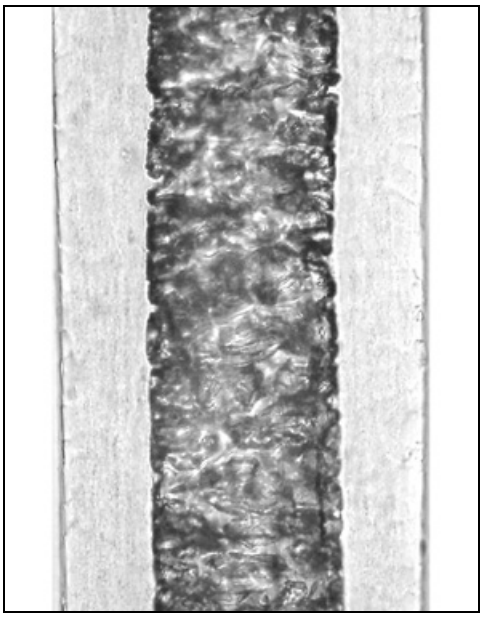


Fig. 55: Medulla at the shield of the Cape clawless otter *Aonyx capensis*

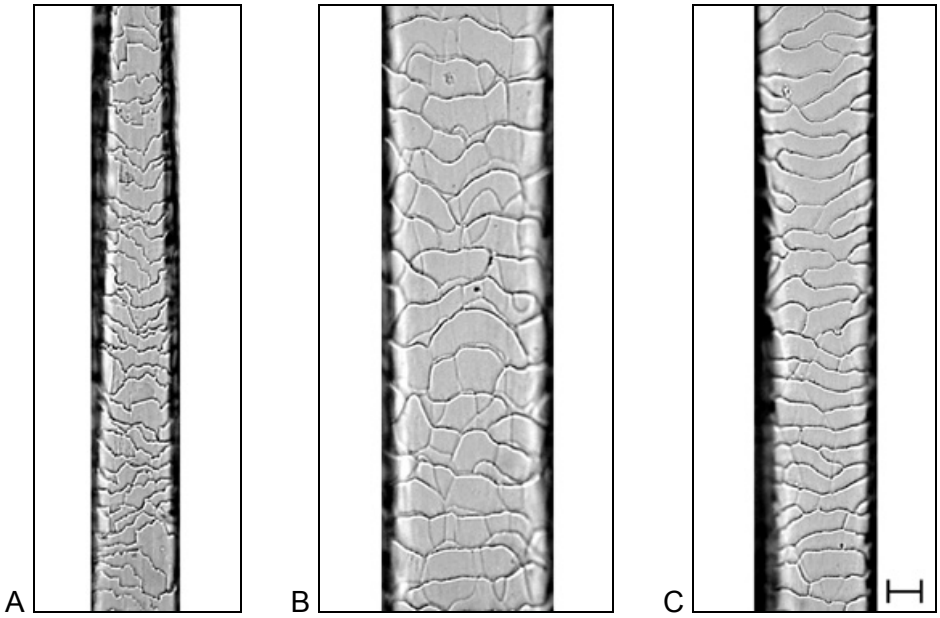


Fig. 56: Hair cuticle at the pars apicalis (A), pars intermedia (B) and pars basalis (C) of the Cape clawless otter *Aonyx capensis* (magnification bar: 10 µm)

Spotted-necked otter *Lutra maculicollis*

Morphology: fusiform
Cross-section: oblong-oval
Colour: beige/light brown, shield mostly dark brown

Hair length: 17 mm (13-22) Shield: 40-50%
Hair width: 131 µm (100-160) Medulla width: 68 µm (47-90) Medullary index: 0.52

Cuticle scales parameters:
Scale area: 324 µm² Scale width Y: 16 µm
Scale perimeter: 96 µm Scale length X: 43 µm
Y-/X-Feret: 0.38

Medulla structure

Wide lattice, tip and base often fragmented. The medulla tended to have quite parallel edges at the lower half of the shield and became visibly thinner immediately above the middle of the shield.
Cortex brown to dark brown at the shield, even at the tip, and beige to light brown at the parallel-edged part of the hair shaft; base whitish

Hair cuticle pattern

Pars apicalis: irregular wave pattern, rippled scale margins, distance between the scale margins: intermediate to close

Pars intermedia: narrow diamond petal pattern with long and narrow scales, smooth scale margins, distance between the scale margins: intermediate

Pars basalis: irregular wave pattern, smooth scale margins, distance between the scale margins: intermediate

N.B. The narrow diamond petal pattern was seen all along the parallel-edged part of the hair shaft, until the irregular wave pattern near the root.

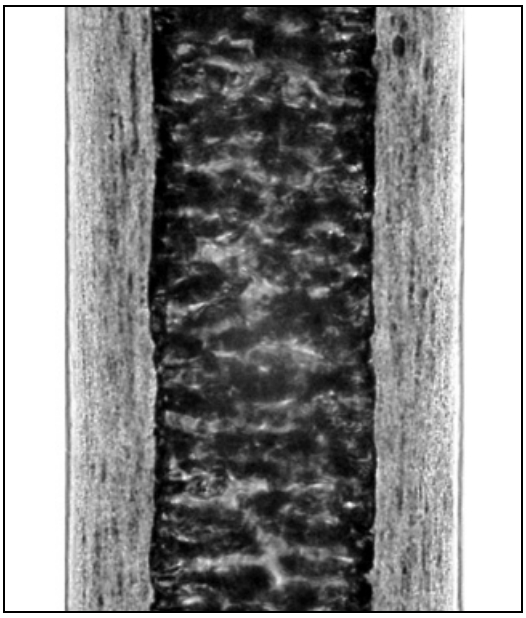


Fig. 57: Medulla at the shield of the Spotted-necked otter *Lutra maculicollis*

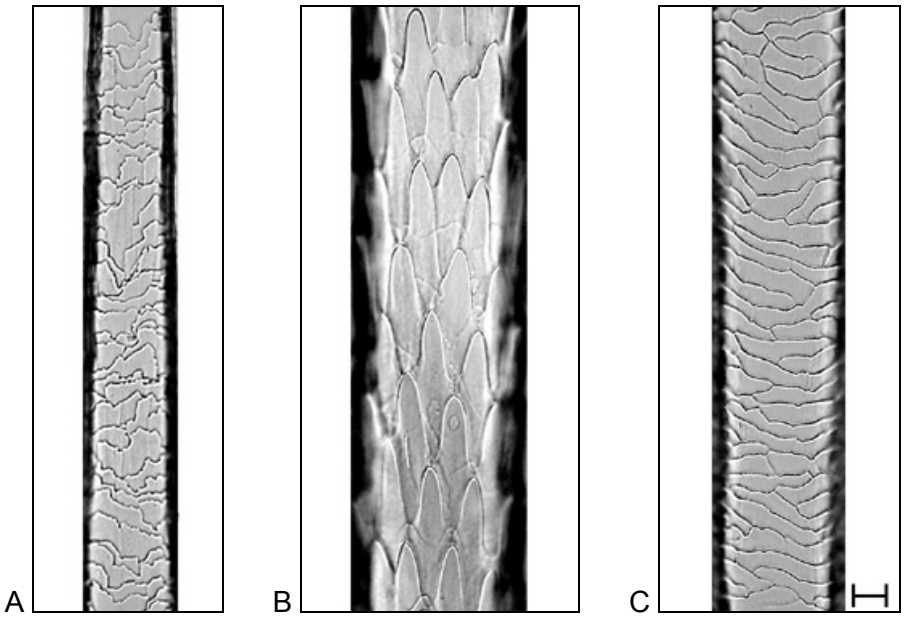


Fig. 58: Hair cuticle at the pars apicalis (A), pars intermedia (B) and pars basalis (C) of the Spotted-necked otter *Lutra maculicollis* (magnification bar: 10 µm)

Small-clawed otter *Amblonyx cinereus*

Morphology: fusiform
Cross-section: oblong-oval
Colour: beige/light brown, shield brown to dark brown

Hair length: 14 mm (11-18) Shield: 40-50%
Hair width: 110 µm (85-135) Medulla width: 68 µm (56-87) Medullary index: 0.62

Cuticle scales parameters:
Scale area: 236 µm² Scale width Y: 17 µm
Scale perimeter: 63 µm Scale length X: 22 µm
Y-/X-Feret: 0.79

Medulla structure

Wide lattice, tip and base often fragmented. Medulla beneath the shield tended to be very thin, sometimes interrupted
Cortex brown to dark brown at the shield, even at the tip, and beige to brown at the parallel-edged part of the hair shaft; base whitish

Hair cuticle pattern

Pars apicalis: irregular wave pattern, rippled scale margins, distance between the scale margins: intermediate to close

Pars intermedia: diamond petal pattern with small scales, often as wide as long, smooth scale margins, distance between the scale margins: intermediate

Pars basalis: irregular wave pattern, smooth scale margins, distance between the scale margins: intermediate

N.B. The diamond petal pattern tended to appear all along the parallel-edged part of the hair shaft. Often the scales got longer near the base, and the pattern turned to a broad diamond or sometimes narrow diamond petal pattern over a short distance (Fig. 60C&D).

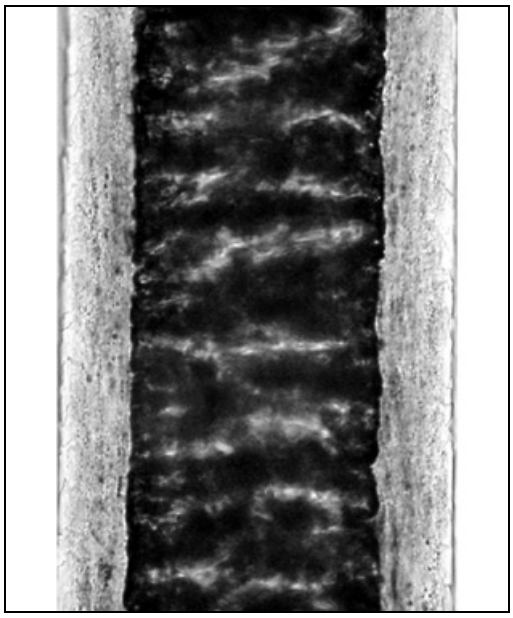


Fig. 59: Medulla at the shield of the Small-clawed otter *Amblonyx cinereus*

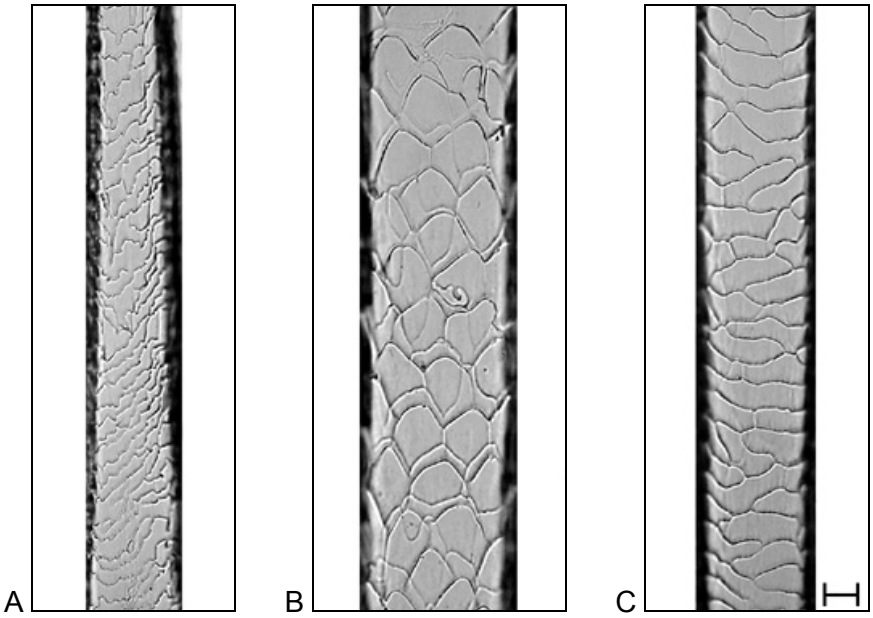


Fig. 60: Hair cuticle at the pars apicalis (A), pars intermedia (B) and pars basalis (C) of the Small-clawed otter *Amblonyx cinereus* (magnification bar: 10 µm)

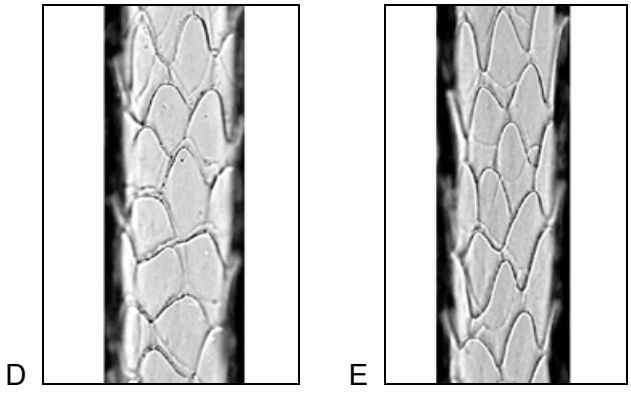


Fig. 60 D&E: Broad diamond petal pattern sometimes encountered beneath the pars intermedia

Smooth otter *Lutrogale perspicillata*

Morphology: fusiform
Cross-section: oblong-oval
Colour: whitish/beige, shield brown to dark brown

Hair length: 12 mm (10-16) Shield: 40-50%
Hair width: 103 µm (72-134) Medulla width: 63 µm (48-78) Medullary index: 0.62

Cuticle scales parameters:
Scale area: 246 µm² Scale width Y: 18 µm
Scale perimeter: 63 µm Scale length X: 23 µm
Y-/X-Feret: 0.84

Medulla structure

Wide lattice, tip and base often fragmented. Medulla beneath the shield tended to be rather thin, rarely interrupted
Cortex brown at the shield and beige at the parallel-edged part of the hair shaft; base whitish

Hair cuticle pattern

Pars apicalis: irregular wave pattern, rippled scale margins, distance between the scale margins: intermediate to close

Pars intermedia: diamond petal pattern with small scales, often as wide as long, smooth scale margins, distance between the scale margins: intermediate

Pars basalis: irregular wave pattern, smooth scale margins, distance between the scale margins: intermediate

N.B. The diamond petal pattern was sometimes seen all along the parallel-edged part of the hair shaft. Often the scales became longer beneath the pars intermedia, and the pattern changed into a broad diamond or sometimes a narrow diamond petal pattern.

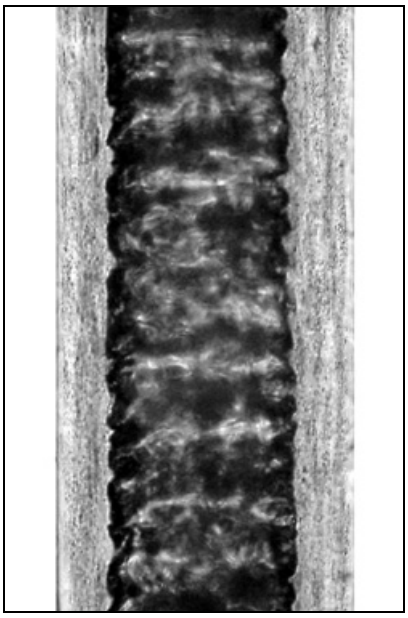


Fig. 61: Medulla at the shield of the Smooth otter *Lutrogale perspicillata*

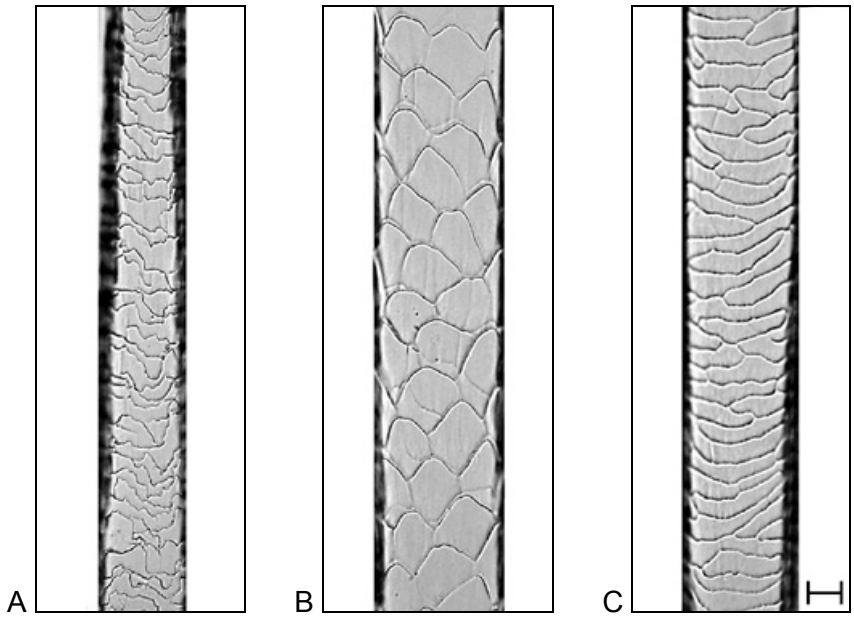


Fig. 62: Hair cuticle at the pars apicalis (A), pars intermedia (B) and pars basalis (C) of the Smooth otter *Lutrogale perspicillata* (magnification bar: 10 µm)

Hairy-nosed otter *Lutra sumatrana*

Morphology: fusiform
Cross-section: oblong-oval
Colour: whitish/beige, shield brown to dark brown

Hair length: 15 mm (12-19) Shield: 40-50%
Hair width: 124 µm (90-163) Medulla width: 78 µm (55-94) Medullary index: 0.62

Cuticle scales parameters:
Scale area: 270 µm² Scale width Y: 15 µm
Scale perimeter: 84 µm Scale length X: 37 µm
Y-/X-Feret: 0.42

Medulla structure

Wide lattice, tip and base often fragmented. Medulla beneath the shield often very thin, sometimes fragmented. Depended on the individual; the 2 Sumatra individuals had interruptions on every hairs, the Borneo individual on half of the hairs and the Thailand individual on no hair (Fig. 63B).
Cortex brown at the shield and beige at the parallel-edged part of the hair shaft; base whitish

Hair cuticle pattern

Pars apicalis: irregular wave pattern, rippled scale margins, distance between the scale margins: intermediate to close

Pars intermedia: narrow diamond petal pattern, smooth scale margins, distance between the scale margins: intermediate

Pars basalis: irregular wave pattern, smooth scale margins, distance between the scale margins: intermediate

N.B. The narrow diamond petal pattern was seen all along the parallel-edged part of the hair shaft, until the irregular wave pattern near the root.

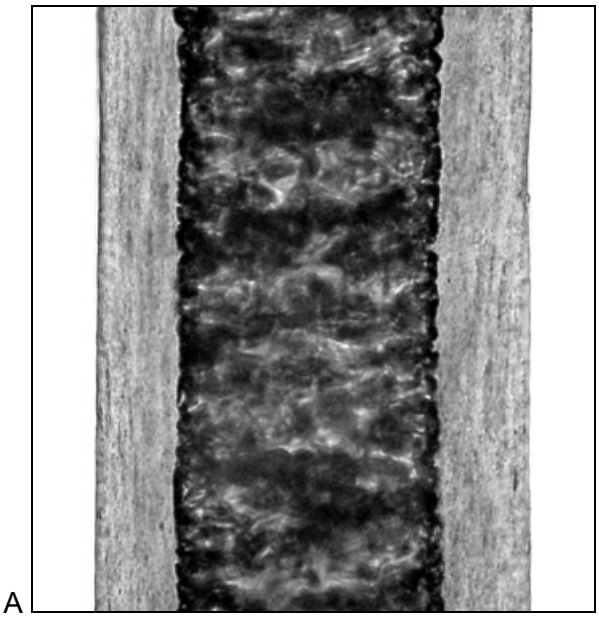


Fig. 63A: Medulla at the shield of the Hairy-nosed otter *Lutra sumatrana*

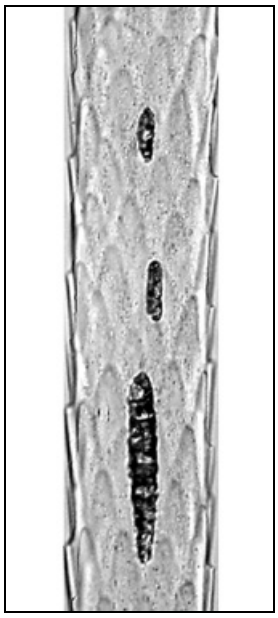


Fig. 63B: Fragmented medulla at the pars intermedia (here of an individual coming from Sumatra)

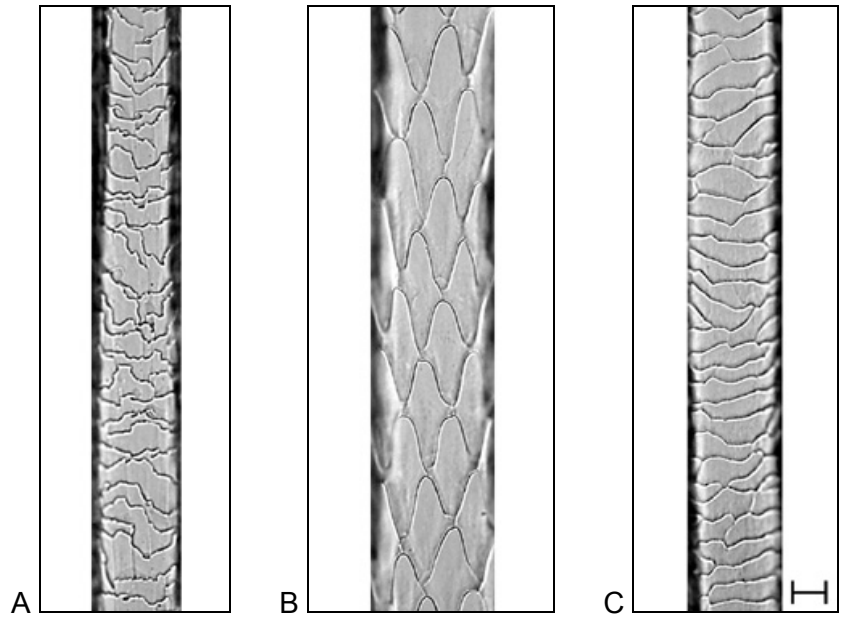


Fig. 64: Hair cuticle at the pars apicalis (A), pars intermedia (B) and pars basalis (C) of the Hairy-nosed otter *Lutra sumatrana* (magnification bar: 10 µm)

Sea otter *Enhydra lutris*

Morphology: slightly fusiform, shield not very distinct. Hair shaft long and thin
Cross-section: oblong-biconvex
Colour: beige/light brown, shield brown to dark brown (sometimes looks black). Tip whitish to light brown (could be 1 cm long)

Hair length: 29 mm (20-40) Shield: about 30%
Hair width: 117 µm (90-143) Medulla width: 27 µm (13-42) Medullary index: 0.23

Cuticle scales parameters:
Scale area: 376 µm² Scale width Y: 15 µm
Scale perimeter: 119 µm Scale length X: 55 µm
Y-/X-Feret: 0.28

Medulla structure

Wide lattice, tip without medulla (upper half of the shield). Medulla at the base of the shield fragmented or absent. Medulla very thin, often fragmented. In many PHs, shield with only slight pieces of medulla or no medulla at all (Fig. 65A&B)
Cortex at the shield light brown to dark brown (sometimes almost black), even at the tip, sometimes grey/whitish. Cortex grey/whitish to light brown at the parallel-edged part of the hair shaft; base whitish

Hair cuticle pattern

Pars apicalis: irregular wave pattern, rippled scale margins, distance between the scale margins: intermediate to close

Pars intermedia: narrow diamond petal pattern, smooth scale margins, distance between the scale margins: intermediate

Pars basalis: irregular wave pattern, smooth scale margins, distance between the scale margins: intermediate

N.B. The narrow diamond petal pattern was seen all along the parallel-edged part of the hair shaft, until the irregular wave pattern near the root.

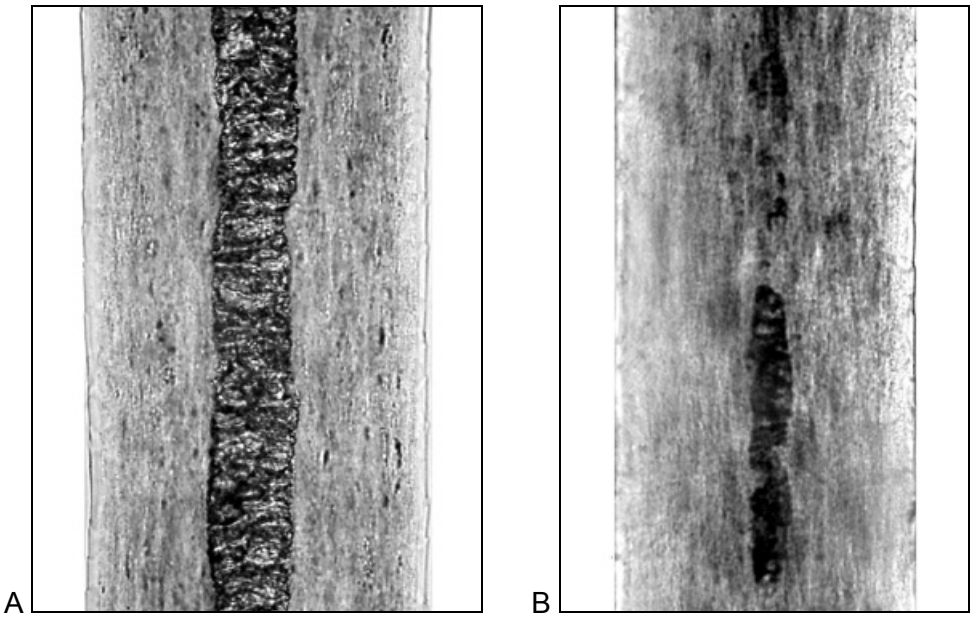


Fig. 65 A&B: Medulla at the middle of the shield of the Sea otter *Enhydra lutris*

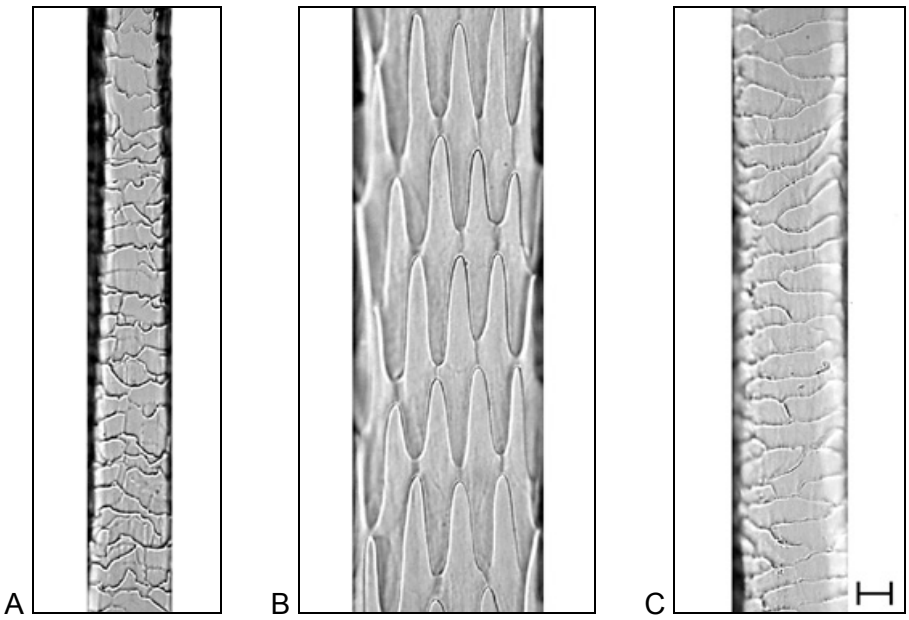


Fig. 66: Hair cuticle at the pars apicalis (A), pars intermedia (B) and pars basalis (C) of the Sea otter *Enhydra lutris* (magnification bar: 10 µm)

North American River otter *Lontra canadensis*

Morphology: fusiform
Cross-section: oblong-oval
Colour: beige/light brown, shield mostly dark brown, shiny

Hair length: 24 mm (17-29) Shield: 30-50%
Hair width: 163 µm (129-191) Medulla width: 89 µm (69-108) Medullary index: 0.54

Cuticle scales parameters:
Scale area: 372 µm² Scale width Y: 14 µm
Scale perimeter: 126 µm Scale length X: 58 µm
Y-/X-Feret: 0.24

Medulla structure
Wide lattice, tip and base often fragmented
Cortex brown to dark brown at the shield, even at the tip, and beige to light brown at the parallel-edged part of the hair shaft; base whitish

Hair cuticle pattern
Pars apicalis: irregular wave pattern, rippled scale margins, distance between the scale margins: intermediate to close

Pars intermedia: narrow diamond petal pattern, smooth scale margins, distance between the scale margins: intermediate

Pars basalis: irregular wave pattern, smooth scale margins, distance between the scale margins: intermediate to close

N.B. The narrow diamond petal pattern was seen all along the parallel-edged part of the hair shaft, until the irregular wave pattern near the root.

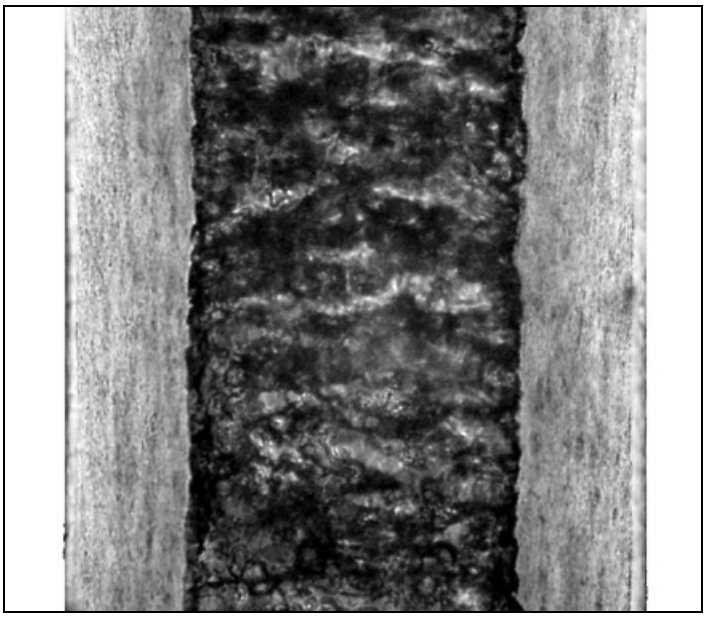


Fig. 67: Medulla at the shield of the North American River otter *Lontra canadensis*

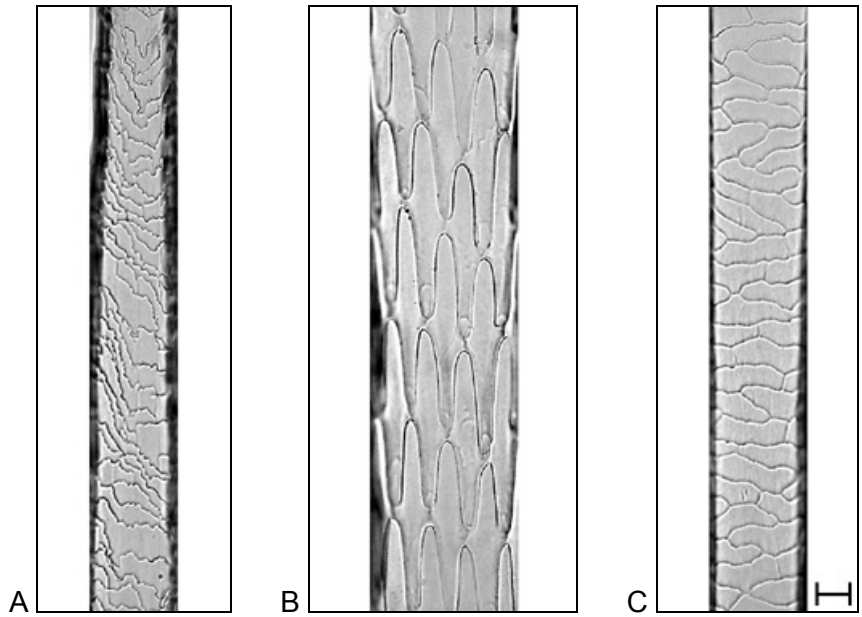


Fig. 68: Hair cuticle at the pars apicalis (A), pars intermedia (B) and pars basalis (C) of the North American River otter *Lontra canadensis* (magnification bar: 10 µm)

Neotropical otter *Lontra longicaudis*

Morphology: fusiform
Cross-section: oblong-oval
Colour: beige/light brown, shield brown to dark brown

Hair length: 16 mm (12-22) Shield: 40-55%
Hair width: 139 µm (107-172) Medulla width: 78 µm (61-99) Medullary index: 0.56

Cuticle scales parameters:
Scale area: 288 µm² Scale width Y: 20 µm
Scale perimeter: 70 µm Scale length X: 26 µm
Y-/X-Feret: 0.79

Medulla structure
Wide lattice, tip and base often fragmented
Cortex brown at the shield and beige to light brown at the parallel-edged part of the hair shaft; base whitish

Hair cuticle pattern
Pars apicalis: irregular wave pattern, rippled scale margins, distance between the scale margins: intermediate to close

Pars intermedia: diamond petal pattern, smooth scale margins, distance between the scale margins: intermediate

Pars basalis: irregular wave pattern, smooth scale margins, distance between the scale margins: intermediate to close

N.B: In the two individuals from Central America (Panama and Mexico), the pattern beneath the shield was clearly diamond petal (scales as wide as long) and observed over quite a long distance on the parallel-edged part of the hair shaft. Then the scales became longer (broad diamond petal pattern). Sometimes a narrow diamond petal pattern could be observed shortly before the final basal pattern. For the three individuals from Guyana, the part beneath the shield with a diamond petal pattern was quite short; a narrow diamond petal pattern followed. In this case it was difficult to say if the pars intermedia began with the diamond petal pattern or the narrow diamond petal pattern.

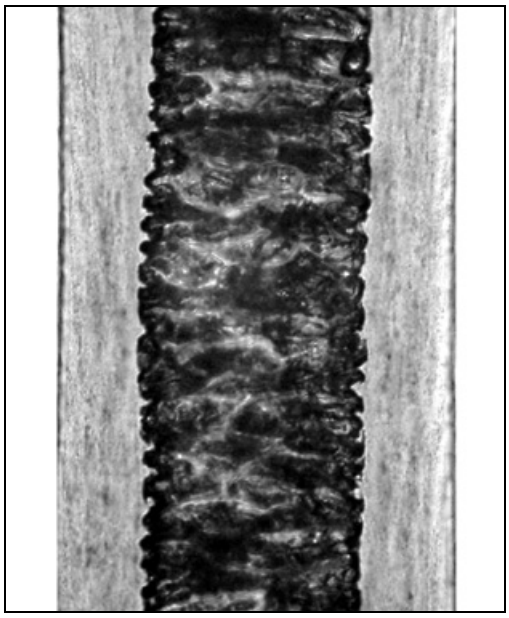


Fig. 69: Medulla at the shield of the Neotropical otter *Lontra longicaudis*

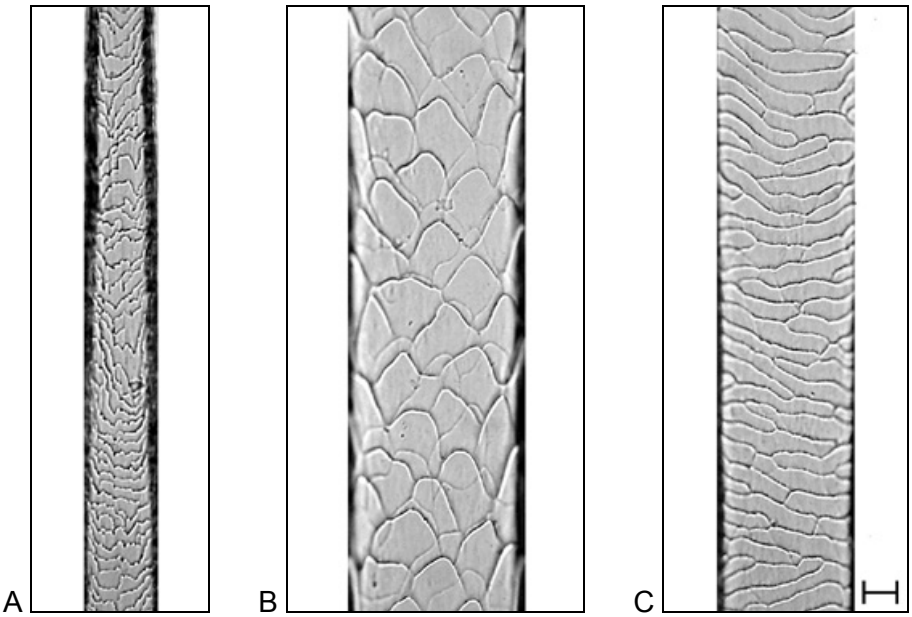


Fig. 70: Hair cuticle at the pars apicalis (A), pars intermedia (B) and pars basalis (C) of the Neotropical otter *Lontra longicaudis* (magnification bar: 10 µm)

Giant otter *Pteronura brasiliensis*

Morphology: slightly fusiform, either parallel-edged hairs with slight apical thickening (shield not really distinct from the rest of the hair shaft)
Cross-section: oval
Colour: beige/light-brown, shield mostly dark brown

Hair length: 11 mm (8-13) Shield: 45-55%
Hair width: 97 µm (68-115) Medulla width: 53 µm (35-75) Medullary index: 0.54

Cuticle scales parameters:
Scale area: 275 µm² Scale width Y: 27 µm
Scale perimeter: 72 µm Scale length X: 13 µm
Y-/X-Feret: 2.10

Medulla structure

Wide lattice, tip and base often fragmented. The proximal end of the medulla was quite distant from the proximal end of the hair shaft (see N.B.). In general, the medulla had an almost constant width all along the hair shaft, except at the tip and base (medulla only slightly thicker at the shield)
Cortex brown at the shield and beige to light brown beneath; base whitish

Hair cuticle pattern

Pars apicalis: irregular wave pattern, rippled scale margins, distance between the scale margins: intermediate to close

Pars intermedia: irregular mosaic pattern, smooth scale margins, distance between the scale margins: intermediate

Pars basalis: irregular wave pattern, smooth scale margins, distance between the scale margins: intermediate to close

N.B: Beneath the pars intermedia, the cuticle scale arrangement turned to a diamond petal pattern. The scales tended to get longer toward the base (broad or narrow diamond petal pattern). The portion of the hair shaft, which exhibited the proximal irregular wave pattern was quite long (sometimes more than 1/5 of the total hair shaft). Only the upper part of this portion contained medulla.

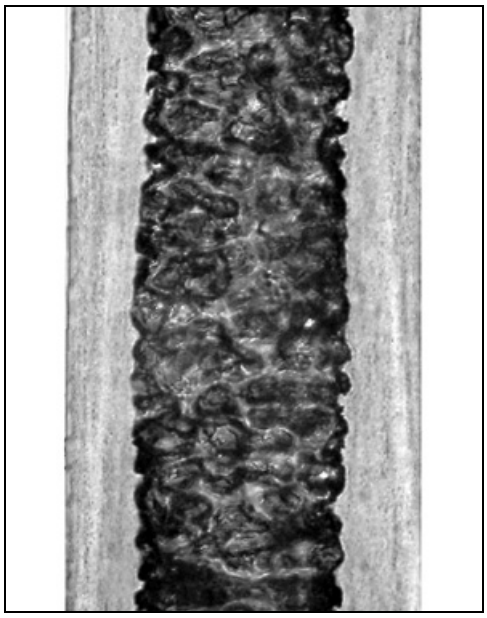


Fig. 71: Medulla at the shield of the Giant otter *Pteronura brasiliensis*

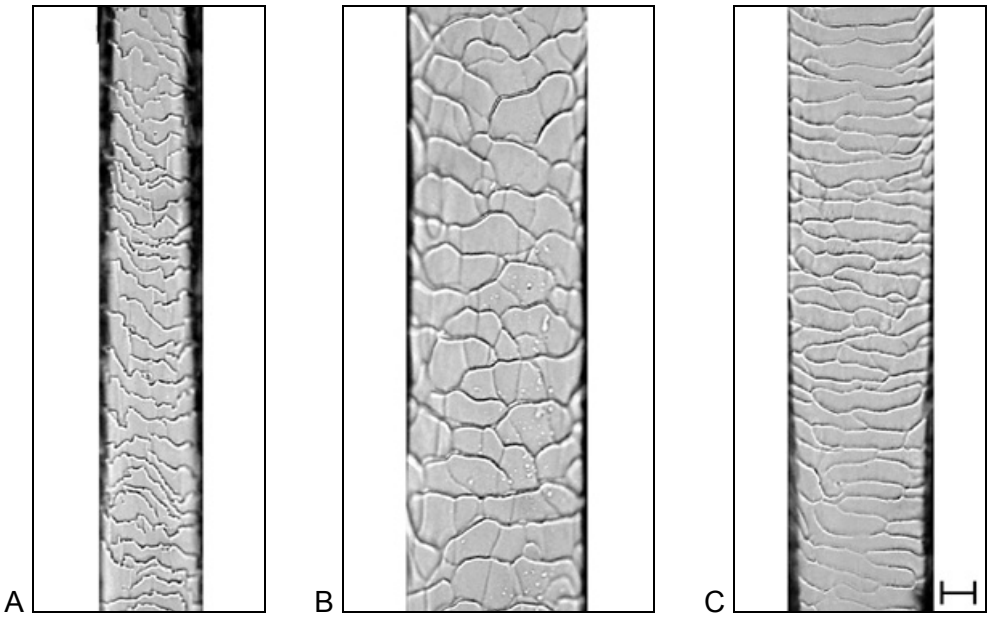


Fig. 72: Hair cuticle at the pars apicalis (A), pars intermedia (B) and pars basalis (C) of the Giant otter *Pteronura brasiliensis* (magnification bar: 10 µm)

Marine otter *Lontra felina*

Morphology: fusiform
Cross-section: oblong-oval
Colour: brown, shield brown to dark brown

Hair length: 20 mm (15-25) Shield: 40-50%
Hair width: 149 µm (130-168) Medulla width: 78 µm (61-88) Medullary index: 0.52

Cuticle scales parameters:
Scale area: 241 µm² Scale width Y: 19 µm
Scale perimeter: 63 µm Scale length X: 21 µm
Y-/X-Feret: 0.92

Medulla structure

Wide lattice, tip and base often fragmented. Sometimes medulla split longitudinally at the pars intermedia and pars basalis (observed in a few hairs by 3 of the 4 individuals studied) (Fig. 73B&C)
Cortex brown at the shield and light brown at the parallel-edged part of the hair shaft; base whitish

Hair cuticle pattern

Pars apicalis: irregular wave pattern, rippled scale margins, distance between the scale margins: intermediate to close

Pars intermedia: diamond petal pattern, smooth scale margins, distance between the scale margins: intermediate

Pars basalis: irregular wave pattern, smooth scale margins, distance between the scale margins: intermediate to close

N.B: The portion of the hair shaft where the diamond petal pattern appeared was not very long. Beneath the pars intermedia, a narrow diamond petal pattern was encountered.

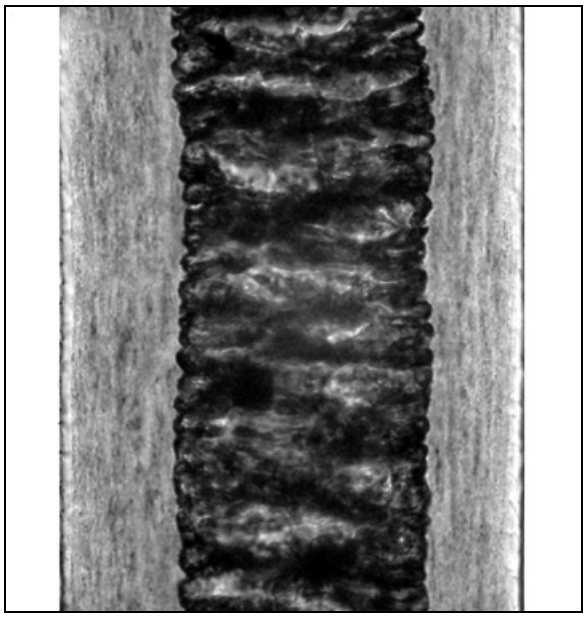


Fig. 73A: Medulla at the shield of the Marine otter *Lontra felina*

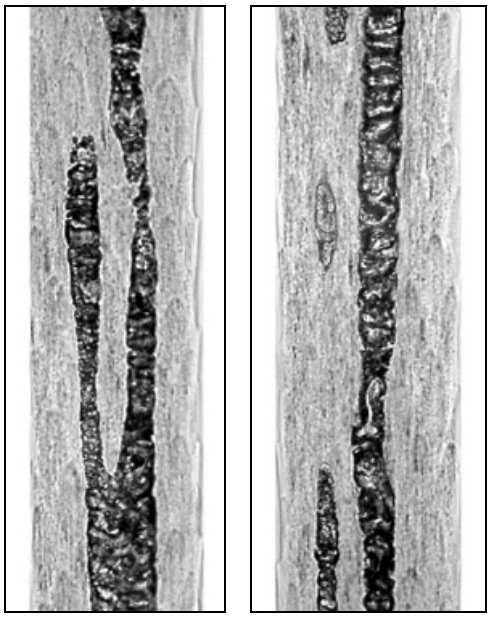


Fig. 73B&C: Medulla split at the pars intermedia

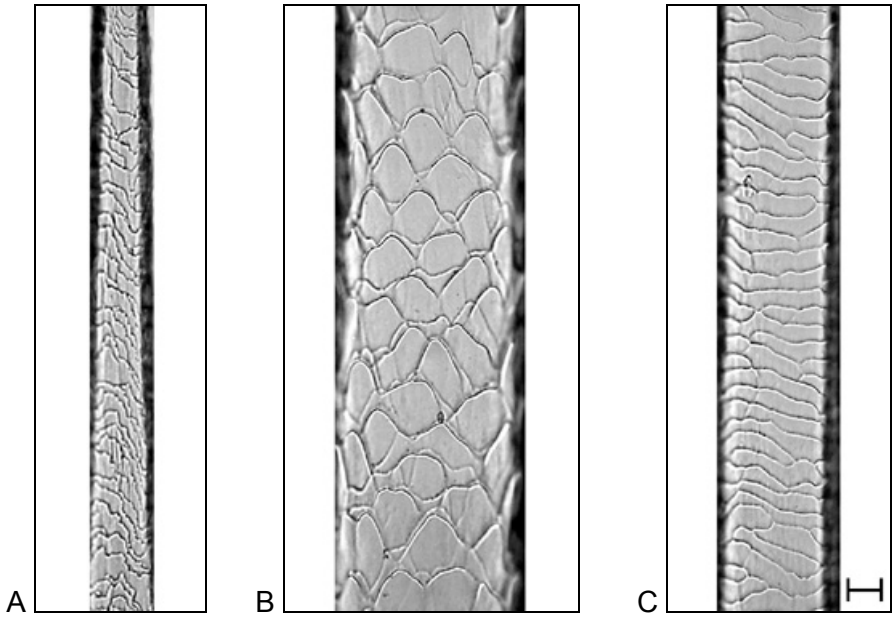


Fig. 74: Hair cuticle at the pars apicalis (A), pars intermedia (B) and pars basalis (C) of the Marine otter *Lontra felina* (magnification bar: 10 µm)

Southern River otter *Lontra provocax*

Morphology: fusiform

Cross-section: oblong-oval

Colour: beige/light brown, shield brown to dark brown

Hair length: 21 mm (17-27) Shield: 40-50%

Hair width: 139 µm (116-174) Medulla width: 82 µm (62-102) Medullary index: 0.59

Cuticle scales parameters:

Scale area: 260 µm² Scale width Y: 19 µm

Scale perimeter: 65 µm Scale length X: 22 µm

Y-/X-Feret: 0.89

Medulla structure

Wide lattice, tip and base often fragmented

Cortex brown at the shield and beige to light brown at the parallel-edged part of the hair shaft; base whitish

Hair cuticle pattern

Pars apicalis: irregular wave pattern, rippled scale margins, distance between the scale margins: intermediate to close

Pars intermedia: diamond petal pattern, smooth scale margins, distance between the scale margins: intermediate

Pars basalis: irregular wave pattern, smooth scale margins, distance between the scale margins: intermediate to close

N.B: The portion of the hair shaft where the diamond petal pattern appeared was not very long. Beneath the pars intermedia, a narrow diamond petal pattern was encountered.

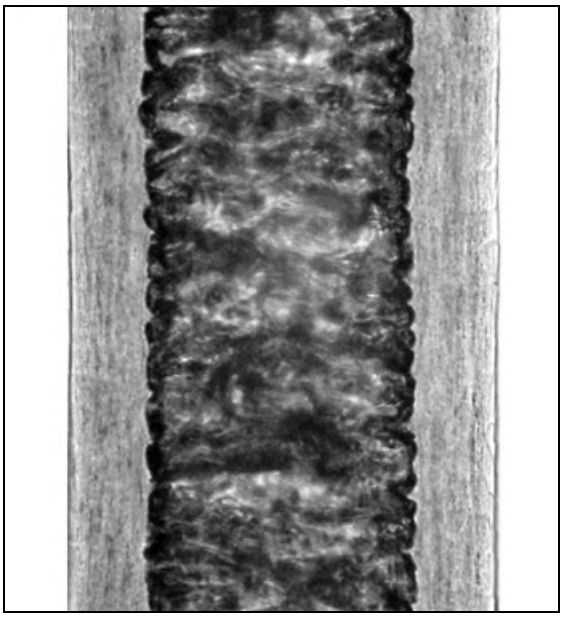


Fig. 75: Medulla at the shield of the Southern River otter *Lontra provocax*

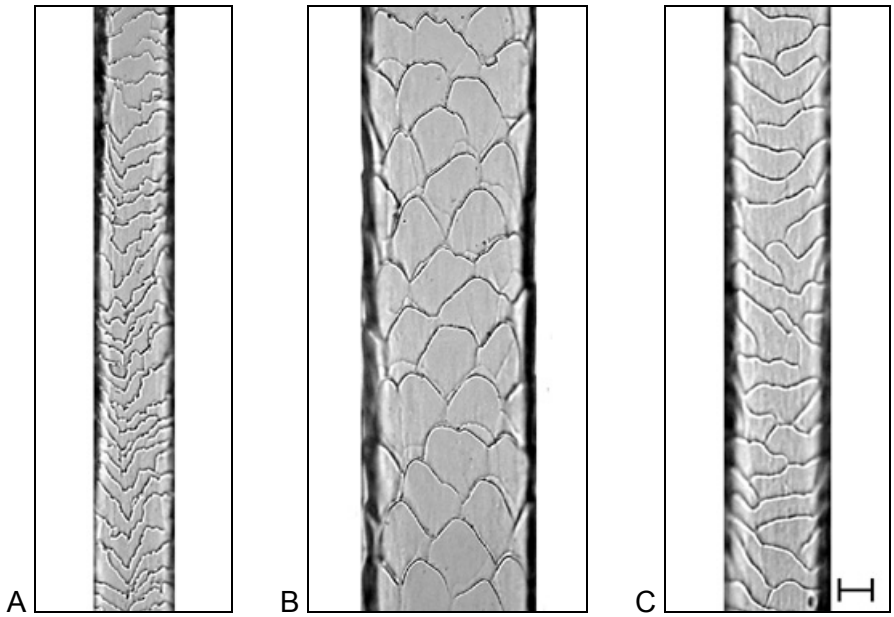


Fig. 76: Hair cuticle at the pars apicalis (A), pars intermedia (B) and pars basalis (C) of the Southern River otter *Lontra provocax* (magnification bar: 10 µm)

2.3.2 Identification of otter species according to primary hair structure

The identification of otter species using primary hair structure analysis was difficult because of the great similarities that exist for medulla and cuticle structure, as seen in the hair atlas, and it was not possible to identify every species confidently. Despite of this, we present a brief determination key for the primary hairs of the Lutrinae, and also compare the PH features of the otter species living sympatrically.

2.3.2.1 Determination key for the primary hairs of the Lutrinae

1.) Straight hairs more or less fusiform, cross-section oval, oblong or biconvex, medulla structure wide lattice. Hair cuticle pattern of the pars intermedia:

- Narrow diamond petal pattern → 2
- Diamond petal pattern with small scales→ 3
- Mosaic pattern→ 4

2.) - Shield does not contain much medulla (medulla thin, often fragmented, upper half and sometimes whole shield without medulla), hair length between 20 and 40 mm, hair width between 90 and 140 µm, very long and narrow scales → *Enhydra lutris*

- Shield with a continuous medulla that takes up more than 50% of the total hair width at the central portion → 5

3.) - Diamond petal pattern apparent all along the parallel-edged part of the hair shaft, eventually broad diamond or narrow diamond petal pattern near the base, hairs between 10 and 18 mm long, and 70 and 135 µm wide → *Amblonyx cinereus*, *Lutrogale perspicillata*

- Distinct narrow diamond petal pattern with long and thin scales beneath the pars intermedia, hairs between 15 and 30 mm long, and between 115 and 175 µm wide → *Lontra felina*, *Lontra provocax*

4.) - Hair shaft with a distinctive shield, distinct narrowing and sometimes interruptions of the medulla at the pars intermedia, hair length between 11 and 25 mm, hair width between 80 and 140 µm, tip sometimes white → *Aonyx congicus, Aonyx capensis*

- Hair shaft parallel-edged with a slight apical thickening, medulla with a more or less constant width (except at the extremities), hair length between 8 and 13 mm, hair width between 70 and 120 µm, shield always uniformly dark brown → *Pteronura brasiliensis*

5.) - Hair length between 15 and 30 mm, hair width between 130 and 180 µm, scale length between 35 and 70 µm, medulla of the shield with quite a constant width (except at the extremities) → *Lutra lutra* (Europe)
- Hair length between 15 and 30 mm, hair width between 130 and 190 µm, scale length between 40 and 80 µm, medulla of the shield with quite a constant width (except at the extremities), hairs particularly shiny → *Lontra canadensis*
- Hair length between 13 and 22 mm, hair width between 100 and 160 µm, scale length between 30 and 60 µm, medulla became visibly thinner immediately above the middle of the shield → *Lutra maculicollis*
- Hair length between 12 and 20 mm, hair width between 90 and 160 µm, scale length between 25 and 50 µm, medulla at the pars intermedia tended to be very thin and sometimes interrupted → *Lutra sumatrana*

2.3.2.2 Identification of otter species living sympatrically

Otter species from Africa

Aonyx congicus, Aonyx capensis, Lutra maculicollis

The PHs of *Lutra maculicollis* could be clearly distinguished from those of the two *Aonyx* by the narrow diamond petal pattern observed at the whole parallel-edged part of the hair shaft. They also tended to be thicker and never had a white tip.

The PHs of *A. congicus* and *A. capensis* were very similar and it was not possible to identify the exact species, except when the hair exhibited a more than 3 mm long white tip, which was typical for *A. congicus*. But the PHs of *A. congicus* could also have shorter white tips or no white tip at all.

Otter species from Asia

Amblonyx cinereus, Lutrogale perspicillata, Lutra sumatrana, Lutra lutra

The PHs of *A. cinereus* and *L. perspicillata* differed from those of *L. sumatrana* and *L. lutra* by the hair cuticle pattern at the pars intermedia, particularly when the diamond petal pattern was encountered all along the parallel-edged part of the hair shaft. The hairs of *L. perspicillata* were more flexible than those of *A. cinereus*, and they also tended to be shorter, thinner and lighter-coloured, but those differences were subtle, and did not allow a really confident identification of the species.

The PHs of *L. sumatrana* and *L. lutra* both exhibited a narrow diamond petal pattern all along the parallel-edged part of the hair shaft, and had a similar length, width and cuticle scale size (if considering the three *L. lutra* from South Asia). The presence of an interrupted medulla depended on the geographic origin of the animal.

Otter species from North America

Enhydra lutris, Lontra canadensis

The PHs of both species were clearly distinct. They both showed a narrow diamond petal pattern at the parallel-edged part of the hair shaft, but the PHs of *Enhydra lutris* were thinner, more flexible, less fusiform and also tended to be longer. Above all, the hairs of *E. lutris* differed clearly from those of every otter species by the thin and often interrupted medulla of the shield.

Otter species from South America

Lontra longicaudis, Pteronura brasiliensis, Lontra felina, Lontra provocax

The PHs of *Pteronura brasiliensis* could be clearly distinguished from those of the other species by their mosaic pattern at the pars intermedia, their morphology, and the fact that they were shorter and thinner. However, *Pteronura* lives sympatrically only with *Lontra longicaudis*.

The PHs of *Lontra felina* and *Lontra provocax* were very similar; nonetheless, the hairs of *L. provocax* were more flexible, and the very apical extremity was thinner. The PHs of *L. felina* were straight, coarse and tended to be darker. A longitudinally split medulla could sometimes be observed in the hairs of *L. felina*.

2.3.3 Comparative analysis of the primary and secondary hair characteristics in the Lutrinae

2.3.3.1 Primary hair length

The primary hair length of every otter species studied is shown in Fig. 77 (mean, minimal and maximal values). The three otters coming from South Asia constitute a separate category (*L.I**). The Sea otter (*Enhydra lutris)* had the longest PHs (highest mean and maximal value), and they differed significantly from the PHs of every other species (ANOVA, $F=52.41$, $p<0.001$, $n=66$, $R^2=0.93$). The Sea otter was followed by *L. canadensis*, *L. lutra*, *L. provocax* and *L. felina*, which all had a mean PH length higher than 20 mm and maximal values higher than 25 mm. The Giant otter (*Pteronura brasiliensis)* and the Smooth otter (*Lutrogale perspicillata)*, which had the shortest PHs, differed significantly from every other species except for the Small clawed otter (*Amblonyx cinereus)*.

Table 15 shows the results of Tukey's HSD test. The individuals within one group do not differ significantly from each other. Fig. 78 indicates the mean value for each individual. The observed intraspecific variations could not be related to the geographic origin of the individuals.

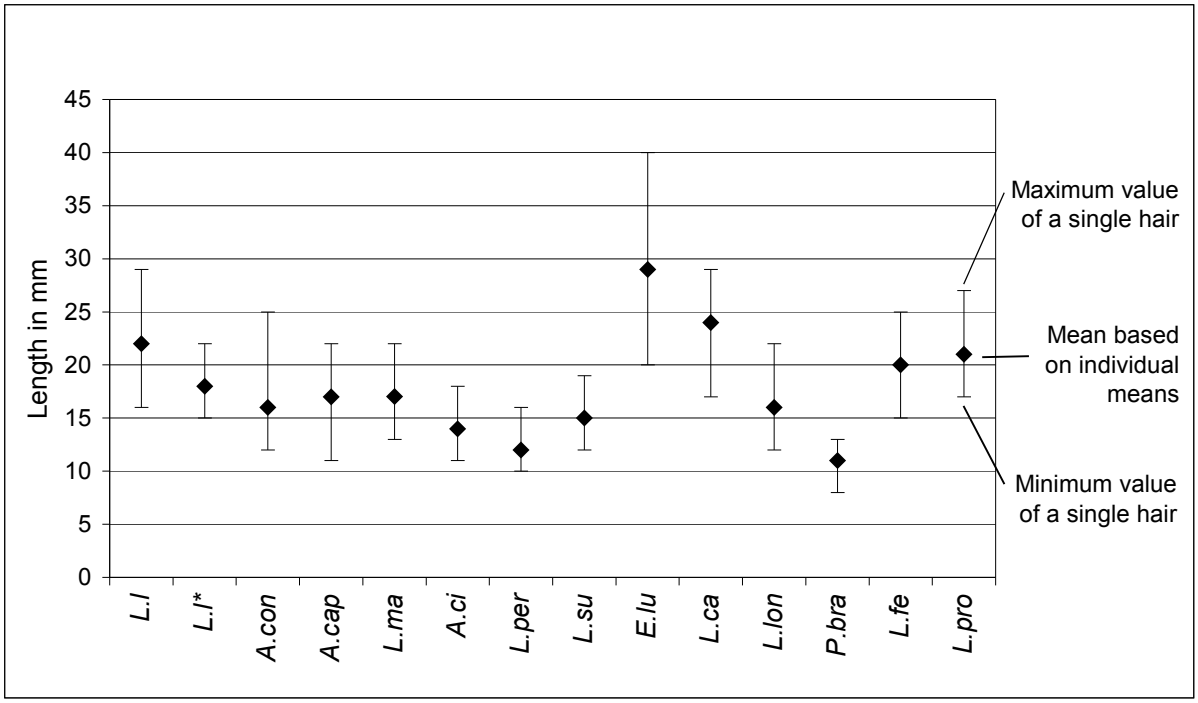


Fig. 77: PH length of the 13 otter species (mean, minimal and maximal values). *L.l** are the three Eurasian otters from South Asia.

Tab. 15: Tukey's HSD test, PH length, grouping of not significantly different species

Species	Mean (mm) ± SD	Groups							
E.lu	29 ± 2.0	A							
L.ca	24 ± 0.9		B						
L.l	22 ± 1.3		B	C					
L.pro	21 ± 0.8		B	C	D				
L.fe	20 ± 1.3			C	D	E			
*L.l**	18 ± 0.8				D	E	F		
L.ma	17 ± 1.8					E	F	G	
A.cap	17 ± 2.0					E	F	G	
A.con	16 ± 2.6						F	G	
L.lon	16 ± 1.5						F	G	
L.su	15 ± 0.9						F	G	
A.ci	14 ± 0.8							G	H
L.per	12 ± 0.9								H
P.bra	11 ± 0.6								H

2.3.3.2 Primary hair width, medulla width and medullary index

The PH width, medulla width and medullary index of the 13 otter species are represented in Figs. 79 and 80. *L. canadensis, L. lutra* and *L. felina* had the thickest PHs. They differed significantly from the two *Aonyx, E. lutris, A. cinereus*, and of course from the two species with the thinnest PHs, *L. perspicillata* and *P. brasiliensis* (ANOVA, $F=14.11$, $p<0.001$, $n=66$, $R^2=0.78$).

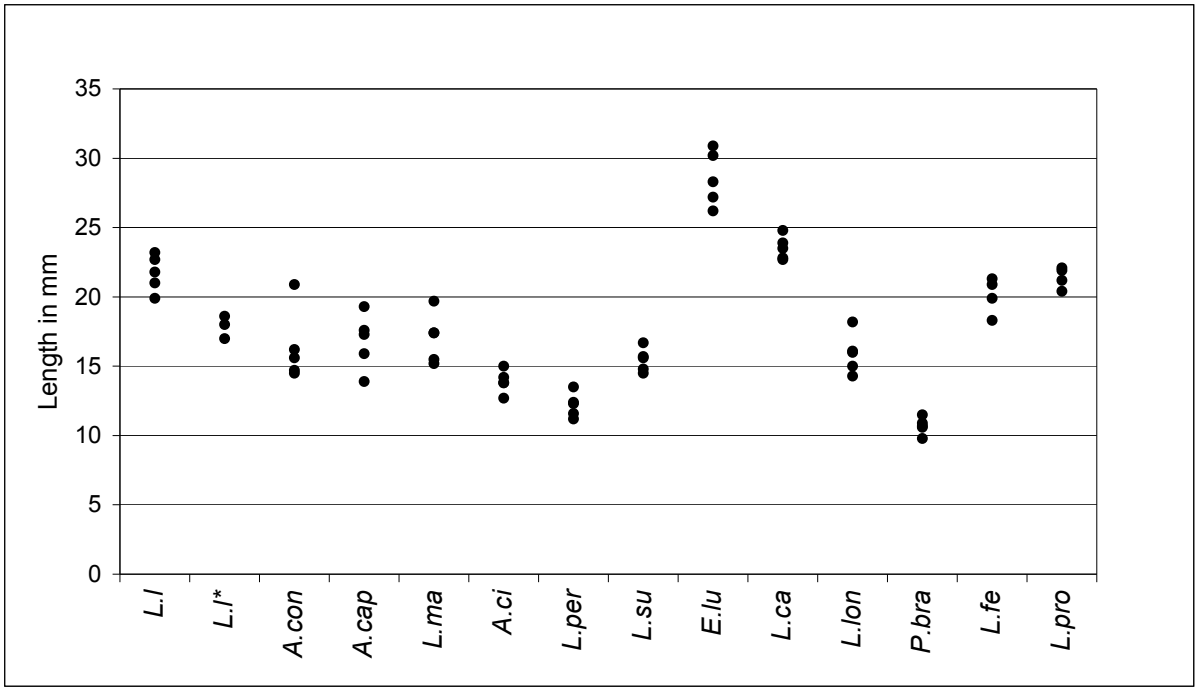


Fig. 78: PH length of the 13 otter species (mean value of each individual)

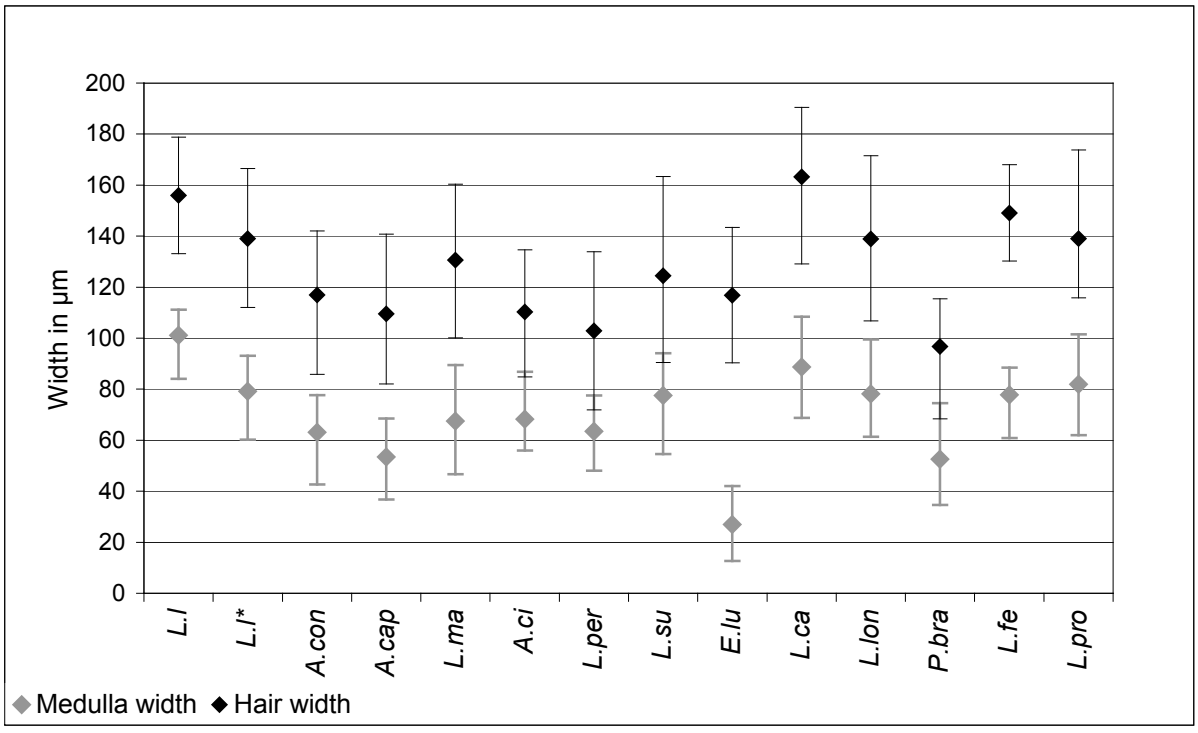


Fig. 79: PH width and medulla width of the 13 otter species (mean, minimal and maximal values). *L.l** are the three Eurasian otters from South Asia.

The ranking of the values of medulla width differed from the ranking of the values of hair width (Tabs. 16 & 17). *L. lutra*, *L. canadensis* and *L. provocax* still had the highest values. *L. lutra* had the thickest , medulla, and differed significantly from every other species, except for *L. canadensis* (ANOVA, F=34.47, p<0.001, n=66, R^2=0.90). *E. lutris* had the most remarkable medulla, which was twice as thin as the second thinnest medulla, observed in the hairs of *P. brasiliensis,* and almost five times as thin as the thickest medulla, found in *L. lutra*.

The calculated medullary index confirmed that medulla thickness was not proportional to hair thickness. The medulla took up 50 to 65% of total hair width, except in *E. lutris*, where it took up about 20% of total hair width (mean value). Thus, the medullary index of *E. lutris* differed significantly from every other (ANOVA, F=47.78, p<0.001, n=66, R^2=0.92). *L. lutra* not only had the thickest medulla and the second thickest PHs, but also the thickest medulla relatively to the hair diameter. But, this was not generally the case. For example, *L. canadensis*, which had the thickest PHs and the second thickest medulla, had a medullary index, which differed significantly from the maximal values, and *L. perspicillata*, which had the second thinnest PHs and also a relatively thin medulla, had one of the highest medullary indices (Tab. 18).

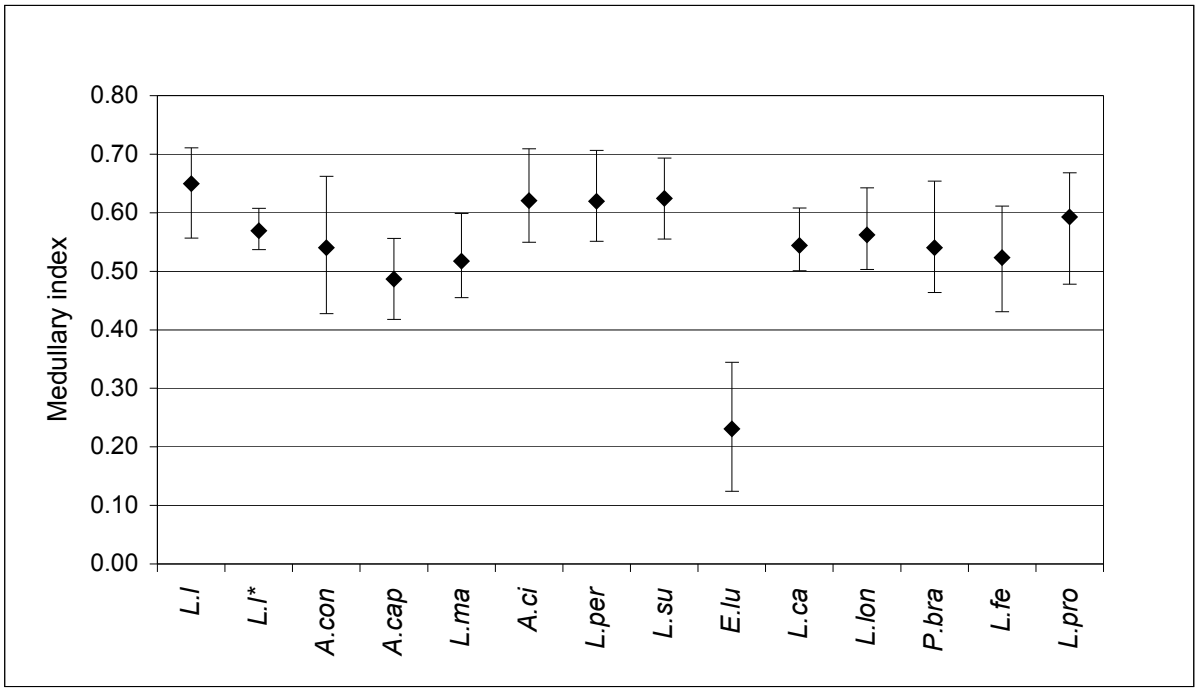


Fig. 80: Medullary index of the 13 otter species (mean, minimal and maximal values)

Tab. 16: Tukey's HSD test, PH width, grouping of not significantly different species

Species	Mean (µm) ± SD	Groups						
L.ca	163 ± 12.7	A						
L.l	156 ± 4.5	A	B					
L.fe	149 ± 4.6	A	B	C				
L.pro	139 ± 17.1	A	B	C	D			
*L.l**	139 ± 12.3	A	B	C	D			
L.lon	139 ± 15.5	A	B	C	D			
L.ma	131 ± 8.8		B	C	D	E		
L.su	124 ± 15.7			C	D	E	F	
A.con	117 ± 13.4				D	E	F	G
E.lu	117 ± 11.0				D	E	F	G
A.ci	110 ± 9.5					E	F	G
A.cap	110 ± 12.5					E	F	G
L.per	103 ± 11.7						F	G
P.bra	97 ± 9.9							G

Tab. 17: Tukey's HSD test, medulla width, grouping of not significantly different species

Species	Mean (µm) ± SD	Groups						
L.l	101 ± 2.3	A						
L.ca	89 ± 7.3	A	B					
L.pro	82 ± 6.3		B	C				
*L.l**	79 ± 6.5		B	C	D			
L.lon	78 ± 10.3		B	C	D			
L.fe	78 ± 3.9		B	C	D			
L.su	78 ± 8.5		B	C	D			
A.ci	68 ± 5.1			C	D	E		
L.ma	68 ± 6.4			C	D	E	F	
L.per	63 ± 5.9				D	E	F	
A.con	63 ± 8.1				D	E	F	
A.cap	53 ± 7.2					E	F	
P.bra	53 ± 8.7						F	
E.lu	27 ± 3.6							G

Tab. 18: Tukey's HSD test, medullary index, grouping of not significantly different species

Species	Mean ± SD	Groups				
L.l	0.65 ± 0.02	A				
L.su	0.62 ± 0.02	A	B			
A.ci	0.62 ± 0.04	A	B			
L.per	0.62 ± 0.02	A	B			
L.pro	0.59 ± 0.05	A	B	C		
*L.l**	0.57 ± 0.01	A	B	C		
L.lon	0.56 ± 0.02		B	C		
L.ca	0.54 ± 0.02			C	D	
A.con	0.54 ± 0.06			C	D	
P.bra	0.54 ± 0.05			C	D	
L.fe	0.52 ± 0.04			C	D	
L.ma	0.52 ± 0.02			C	D	
A.cap	0.49 ± 0.02				D	
E.lu	0.23 ± 0.03					E

Intraspecific variations:

The Fig. 81 indicates the mean hair and medulla width of every individual. In the *Lutra lutra* South Asia group, the individual with a visibly higher value (both for the hair and medulla width) was the one from India, whereas the two individuals from Sri Lanka had very close values. The medullary indices of all three individuals were almost similar (see Fig. 82). The *L. sumatrana* with the lowest value (both for the hair and medulla width) was the one from Thailand. The thickest PHs, which had the thickest medulla, belonged to the two individuals from Sumatra. The two *L. longicaudis* with the lowest value for both, the hair and medulla width were the two individuals from Central America (remember the three other individuals came from French Guiana). The two *L. provocax* with a distinct higher mean PH width came from the Lake Todos Los Santos and Temuco, two inland areas situated about 70 km away from the coast, whereas the two other individuals came from coastal areas. The individual from the Lake Todos Los Santos also had a visibly thicker medulla. Further investigations would be necessary to see whether the correlations observed are just coincidences or not. The intraspecific variability observed in the other species was not related to the origin of the species. Note that the Eurasian otters, which all came from the same region of Germany, had very close values (for every PH feature). The variability observed in *A. congicus* was quite surprising because all the individuals came from quite contiguous regions (*A. congicus* has a relatively small distribution range compared to other species). The medullary index showed less intraspecific variability, with some exceptions, but these could not be related to the origin of the individuals.

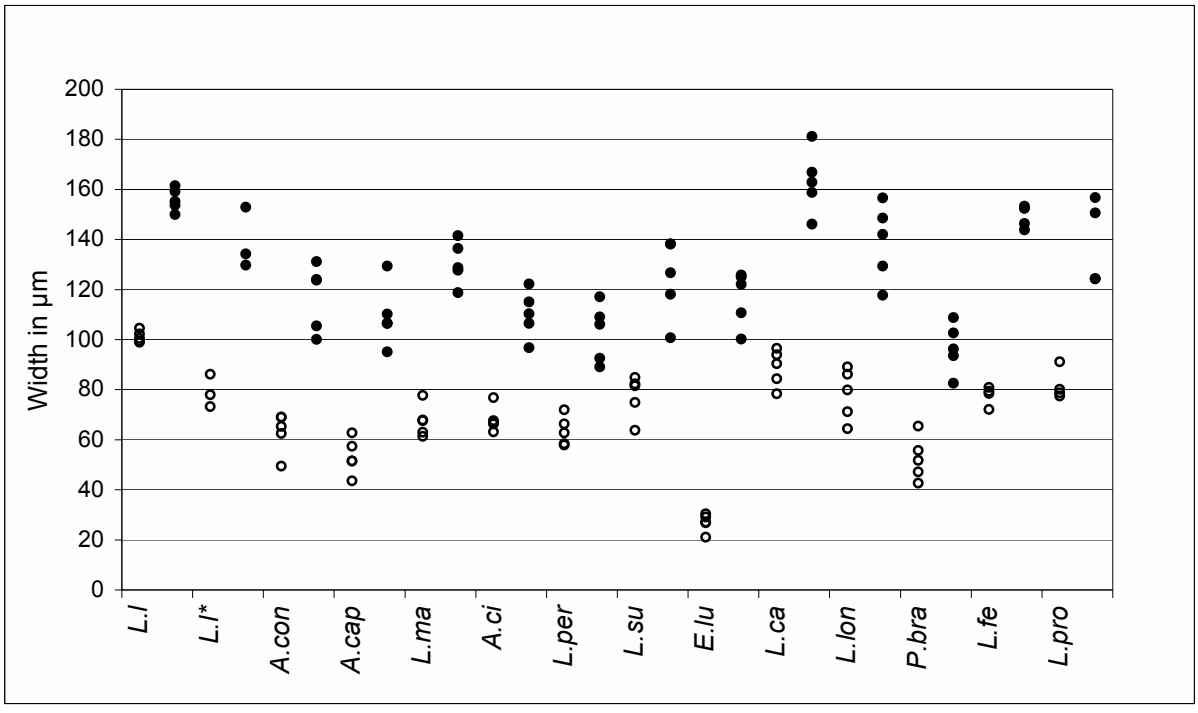


Fig. 81: PH width (black circles) and medulla width (clear circles) of the 13 otter species (mean value of each individual)

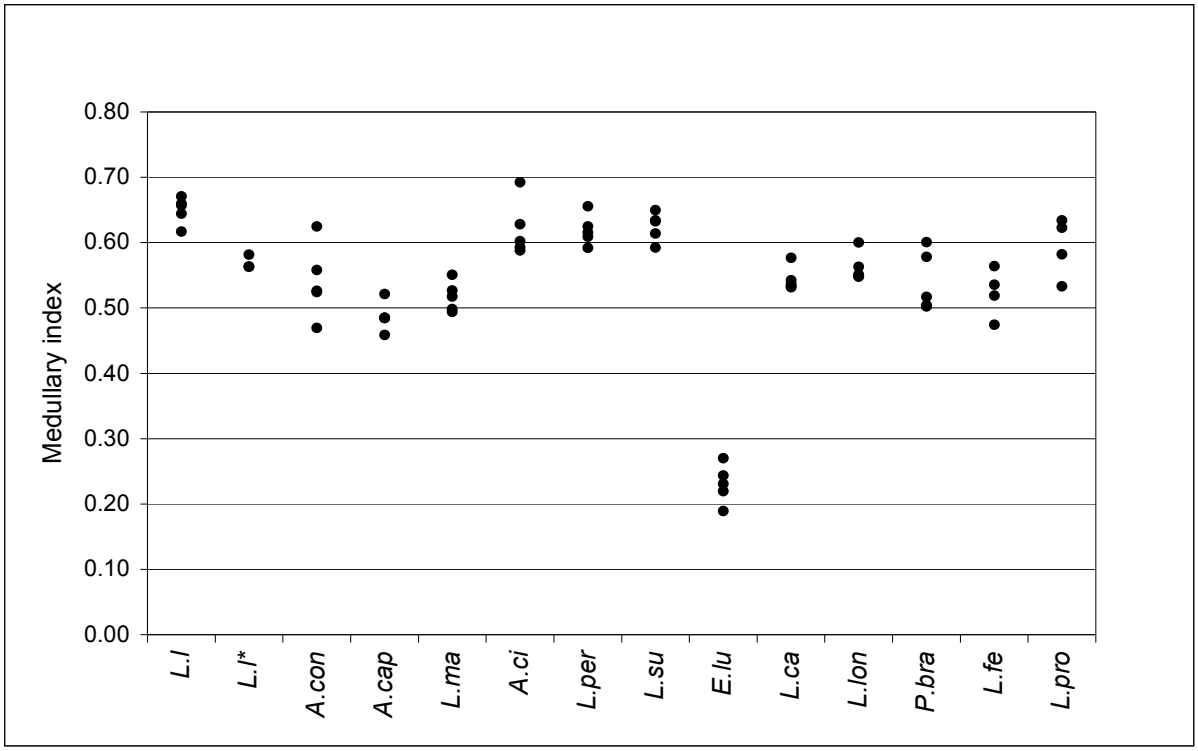


Fig. 82: Medullary index of the 13 otter species (mean value of each individual)

2.3.3.3 Cuticle scales parameters (area, perimeter, length and Y-/X-Feret)

The graphs in Figs. 83, 84, 85, 86 show cuticle scale area, perimeter, length and Y-/X-Feret (ratio of scale width to length) of the 13 otter species.

Scale area: *L. lutra, E. lutris* and *L. canadensis* had the highest scale area and differed significantly from every other species, with the exception of *L. maculicollis*, which differed from *L. lutra* but not from *E. lutris* and *L. canadensis* (ANOVA, $F=26.02$, $p<0.001$, $n=66$, $R^2=0.87$). These four species with the largest scales all had PHs that exhibited a narrow diamond petal pattern at the pars intermedia, but in the species with smaller scales, there was no correlation between scale area and scale shape. The long, narrow petal like scales of *L. sumatrana* had a smaller area than the scales of *L. longicaudis* and *P. brasiliensis,* which were in a diamond petal (short scales, as wide as long) and mosaic arrangement respectively. All species with a diamond petal and a mosaic pattern did not differ significantly from each other, and also not from *L. lutra** (South Asia) and *L. sumatrana*.

Perimeter: In a ranking from the highest to the lowest value, the six first places were occupied by the species exhibiting a narrow diamond petal pattern. They all differed significantly from the species showing a diamond petal and a mosaic pattern (ANOVA, $F=107.73$, $p<0.001$, $n=66$, $R^2=0.96$). Within the first group, *L. canadensis, E. lutris* and *L. lutra* differed significantly from *L. maculicollis*, *L. lutra** (South Asia) and *L. sumatrana*. Within the second group (diamond petal and mosaic pattern), there were no significant differences. *A. cinereus* was the species with the smallest scales (area and perimeter).

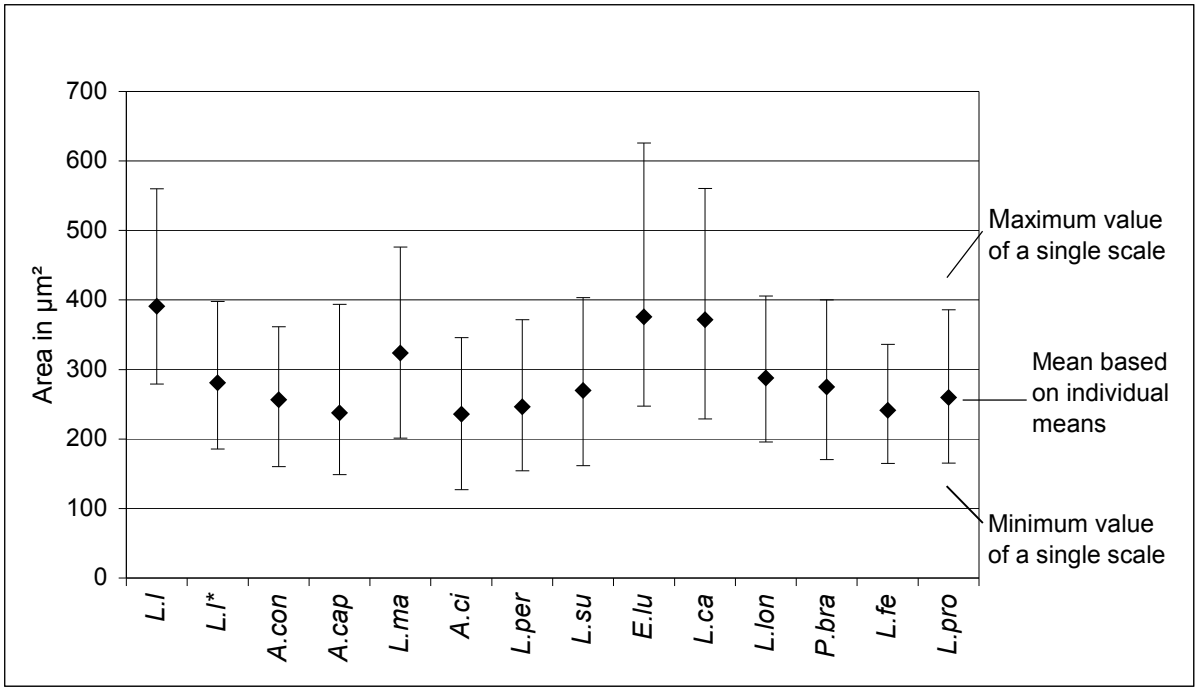


Fig. 83: Cuticle scale area of the 13 otter species (mean, minimal and maximal values)

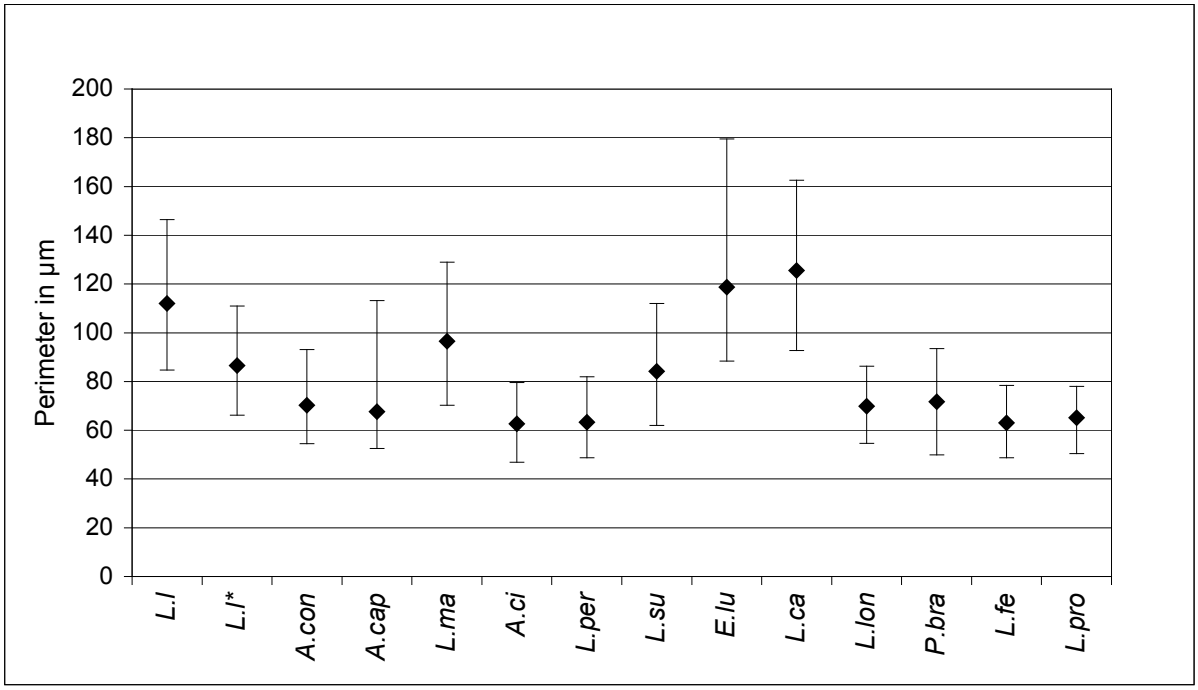


Fig. 84: Cuticle scale perimeter of the 13 otter species (mean, minimal and maximal values)

Scale length: The species showing a narrow diamond petal pattern had the longest scales. All the otter species differed significantly from the species exhibiting a different scale pattern. Within the first group (narrow diamond petal), *L. canadensis, E. lutris* and *L. lutra* differed significantly from *L. maculicollis*, *L. lutra** (South Asia) and *L. sumatrana* (ANOVA, F=244.19,

p<0.001, n=66, R^2=0.98). There were no significant differences within the group of species the scales of which were in a diamond petal arrangement (*L. longicaudis, L. perspicillata, A. cinereus*, *L. provocax* and *L. felina*) and also within the group of species showing a mosaic pattern (*P. brasiliensis*, *A. congicus* and *A. capensis*).

Y-/X-Feret: Here again, all the species differed significantly from the species exhibiting a different scale pattern (ANOVA, F=507.89, p<0.001, R^2=0.99). The species the scales of which were in a mosaic pattern, had a Y-/X-Feret close to 2. The species the scales of which were in a diamond petal pattern had a Y-/X-Feret between 0.8 and 1. There were no significant differences within these two groups. Within the third group, *L. sumatrana* and *L. lutra** (Y-/X-Feret=0.42) differed significantly from *L. canadensis*, which had the slimmest scales (Y-/X-Feret=0.25). Note the important difference between the minimal and maximal values in the species showing a diamond petal and, particularly, in those showing a mosaic pattern. This was due to the irregular nature of those patterns.

Intraspecific variability:

The Figs. 87, 88, 89 and 90 indicate the mean scale area, perimeter, length and Y-/X-Feret of each individual. In the *L. lutra** (South Asia) group, the individual from India had a higher scale area, perimeter and length than the two individuals from Sri Lanka. The two *L. longicaudis* coming from Central America had a lower scale area than the individuals from French Guiana, but this divergence was not observed in the other scale parameters. The same for *L. provocax*; indeed the two inland individuals had lower values than the coastal individuals for the scale area, but not for the other parameters. The intraspecific variability observed in the other species could not be related to the geographic origin of the individuals.

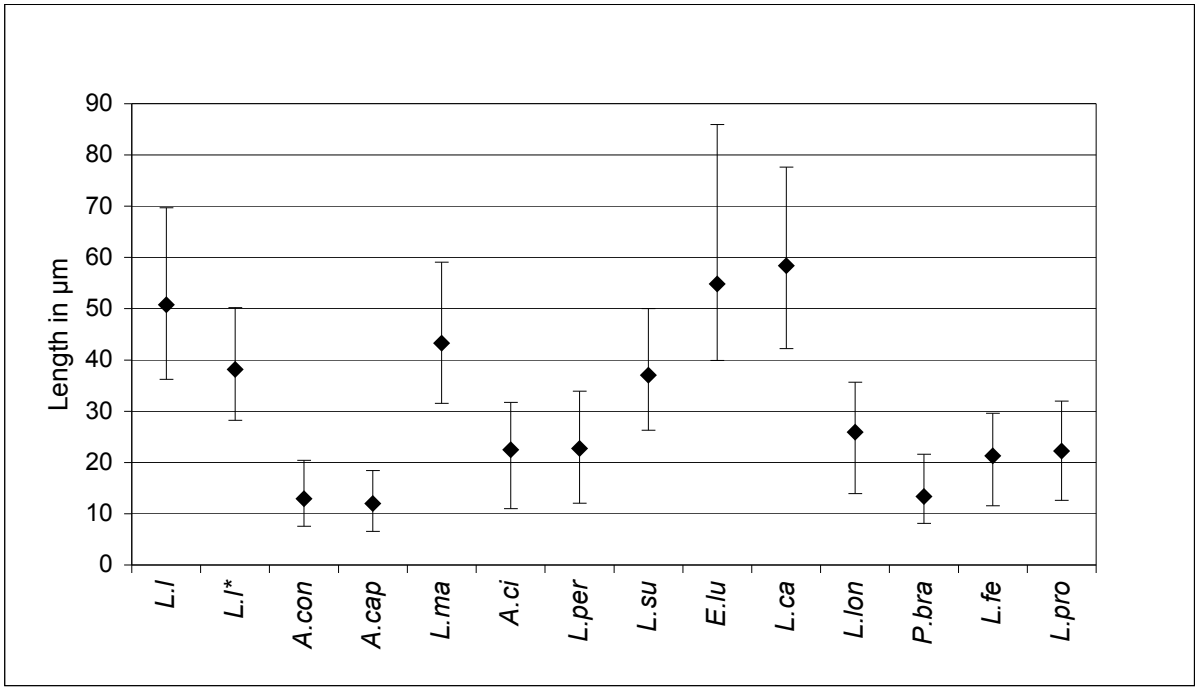


Fig. 85: Cuticle scale length of the 13 otter species (mean, minimal and maximal values)

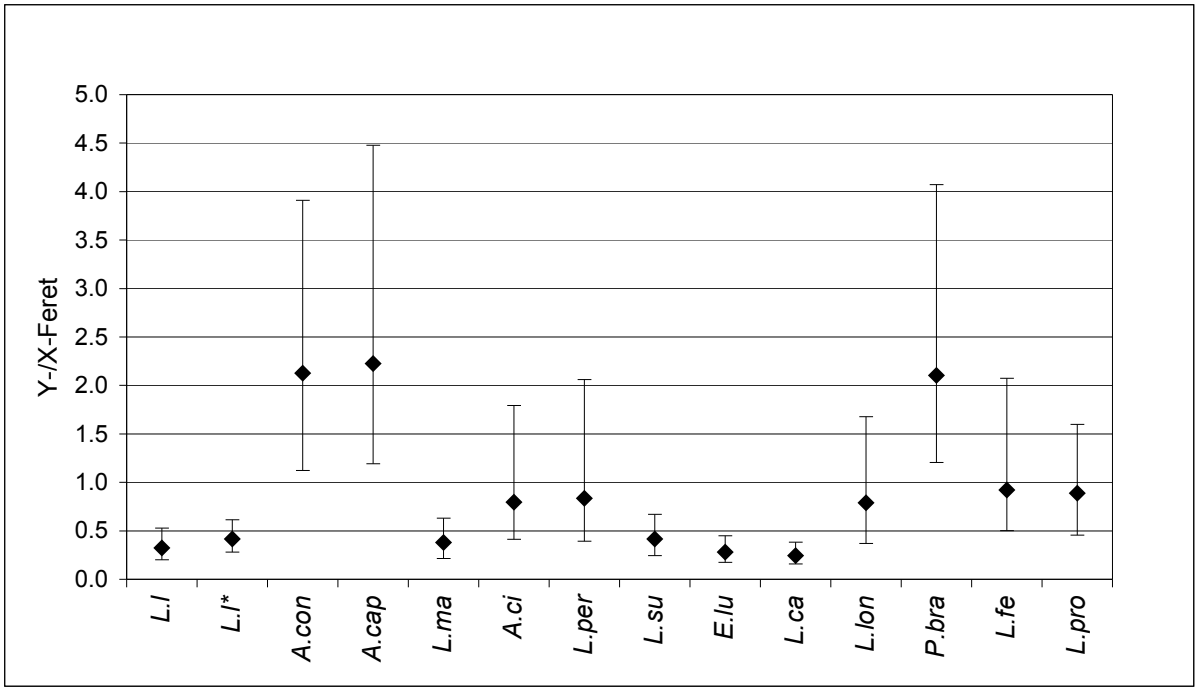


Fig. 86: Y-/X-Feret of the 13 otter species (mean, minimal and maximal values)

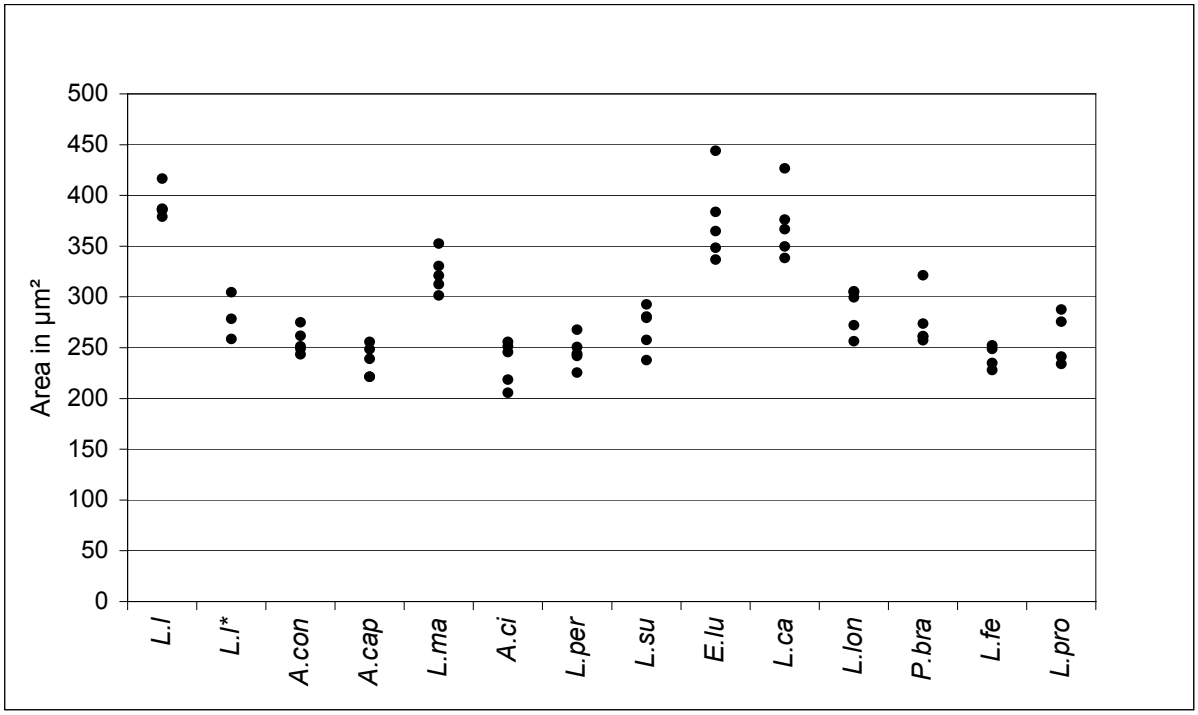


Fig. 87: Cuticle scale area of the 13 otter species (mean value of each individual)

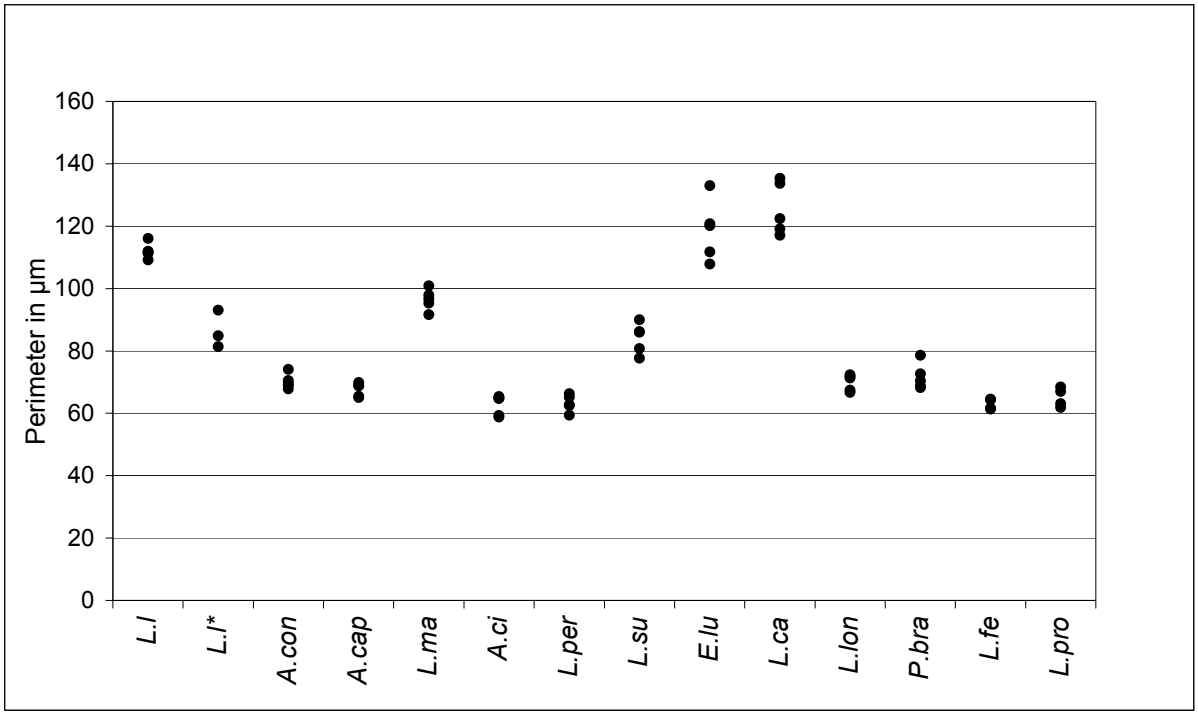


Fig. 88: Cuticle scale perimeter of the 13 otter species (mean value for each individual)

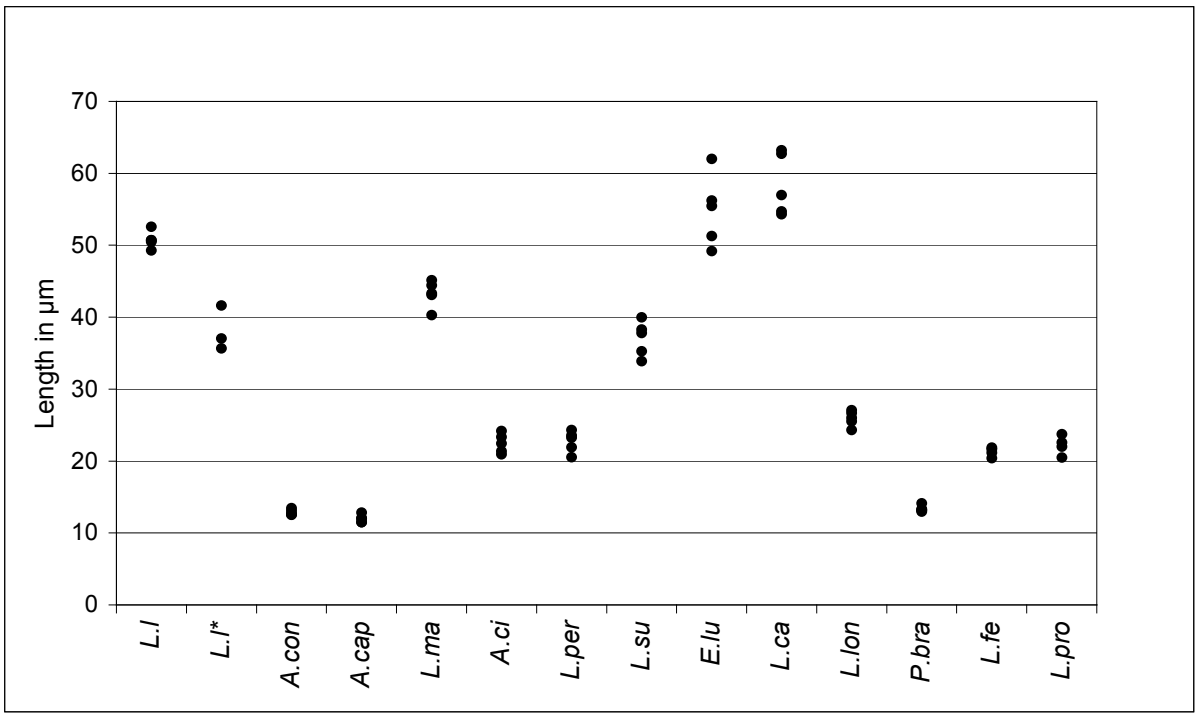


Fig. 89: Cuticle scale length of the 13 otter species (mean value for each individual)

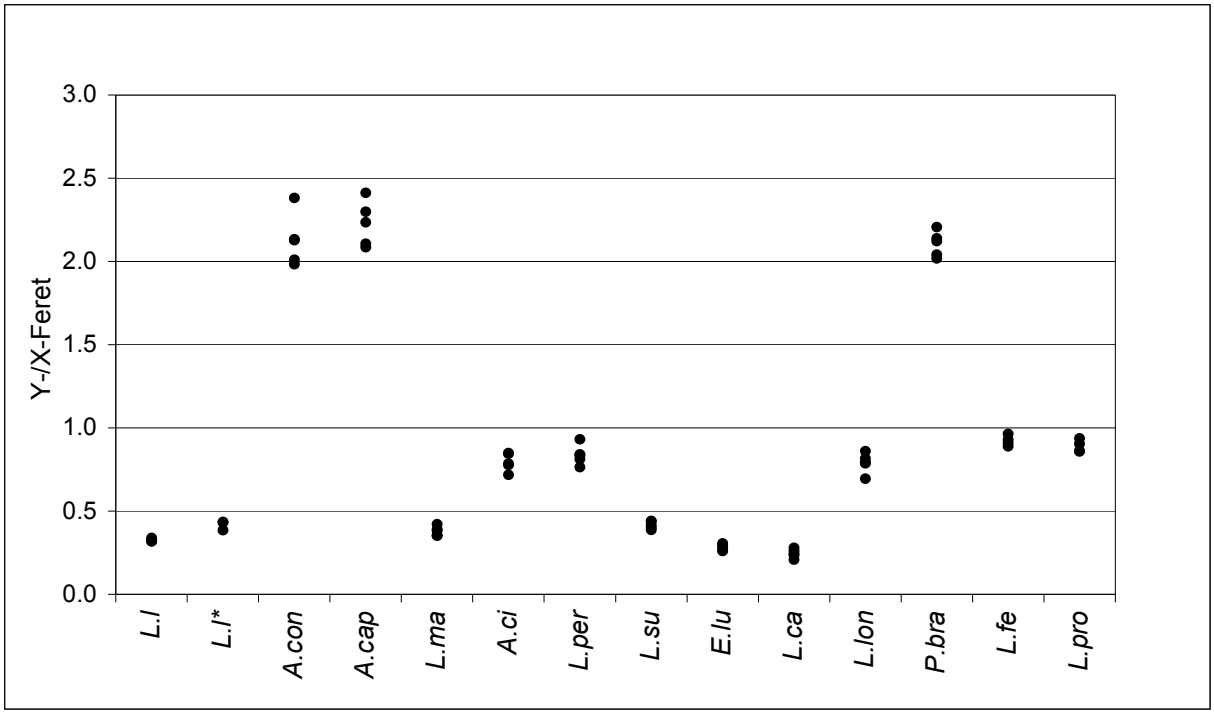


Fig. 90: Y-/X-Feret of the 13 otter species (mean value for each individual)

2.3.3.4 Secondary hair length

The graph in Fig. 91 indicates the secondary hair (or underhair) length of the 13 otter species. The three otters coming from South Asia constituted a separate category (*L.l**). The Sea otter *E. lutris*, which had the longest SHs, differed significantly from every other species (ANOVA, $F=60.70$, $p<0.001$, $n=63$, $R^2=0.94$). All the species with a mean SH length higher than 12 mm (*E. lutris, L. canadensis, L. lutra, L. provocax* and *L. felina*) differed significantly from the species with shorter SHs, including the *L. lutra** (South Asia) group. *P. brasiliensis,* which had the shortest SHs, differed significantly from every other species, except from *L. perspicillata* (see Tab. 19).

Intraspecific variability: The mean individual SH length varied only slightly in most of the species. The difference between the maximal and the minimal value exceeded 2 mm only in *E. lutris* (5 mm), *L. canadensis* (3 mm) and *L. provocax* (5 mm). In the first two species, the variability could not be related to the geographic origin of the animals, but in *L. provocax*, the two individuals coming from inland areas had a mean SH length of 10 and 11 mm, whereas the two coastal otters showed a mean SH length of 14 and 15 mm.

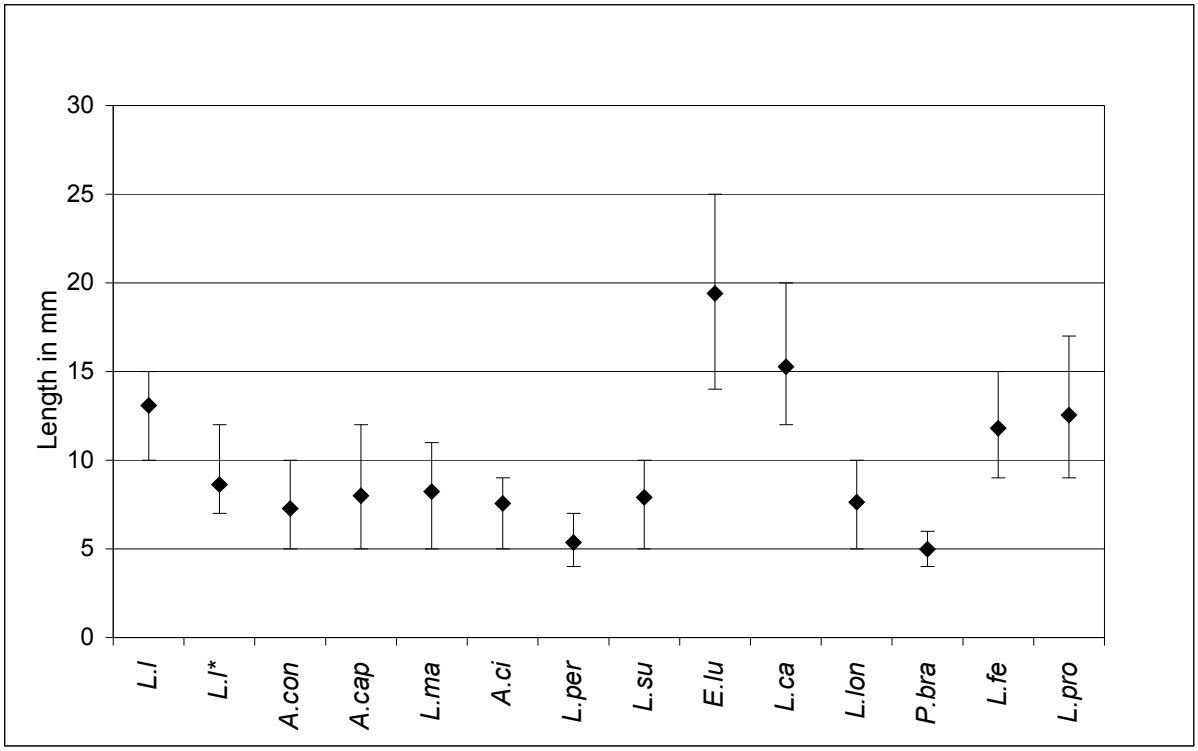


Fig. 91: Secondary hair length of the 13 otter species (mean, minimal and maximal values)

Tab. 19: Tukey's HSD test, SH length, grouping of not significantly different species

Species	Mean (mm) ± SD	Groups					
E.lu	19 ± 2.7	A					
L.ca	15 ± 1.3		B				
L.l	13 ± 0.5		B	C			
L.pro	13 ± 2.5			C			
L.fe	12 ± 0.9			C			
*L.l**	9 ± 0.3				D		
L.ma	8 ± 0.7				D		
A.cap	8 ± 0.4				D		
L.su	8 ± 0.4				D		
L.lon	8 ± 0.6				D	E	
A.ci	8 ± 0.5				D	E	
A.con.	7 ± 0.8				D	E	
L.per	5 ± 0.5					E	F
P.bra	5 ± 0.2						F

Ratio of SH to PH length: The graph in Fig. 92 shows the ratio of the secondary to primary hair length of the 13 otter species. The Sea otter *E. lutris* not only had the longest PHs and the longest SHs, but also the longest SHs relatively to PH length. The SH/PH length ratio of *E. lutris* differed significantly from the SH/PH length ratio of most of the species, except for *L. canadensis*, *L. lutra, L. felina* and *L. provocax*, which all had a ratio higher than 0.58 (ANOVA, $F=7.61$, $p<0.001$, $n=63$, $R^2=0.67$). *L. canadensis* also differed significantly from

most of the species except for *E. lutris*, *L. lutra*, *L. felina*, *L. provocax* and also *A. cinereus,* the SH length of which equalled 55% of the PH length. *E. lutris* and *L. canadensis* were the only species, which differed from every species having a ratio lower than 0.51. Those species (from *L. sumatrana* to *L. perspicillata* when ordering the ratios from the highest to lowest value) did not differ significantly from each other. *L. perspicillata*, the SH length of which equalled only 44% of the PH length, differed significantly from all the species having a ratio higher than 0.58.

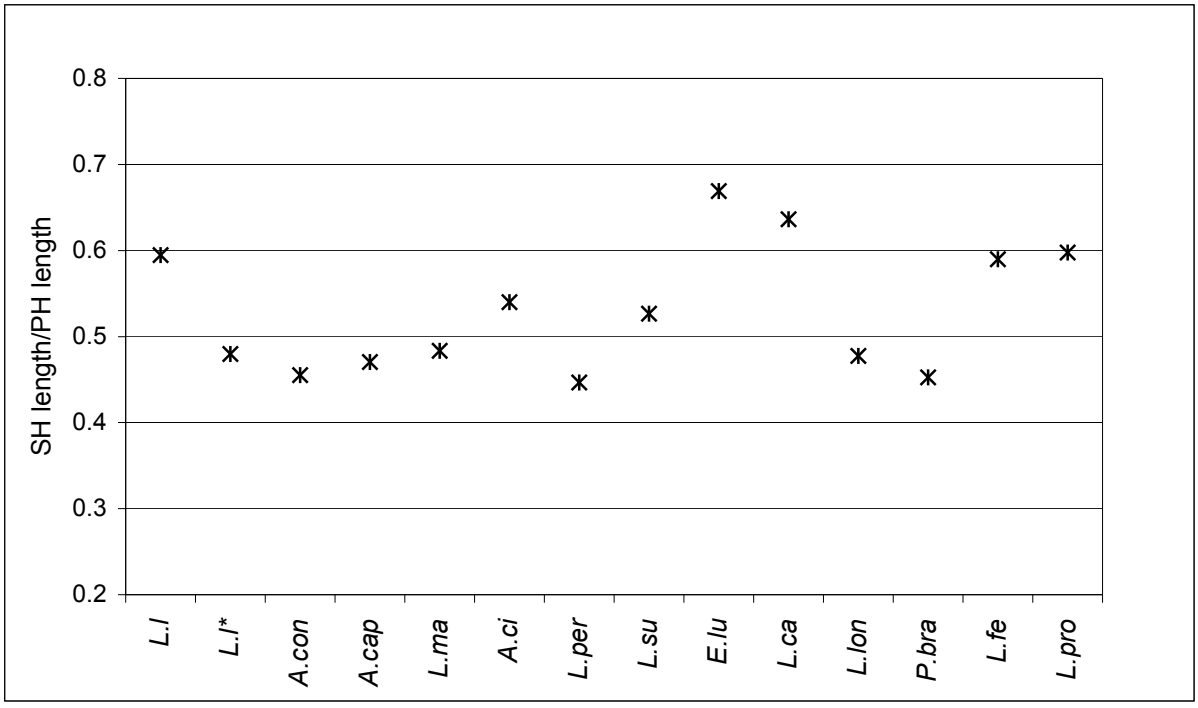


Fig. 92: Ratio of secondary to primary hair length of the 13 otter species

2.3.3.5 Secondary hair width

The secondary hair width ranged between 10 and 12 µm (see Fig. 93). *E. lutris* still was the species with the highest value, and *L. perspicillata* the one with the lowest value, but aside from that, the rankings of the values differed from those observed previously (SH length and SH/PH length ratio). For example, *E. lutris* differed significantly from *L. canadensis* and from *L. lutra* but not from *A. congicus, A. capensis, L. felina, L. provocax, L. sumatrana* and *A. cinereus* (ANOVA, $F=8.28$, $p<0.001$, $n=63$, $R^2=0.69$). *L. perspicillata* significantly differed from all those species having SHs thicker than 11 µm (mean value). When the three categories with the highest values (*E. lutris, A. congicus, A. capensis*) and the two with the lowest values (*L. lutra*, L. perspicillata*) were excluded, no significant differences were observed between the remaining species.

Intraspecific variability: The difference between minimal and maximal individual mean, within a species, never exceeded 2 µm.

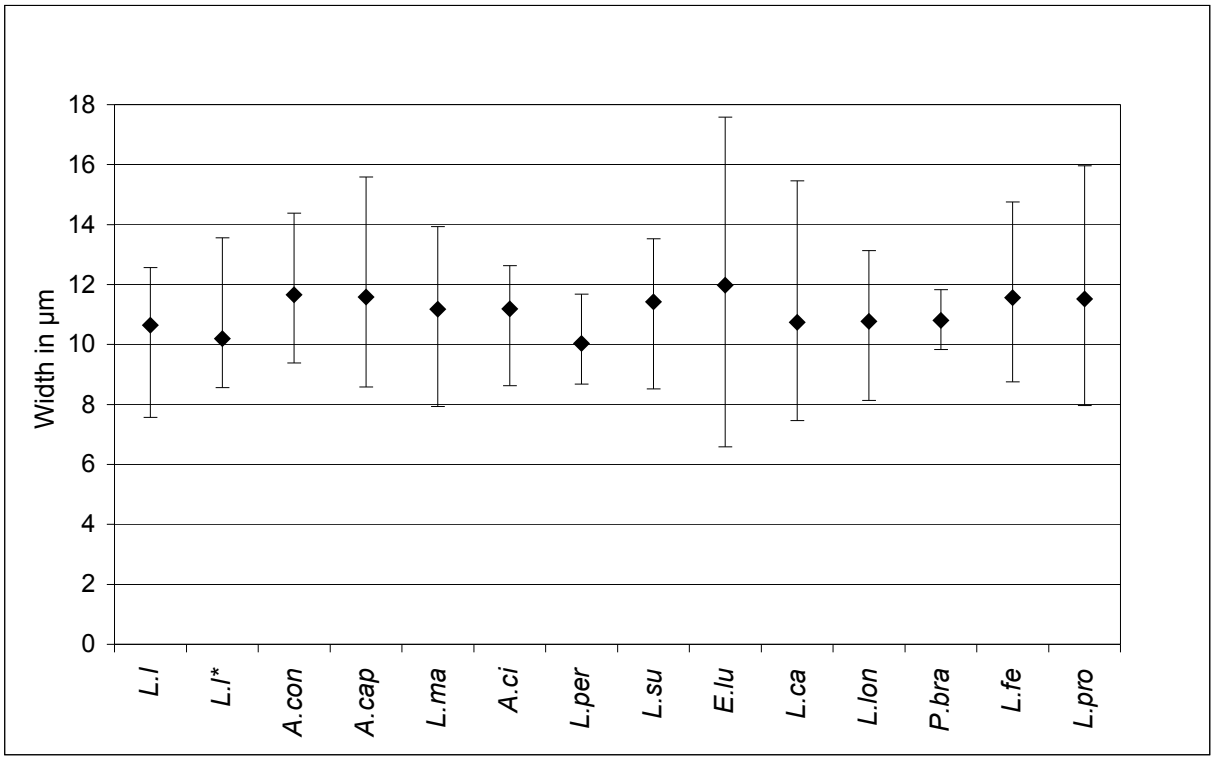


Fig. 93: Secondary hair width of the 13 otter species (mean, minimal and maximal values)

2.3.3.6 Subgroup differentiation in the Lutrinae by hair analysis

The results of the measurements presented previously, were subjected to a regression analysis in order to see if there is a correlation between hair parameters and specific interdependences (zoosystematical or adaptive) within the Lutrinae subfamily.

According to the regression analysis performed, relationships could be demonstrated for several hair parameters. First, a relationship could be demonstrated between PH length and PH width for the Lutrinae, when excluding the Sea otter *Enhydra lutris* (R^2=0.84, p<0.01, see Fig. 94).

A clear relationship between cuticle scale length and PH width could be demonstrated for a group made of the *Lutra* species, *L. perspicillata*, *L. canadensis* and *P. brasiliensis*, (R^2=0.94, p<0.01, see Fig. 95). All the most aquatic otters are found in this group, except *E. lutris* and the South American *Lontras*. A significant relationship could also be observed for a group made of all the Old World otters (R^2=0.72, p<0.01).

Between cuticle scale perimeter and PH width, relationships could be demonstrated for all the Old World species (R^2=0.90, p<0.01, see Fig. 96), and also for the group previously mentioned, *Lutra+L.per+L.ca+P.bra* (R^2=0.89, p<0.01).

A relationship between cuticle scale area and PH width could be observed for all the Old World otters (R^2=0.82, p<0.01), and for the group *Lutra+L.per+L.ca+P.bra* (R^2=0.72, p<0.05). Less relationship could be observed between the cuticle scale parameters and PH length, except for scale length and PH length (*Lutra+L.per+L.ca+P.bra*: R^2=0.93, p<0.01, see Fig. 97). A relation could also be demonstrated for the *Lutra+L.per+L.ca+P.bra* group concerning scale perimeter and PH length (R^2=0.93, p<0.01), and between cuticle scale area and PH length (R^2=0.79, p<0.05).

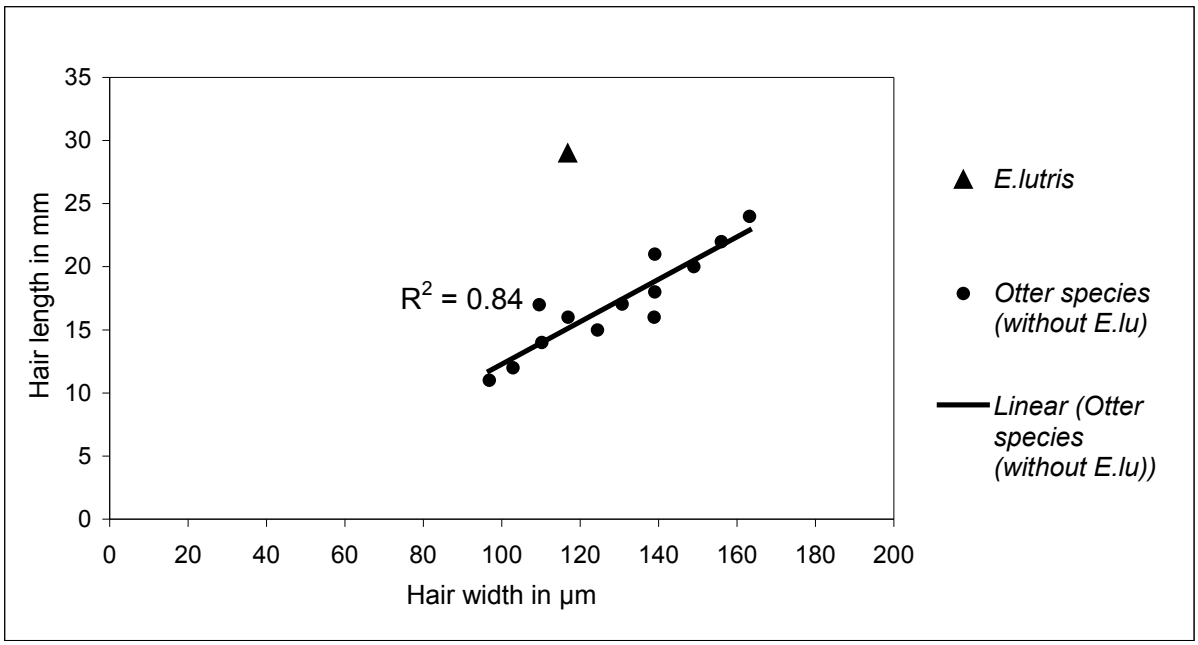


Fig. 94: Relationship between PH length and PH width

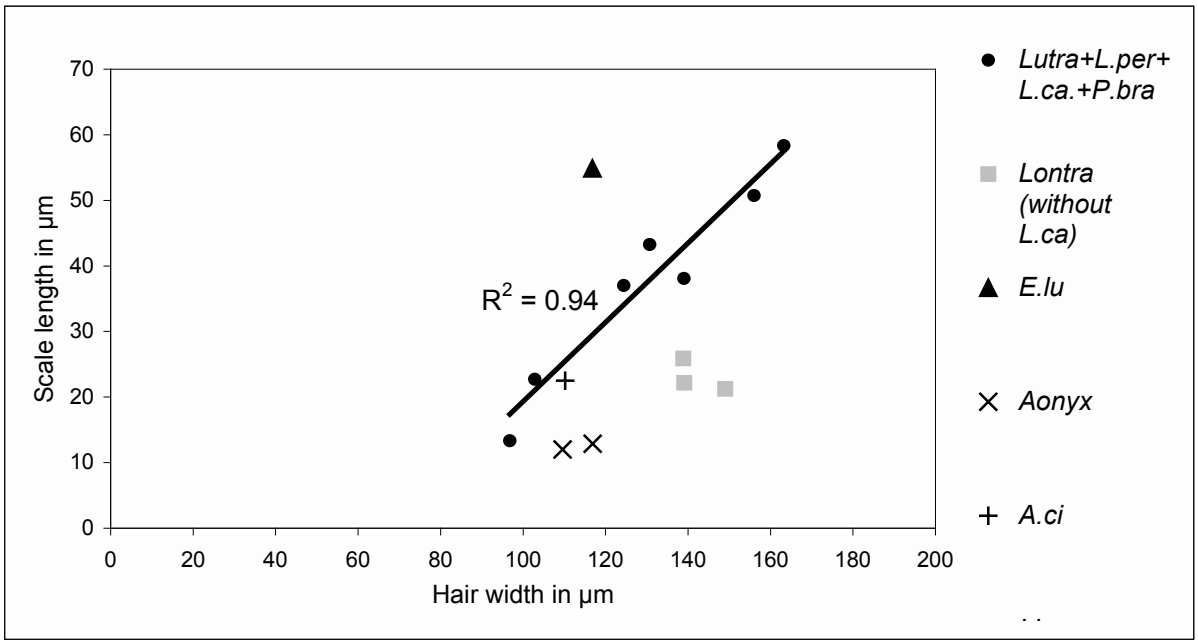


Fig. 95: Relationship between cuticle scale length and PH width

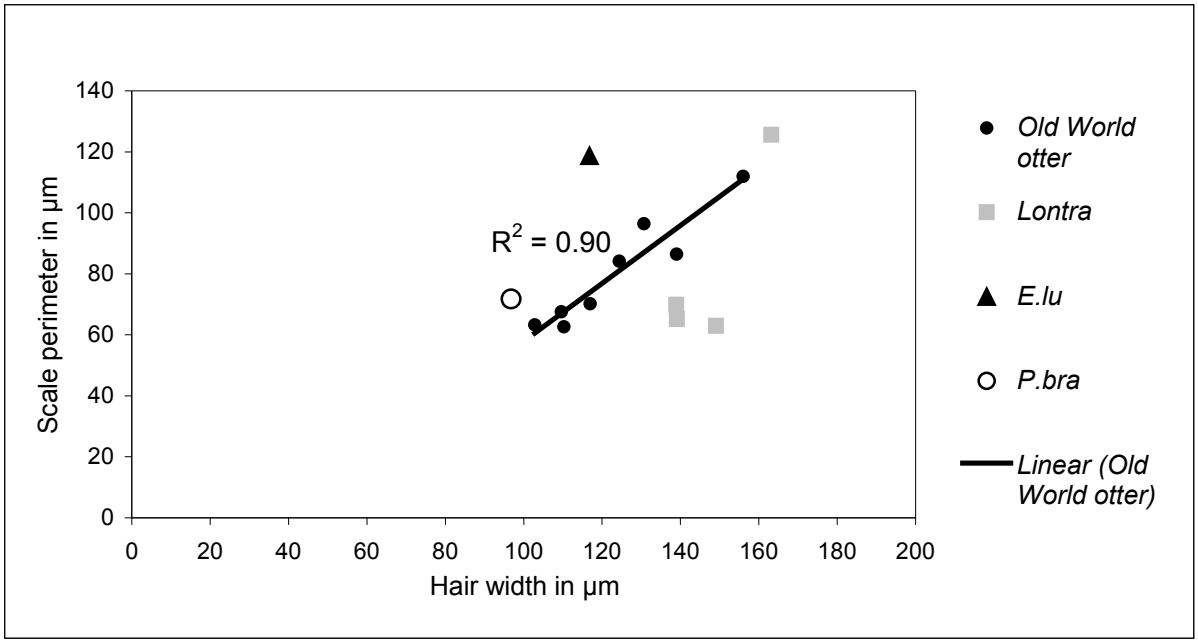


Fig. 96: Relation between cuticle scale perimeter and PH length

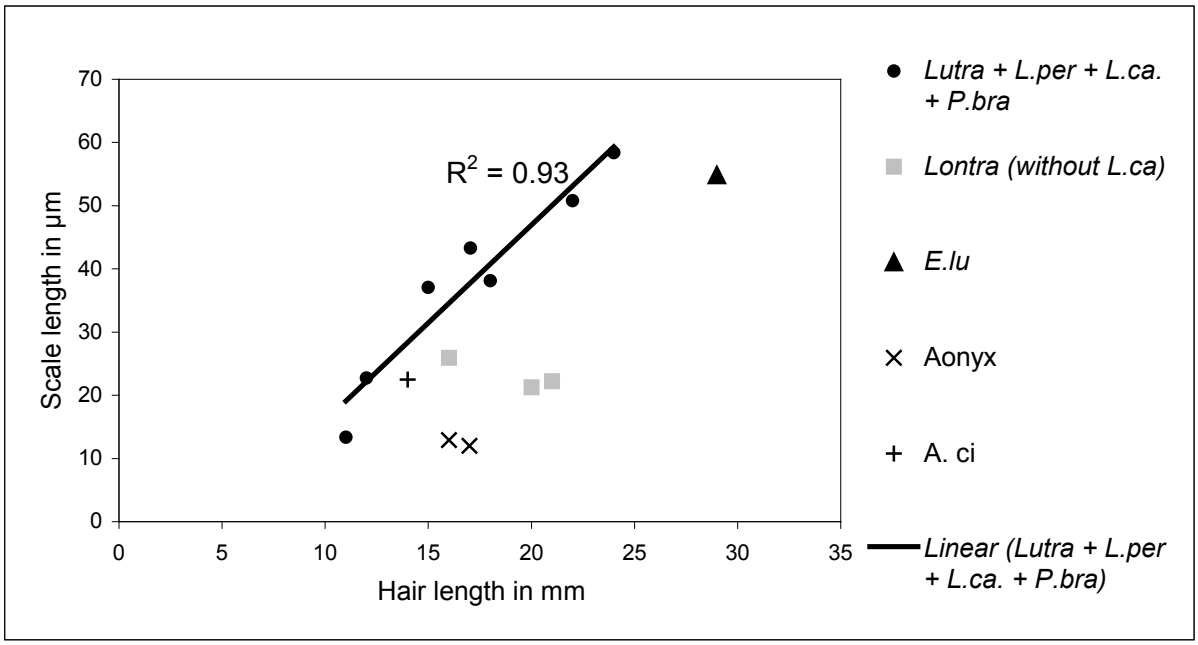


Fig. 97: Relation between cuticle scale length and PH length

2.4 Discussion

2.4.1 Hair structure in the Lutrinae

We have already recognized (chap. 1) that the characteristics of the cross-section, medulla structure and hair cuticle pattern, including metrical data, of the Eurasian otter (*Lutra lutra*) corresponded to former descriptions (HAUSMAN 1920ab, 1930, APPLEYARD 1960, FALIU et al. 1979, 1980, KELLER 1981, TEERINK 1991, MEYER et al. 2002, TÖTH 2002). The descriptions of the PH structure of the North American River otter (*Lontra canadensis*) and Sea otter (*Enhydra lutris*) given by HAUSMAN (1920a, 1930), BROWN (1942), BARABASCH-NIKIFOROW (1947), TARASOFF (1974), MOORE et al. (1974), WILLIAMS et al. (1992) and ZAGREBELNY (1998) also fit in with our observations, with the exception of some discordances in the data for hair length and width. For example, BARABASCH-NIKIFOROW (1947), BROWN (1942) and WILLIAMS (1992) found a mean PH width similar to ours for *E. lutris*, but BROWN measured a much higher value for the hair width of *L. canadensis* (between 235 and 330 µm, mean: 280). TARASOFF (1974) published a somewhat higher value for *L. canadensis* (198 µm) and a somewhat lower value for *E. lutris* (97 µm). MOORE et al. (1974) found a PH width for *L. canadensis* quite similar to that found in this study (max: 185 µm). The values for the hair length are more or less similar to ours, but tend to be a little bit underestimated, particularly in *E. lutris.*

The mean PH length in the Lutrinae ranged between 10 and 30 mm. The Sea otter (*Enhydra lutris*) had significantly longer PHs than any other otter species (up to 40 mm). It was followed by the North American River otter (*Lontra canadensis*), the Eurasian otter (*Lutra lutra*), the Southern River otter (*Lontra provocax*) and the Marine otter (*Lontra felina*). The Giant otter (*Pteronura brasiliensis*) had the shortest PHs, followed by the Smooth otter (*Lutrogale perspicillata*).

The mean PH width ranged between 97 µm and 163 µm. *Lontra canadensis* had the thickest PHs, followed by *Lutra lutra* and *Lontra felina*. *Pteronura brasiliensis* had the thinnest PHs, again followed by the Smooth otter (*Lutrogale perspicillata*). The Sea otter (*Enhydra lutris*) had relatively thin PHs compared to their length.

The PHs of all the otter species were more or less fusiform. The morphology differed the most in the hairs of the Sea otter (*Enhydra lutris*) and Giant otter (*Pteronura brasiliensis*), which had less fusiform hairs. In the Sea otter, the hair shaft was long and thin with a rather inconspicuous shield in the apical third. In the Giant otter, the hairs were short, rather parallel and became only slightly and very gradually thicker toward the apical part.

The shape of the cross section was oblong-oval in all the species, except for, again, the Sea and Giant otter. In the Giant otter, the cross section was less elongated and rather oval. In the Sea otter, the cross-section was rather biconvex; the edges were less rounded and the

elongated cross section looked somewhat like an almond. However, the differences between the shapes of the cross-section were subtle.

The medulla varied in thickness but not in structure, which was wide lattice in every species. A short ladder-like structure could be observed where the medulla becomes thinner, for example near the base or before interruptions. The Sea otter had an exceptionally thin medulla compared to the other species and, thus, a mean medullary index of 0.2, whereas the other species had a medullary index between 0.5 and 0.6. If the Sea otter is excluded, the medulla width ranged between 50 and 100 µm. The two species with the thickest PHs (*L. canadensis* and *L. lutra*) also had the thickest medulla, whereas the species with the thinnest PHs (*P. brasiliensis*) had the thinnest medulla. However, the medullary width was not proportional to the hair width, as shown by the variation of the medullary index. MAYER (1952) mentioned that *L. canadensis* and *E. lutris* both have a narrow medulla. Actually, the medulla of this two species was very different, the medulla of *L. canadensis* being more than three times as thick as the medulla of *E. lutris.* The medulla of the Sea otter was not only very thin, but also strongly fragmented, and almost absent from the shield of some hairs. BROWN (1942) also noted the fragmentary medulla and found the same medullary index as in this study. He also calculated the same medullary index as we did for *L. canadensis.*

The medulla of the two clawless *Aonyx* became very thin at the pars intermedia and was sometimes interrupted. This could also happen in the PHs of the Small-clawed *Amblonyx.* A stricture of the medulla could be observed in the Smooth otter (*Lutrogale perspicillata*) too, but interruptions were rare. In the PHs of the Hairy–nosed otter (*Lutra sumatrana*), the presence of an interrupted medulla at the pars intermedia depended on the geographic area from where the individuals come from. A curious longitudinally split medulla was observed in some hairs of the Marine otter (*Lontra felina*).

The hair cuticle pattern of the pars basalis and pars apicalis was similar for all the otter species; irregular wave with rippled scale margins at the apical, and irregular wave with smooth scale margins at the most basal part. The pattern of the tip was constant over the whole shield, with closer margins at the middle. A narrow diamond petal pattern appeared at the pars intermedia of all the *Lutra* species, *Lontra canadensis* and *Enhydra lutris*. The two *Aonyx* and *Pteronura brasiliensis* showed a mosaic pattern, whereas *A. cinereus*, *L. perspicillata* and the three other *Lontra* (*L. longicaudis*, *L. provocax* and *L. felina*) exhibited a diamond petal pattern at the pars intermedia with short scales, which were as wide as long. In the PHs where a narrow diamond petal pattern was seen at the pars intermedia, this pattern remained constant all along the lower shaft (parallel-edged part of the hair shaft), whereas in the other PHs, the pattern turned into a diamond petal, broad or narrow diamond petal toward the base. So, petal like scales were always observed in the PHs of the Lutrinae, but the distance over which this pattern appeared changed from one species to the other,

and the size and shape of the scales (square like or more or less elongated petals) also changed between species. In the *Lontra* genus, the scales were long and narrow from the pars intermedia to the base in *L. canadensis*, whereas they were first shorter and then became longer and more narrow beneath the pars intermedia in the other species. Curiously, in the PHs of the Neotropical otters (*L. longicaudis*) coming from French Guiana, the scales became longer almost directly beneath the shield, and it was ambiguous to say which pattern was the “pars intermedia” pattern, whereas in the individuals from Central America, a diamond petal pattern with short and wide scales was seen over a considerable distance beneath the shield. The Central American otters belong to the subspecies *L. longicaudis annectens*, whereas those from the Orinoco and Amazon Basin belong to the subspecies *L. longicaudis enudris*. Both subspecies differ by some morphological characters like the shape of the rhinarium, and used to be classified as different species (HARRIS 1968). Actually, it is surprising that between the PHs from the Central American subspecies and the Guianan subspecies, those from the Guiana population resemble the most the PHs from *L. canadensis*, despite of the Central American otters being nearer to the distribution range of the North American River otter. More samples should be examined to confirm this tendency. The variation of the rhinarium for example follows the opposite direction. Indeed, the rhinarium of the Central American otters resembles that one of *L. canadensis*, whereas the one from the Amazon region is completely different. Influence of the environment could provide an explanation and will be discussed in 2.4.5. VÁZQUEZ et al. (2000) described lanceolate rhomboid scales in the proximal region of the hairs of *Lontra longicaudis* in Argentina, which would correspond to a broad/narrow diamond pattern, and thus fits in with our observations. The medulla of the PHs analysed by these authors also had a wide lattice structure. CHEHÉBAR & MARTIN (1989) analysed the PHs of Southern River otter (*L. provocax*) from Patagonia, and observed a narrow diamond petal pattern at the lower shaft, a lattice medulla and a hair length of about 20 mm, which corresponds to our findings. The only other “exotic” otter for which data on hair structure are available is the Cape clawless otter (*Aonyx capensis*). PERRIN & CAMPBELL (1980) described a “fine waved flattened mosaic pattern” followed proximally by a “pectinate pattern” (diamond petal in our nomenclature), which changes to a “petal pattern” (broad or narrow diamond in our nomenclature), a description similar to ours.

The mean secondary hair (SH) length ranged between 5 mm and 20 mm. The longest SHs were found in the same species as for the PHs, which were *E. lutris*, *L. canadensis*, *L. lutra*, *L. provocax* and *L. felina*. The shortest SHs were also found in the species, which had the shortest PHs, *L. perspicillata* and *P. brasiliensis*. SH width did not follow this rule; the thickest SHs were still found in *E. lutris* and the thinnest in *L. perspicillata,* whereas *L. lutra*, *L. canadensis* and *P. brasiliensis* showed intermediary values. However, the SHs of all the species studied had a mean value between 10 and 12 µm, so despite of significant statistic differ-

ences, we can consider that SH width is rather similar in all otter species. FISH et al. (2002) found SH length values similar to ours for *E. lutris* (18.1 mm) and for *L. canadensis* (13.8 mm) and a SH width only slightly lower than ours (9 µm in *L. canadensis* and 8 µm in *E. lutris*). BROWN (1942) published for *L. canadensis* a SH length between 10 and 15 mm and a width between 12 and 24 µm (mean 16 µm), and for *E. lutris* also a length between 10 and 15 mm and a width between 20 and 56 µm (mean 31 µm).

2.4.2 Identification value of the otter primary hairs

The PH structure of mammals is usually studied for the purpose of species identification in the wild, for example when hairs are collected from scats (DAY 1966, OLI 1993, MUKHERJEE et al. 1994ab) or using hair traps (BAKER 1980, DICKMAN & DONCASTER 1987, MOWAT & STROBECK 2000). The identification of otter species using PH structure analysis was difficult because of great similarities in medulla and cuticle structure, and it was not possible to identify every species confidently. The species which could be identified with the greatest confidence was the Sea otter, because of the long and narrow scales, the length and morphology, and particularly because of the thin interrupted and sometimes almost absent medulla. DOVE & PEURACH (2002) could not distinguish between Sea otter and River otter when analysing hairs associated with a human mummy. The Sea otter shares a similar cuticle structure with *L. canadensis* and also with *L. lutra* (from Europe) but its medulla is unique. BROWN (1942) also considers that the medulla separates the PHs of *L. canadensis* and *E. lutris,* and moreover the long and very flexible hairs are typical for *E. lutris.* The second species, the PHs of which really differed from those of the other Lutrinae was the Giant otter (*P. brasiliensis*). Its PHs were really different from those of *L. longicaudis*, which occurs in the same area, and anyway, they can be easily identified, even if their geographic origin is unknown.

The PHs of *L. canadensis* and *L. lutra* were very similar, and it was not possible to differentiate them using medulla and cuticle structure, despite of cuticle scales which tended to be longer in *L. canadensis*. The very shiny look of the PHs of *L. canadensis* is probably the only good “clue” to identify them. However, both species are not sympatric, so criteria to distinguish between them are only of limited use. *L. lutra lutra* differed from the South Asian subspecies and from the other *Lutra* species by the longer and thicker PHs with longer and narrower scales.

When looking at sympatric species - we did not analyse PHs from *L. lutra* coming from South-East Asia, but we suppose that those PHs are rather similar to the PHs of animals from India and Sri Lanka than from Europe - we consider that in South-East Asia it is difficult to distinguish between the PHs from *L. lutra* and from *L. sumatrana*. The tracks and spraints of these two species are also similar (KRUKK 2006), and, unfortunately, hair identification

will not really help to improve the knowledge of the geographical range of the few known and very elusive Hairy-nosed otter.

The PHs from the two *Lutra* species could be distinguished from those of the Small-clawed otter (*Amblonyx cinereus*) and the Smooth otter (*Lutrogale perspicillata*). The PHs of the last two species differed slightly, the hair cuticle of *Amblonyx* tending to exhibit a diamond petal pattern over the whole lower shaft, whereas in *Lutrogale* the scales showed a greater tendency to get longer toward the base, but this criterion is quite hazardous. The PHs of *Lutrogale* tended to be shorter, thinner and much more flexible than those from *Amblonyx*. This may be a rather reliable criterion when several hairs are analysed, but when only one or two hairs are examined, then the risk of error is important.

In Africa, the PHs of *L. maculicollis* totally differed from those of the *Aonyx* species, whereas in the latter the PHs were identical. The presence of a white tip of 4 or 5 mm length assigns the hair to *A. congicus,* but a shorter or missing white tip is not a criterion for *A. capensi*s. Anyway, the two species do not live in the same areas.

In South America, the Southern River otter (*Lontra provocax*) and Marine otter (*Lontra felina*) shared many PH features and could easily be confused. *L. provocax* had thinner, particularly at the tip, and more flexible PHs than *L. felina*, but again, this criterion can allow a reliable identification only when many hairs are examined. The presence of a longitudinally split medulla can assign the PH to *L. felina*, but this particularity has been observed in only a few hairs.

To summarize, the PHs of otters are as similar to each other as the animals they belong to, and even to identify species which have some more specific hairs, we recommend to examine every macroscopic and microscopic characteristic, also to exclude confusion with non-lutrine species. Many previous authors consider that only a combination of criteria can allow a confident identification to species level (DAY 1966, MUKHERJEE et al. 1994b), and we agree with MOORE (1988) who emphasizes that “a considerable amount of experience is required before the identification of animal hairs can be carried out with confidence”. Even very experienced practitioners can make a considerable number of mistakes (LOBERT et al. 2001). We also want to add that an atlas like the one we have presented here is aimed to support the work of the examiner, and will never supplant direct comparison. Particularly to differentiate species that have a resembling hair structure, examination of reference PHs should be done.

2.4.3 Hair structure in Mustelidae

Mustelids have fusiform PHs with a more or less flattened shield. Otter PHs have a strong flattened shield, compared for example to the PHs from the Stoat (*Mustela erminea*), whose cross-section has almost a circular shape (TEERINK 1991). When looking at European mus-

telids, the PHs the cross-section of which is the closest to that of *Lutra lutra* are from the Mink (*Mustela vison*) and to a lesser extent from the European polecat (*Mustela putorius*). KELLER (1981) and TÖTH (2002) also noted that the cross-section is more elongated in *L. lutra* than in other European mustelids.

Compared to other mustelids, otters have intermediary PH length and width. However, wolverines and badgers, which like the otters belong to the largest mustelids, have PH length up to 8 cm on the dorsum, with diameters close to that of *L. lutra* and *L. canadensis*, and even the small skunks have such long hairs (MOORE et al. 1974). Stoats and weasels have a PH length similar to that of the much larger Giant otter. Martens have PH length up to 4 cm. The mink has PHs up to 3 cm and a hair width of about 140-150 µm, which is similar to *L. lutra* and *L. canadensis*.

In many Mustelinae, the secondary hairs have a length close to that of otters, for example about 2 cm in *Martes* and 1.5 cm in *Mustela*, which correspond to the mean SH length in *E. lutris* and *L. canadensis* (BROWN 1942). The SHs are thicker than in otters (mean between 20 and 30 µm). The American badger (*Taxidea taxus*) has SHs up to 3 cm, and the Wolverine (*Gulo gulo*) up to 4 cm.

Mustelids exhibit a cloisonné or nodule like lattice medulla, except otters, which show a wide lattice pattern. TÖTH (2002) also mentioned that the terrestrial mustelids he has studied show a different medulla structure than *L. lutra.* He found a mean medullary index between 0.70 and 0.77 (max value 0.88) in the genera *Martes* and *Mustela*, whereas the European badger (*Meles meles*) has a mean medullary index of about 0.5. To compare with a terrestrial non-mustelid small carnivore, the Red fox (*Vulpes vulpes*) shows a mean medullary index of 0.74. BROWN (1942) found close values, between 0.6 and 0.8 (mean 0.67), in North American martens, and between 0.56 and 0.87 (mean 0.8) in the genus *Mustela*, including the mink. He noted values between 0.3 and 0.8 (means between 0.5 and 0.6) in skunks (Mephitinae) and between 0.5 and 0.9 (mean 0.75) in the American badger (*Taxidea taxus*). The European mink (*Mustela lutreola*) has a medullary index of 0.66 (SOKOLOV 1962).

Petal like cuticle scales are observed not only in Lutrinea, but also in Mustelinae, whereas badgers and skunks exhibit a totally different structure, mostly waved with rippled margins all along the hair shaft (MOORE et al. 1974, KELLER 1981, TEERINK 1991, MEYER et al. 2002, GONZÁLEZ-ESTEBAN et al. 2006). However, the distance over which a broad or narrow diamond petal pattern appears is shorter than in some Lutrinae. Indeed, *Lutra lutra* and *Lontra canadensis* show a narrow diamond petal pattern at the mid-shaft, whereas in Mustelinae from the same area, the patterns at the mid-shaft are either diamond petal, mosaic or even waved (MOORE et al. 1974, MEYER et al. 2002). Those patterns then turn to a broad or narrow diamond petal pattern toward the base.

In some species, a narrow diamond petal pattern already appears directly beneath the shield, but with shorter and more irregularly shaped scales. Elongated, very symmetrical scales forming a very regular arrangement are typical for *Lutra*, *Lontra* and *Enhydra lutris*. HAUSMAN (1920b) measured petal like scales of different mammal species, also from non-mustelid, and found that the scales reached their highest length values in otter species. VÁZQUEZ et al. (2000) describes lanceolate rhomboide scales in the proximal region of the PHs from the Neotropical otter (*Lontra longicaudis*), and a mosaic pattern in the PHs of other mustelids from the same area, the Tayra (*Eira barbara*) and the Lesser grison (*Galictis cuja*), which are both Mustelinae. The diamond, broad or narrow diamond pattern characteristic for Mustelinae, probably also appears on the hair shaft of these species, but is apparently not the dominant pattern. However, we could not find further data on the hair structure of these species. CHEHÉBAR & MARTIN (1989) observed a narrow diamond petal pattern in the PHs of the Southern River otter (*Lontra provocax*) and found a broad diamond or mosaic pattern at the corresponding part of the hair shaft in other mustelids from the same area.

The mustelid the PHs of which resemble those of *Lutra* and *Lontra* the most is the mink. The narrow diamond petal pattern appears later in this species than in *L. lutra* and *L. canadensis* (when looking from the distal to the proximal part), but the scales are very elongated, symmetrical and regular. Metrical data of the scales are not available but we could measure from pictures of the Atlas of TEERINK (1991), that they have a length between 50 and 60 µm, which is within the values of the otter species showing the longest scales. GONZÁLEZ-ESTEBAN et al. (2006) compared the cuticle structure of the European and American mink and the polecat, and noted that the two mink species have long and narrow scales with almost parallel edges, whereas the polecat has triangle shaped scales. KELLER (1981) observed that the scales of *Lutra lutra* have rounded extremities, whereas the extremities of the scales of the *Martes* und *Mustela* species (except the minks) are triangle shaped and pointed. This is actually a simple and precise description of the difference between the cuticle scales of the Mustelinae and the Lutrinae.

2.4.4 Hair structure in semi-aquatic mammals

The European beaver (*Castor fiber*), Coypu (*Myocastor coypus*) and Muskrat (*Ondatra zibethicus*), three semi-aquatic rodents, have fusiform hairs with an elongated cross-section, particularly the two last species. Seals also have a flattened cross-section (SCHEFFER 1964b). TOLDT (1933) remarked that an oval cross-section is a common characteristic of semi-aquatic mammals. An oval or oblong cross-section can also be observed in terrestrial mammals, particularly in mustelids, but the cross-section is more elongated in aquatic forms (SOKOLOV 1962, HEPTNER & NAUMOV 1974). This feature allows the hairs to lie flat against the body.

Another particular cross-section is observed in water shrews. The shrews of the monophyletic Soricinae subfamily all have a very particular and unique H-shaped cross-section (VOGEL & KÖPCHEN 1978). In the aquatic forms of the subfamily (genera *Sorex*, *Neomys*, *Chimarrogale* and *Nectogale*) this H-shaped profile is more complex with the margins of the hairs being indented. The number and depth of the indentations increase from one species to the other, parallel to the increasing aquatic nature of the species, being maximal in the most specialized water shrew, *Nectogale elegans* (HUTTERER & HÜRTER 1980). This specific structure is thought to better trap the air in the hair coat. Shrews have elongated petal like scales in a coronal arrangement (MEYER et al. 2002), without a particular development in water shrews. The medulla is in a ladder-like arrangement as in other Insectivora and in Gliridae, and there is no evidence that it takes a more or less important part of the hair width in aquatic forms.

KENNEDY & CARBYN (1981) remarked that the medulla is absent in the shield region of the beaver, which is not the case in the muskrat. Actually, the beaver has a thinned and interrupted medulla all along the hair shaft (TEERINK 1991, MEYER et al. 2002). In the medulla of the muskrat, the gas spaces are in a lattice or nodule-like arrangement. In the coypu, the gas spaces are in a nodule-like interwoven or ladder-like arrangement. In the muskrat, the medullary index is of about 0.55, the PHs are between 2 and 4 cm long and between 50 and 120 µm thick (in thickest part). In the coypu, the medullary index is of about 0.7, the PHs are between 3 and 6 cm long and between 50 and 200 µm thick. The beaver has PHs between 3 and 9 cm long and between 50 and 200 µm thick. SOKOLOV (1962) remarked that a slighter development of the medulla is characteristic in semi-aquatics, and considers that it makes the hairs more solid and flexible in swimming. Indeed, we never observed a medullary index higher than 0.7 in semi-aquatics, whereas terrestrial mammals can have a medullary index close to 1.

Beaver, muskrat and coypu, all have a more or less regular wave cuticle pattern all along the hair shaft (HARDY & PLITT 1940, MOORE et al. 1974, TEERINK 1991, MEYER et al. 2002). A broad diamond petal pattern appears on the lower shaft of the platypus (*Ornitorhynchus anatinus*) (HAUSMAN 1920b). The less aquatic, but also associated to wetlands, Crab-eating mongoose (*Herpestes urva*) and Indian Marsh mongoose (*Herpestes palustris*), have PHs which exhibit an irregular wave pattern with rippled scale margins, typical for mongooses (DE & CHAKRABORTY 1995, DE et al. 1998). The Raccoon (*Procyon lotor*) also exhibits an irregular wave pattern with rippled or smooth margins.

Little information is available on the hair structure in seals. Primary hairs from several European Phocidae exhibit wave and mosaic cuticle patterns (MEYER et al. 2002). SCHEFFER (1964b) observed that the two seal species with the shortest hairs live in warm waters, but he did not consider that temperature may influence the hair length, because two species with

nearly the shortest hairs, the Ribbon seal (*Phoca fasciata*) and Leopard seal (*Hydrurga leptonyx*) are both adapted to life on ice. However, the fur has not the same importance in seals than in semi-aquatic mammals, because they rely on their blubber for thermo-insulation in water.

In general, the PHs tend to be shorter and thinner in semi-aquatic mammals than in terrestrial mammals (FISH et al. 2002). Thinner hairs are often associated with higher hair density and may be advantageous for thermo-insulation, whereas thicker hairs increase the airflow, and thus heat loss through the hair coat (JOHNSON 1970). Terrestrial mammals that grow a denser coat in winter, get thinner and also longer hairs at this time of the year (MEYER et al. 1982). Longer hairs provide a better insulation (SCHOLANDER et al. 1950), that is why many mammals grow longer hairs in winter and also why within a species, populations living in colder climate get longer hairs (TÄNZER 1932). For semi-aquatic mammals, this is only partly true, because too long hairs would induce conspicuous openings of the fur in water. Semi-aquatics have hairs with a length usually up to 4 cm, with the exception of the coypu and beaver, the PHs of which can be up to 6 cm and up to 9 cm long, respectively.

The underhairs of semi-aquatic mammals are finer and typified by a large number of kinks. TOLDT (1933) and SOKOLOV (1962) noted that the difference between the secondary and primary hairs is more important in semi-aquatic than in terrestrial species, whereas FISH et al. (2002) observed the contrary. Indeed, FISH et al. measured SH/PH length ratio of 0.59 in *M. vison*, 0.57 in *C. canadensis*, 0.51 in *O. zibethicus* and 0.63 in *O. anatinus*, whereas in the terrestrial *Didelphis virginiana* and *Rattus norvegicus*, the ratios are 0.4 and 0.25 respectively. BROWN (1942) found that the PHs are twice as long as the SHs in *Martes*, three times as long in *Gulo*, and three to four times as long in different fox species. This observations corroborate the theory from FISH et al. (2002). One should not forget, that in terrestrial mammals which grow a winter coat, there is an important difference in the ratio of PH to SH length between summer and winter because the SH length increases much more than the PH length. For example, in the Wild cat (*Felis silvestris*) the PHs are between 1 and 4 mm longer in winter, whereas the SH length increases by 9 mm on the back (MEYER et al. 1982). Thus the SH/PH length ratio in the Wild cat is of 0.6 in summer and 0.7 in winter. In the Red deer (*Cervus elaphus*), the outer coat of the males reaches a length of 50 mm in summer and 60 mm in winter, whereas the underhairs grow from a length of a few mm in summer to 20 mm in winter (RYDER 1977). In our study, the SH/PH length ratio ranges between 0.45 and 0.67. The species with the highest need for a good insulating fur, particularly the Sea otter, have the highest ratios. This also speaks for the fact, that a smaller difference between SH and PH length is advantageous for thermo-insulation in water. Actually both theories would make sense, because in semi-aquatic mammals, PHs are supposed to protect SHs from getting wet, so they should be much longer than the SHs. On the other hand,

the SHs trap air, and a thicker air layer provides a better insulation. So longer SHs are advantageous, but we saw that in semi-aquatics the PH length is limited, so the difference between the SH length and the PH length cannot be important. Apparently, there is no clear tendency which distinguishes the SH/PH length ratio in terrestrial mammals from the ratio in semi-aquatics, because Wild cats have a ratio similar to that of the otter species that show the highest values, and martens also have a ratio within the values of the Lutrinae. However, according to our information, SH/PH lower than 0.4 are observed only in some terrestrial mammals and never in semi-aquatic. Moreover, within a group of semi-aquatics like the Lutrinae, the species with the highest need of a good insulating fur, also have the highest SH/PH ratio.

2.4.5 Adaptive value of microscopic hair features

Different interpretations of the role of the medulla have been made. RYDER (1973) considers that the air trapped within the medulla of PHs must provide insulation. LING (1970) shares this opinion and quotes FLEROV (1952) as writing that the medulla of the Reindeer (*Rangifer tarandus)*, which occupies the greatest portion of the hair fibers, provides both an efficient insulating medium and considerable buoyancy, which probably facilitates the crossing of rivers and lakes during seasonal migrations.

As already noted before, semi-aquatic mammals do not have medullary indices over 0.7, which, according to SOKOLOV (1962) makes the hair more solid and flexible. Several authors observed that the medulla is a fragile structure, and that PHs in which the medulla occupies almost the whole hair width, like in deers, break easily (TOLDT 1933, CEREVITINOV 1958 in HEPTNER & NAUMOV 1974, SOKOLOV 1982, CHERNOVA 2002). Observations made by HAUSMAN (1920a) corroborate this idea because he observed that the Sea otter has the most durable fur and that the durability of the fur stands in inverse relationship to the expanse of the medulla. These species follow this rule:

Sea otter>Raccon>Lynx>Chinchilla>European mole>Rabbit or Hare. Two other species, which proved to have a durable fur are the beaver and the bear (black or brown), with a durability value of 90 and 94 (the Sea otter has a durability value of 100), and these two species have a medulla, that takes less than the half of hair width. HAUSMAN found that River otter fur also has a durability of 100. Other values for the durability are 70 in the mink, 45 in the muskrat, 25 in the nutria (or coypu), 40 in the Red fox and 25 in the stoat. We can see here that the hair coat of the coypu has not a good durability; remember that the coypu has a medullary index of 0.7, which is quite high for a semi-aquatic mammal. However, the fox as a similar medullary index but a higher durability, and the mink has a higher durability than the muskrat, but also a slightly higher medullary index.

Two aspects should be taken into consideration. First, the medullary index expresses only partly the development of the medulla. Indeed, the shape of the medulla tends to follow the shape of the hair shaft, so circular hairs tend to have a circular medulla and flattened hairs have a flattened medulla, which can be wide but very thin. The width of the medulla is measured parallel to the shield, but the depth of the medulla is not measured. So different hairs can have the same medullary index but a more or less developed medulla, depending on the shape of the medulla.

Secondly, the structure of the medulla, more precisely the way the cell residues are fused and how much gas is in the medulla, may be related to its specific properties. We could not find precise information on the relation between medulla structure and mechanical properties of the hair shaft, but one can imagine, that a structure where the gas spaces surround the cell residues and separate them from each other, which is the case in otter hairs (only a part of the cell residues are connected together, mostly at the margins), may confer a higher flexibility to the hair shaft than a structure where the cell residues surround the gas spaces and build a more or less continuous network. To sum up, a structure where the solid elements are within a gas may be more flexible and thus also less breakable, than a structure where the solid elements are all more or less connected together in a network that surrounds the gas spaces.

The most surprising feature of the values published by HAUSMAN (1920a) is that Sea and River otters have the same durability index, despite of the large difference between their medullary indices. However, the author did not indicate how he has tested the durability, and it is highly possible that otters, at least some species, have close durability indices, all superior to that of non-lutrine species. The durability of the fur may also be influenced by other factors than medulla width.

Less attention has been given to the properties of the cuticle structure. Two studies looked at the adaptive value of cuticle structure in bats. MEYER et al. (1995) observed a cuticle scale structure in some bats, which could increase the flight efficiency by influencing the airflow over the body. HOWELL & HODGKIN (1976) found a scale structure, which may help in collecting pollen in nectar-feeding bats. PERRIN & CAMPBELL (1980) observed a similarity between the hair structure of fossorial rodents and fossorial insectivores, which both show a petal scale pattern. WILLIAMS (1938) also suggested that the petal scale pattern is an adaptation to the fossorial mode of life because this form permits movement of the animal with a minimum of friction. He also put forward the hypothesis of a waterproofing role of this cuticle structure.

2.4.6 Adaptive and phylogenetic value of hair structure in the Lutrinae

Several adaptive features can be recognized in otter PHs and SHs. These hair types all have a flattened cross-section, which is characteristic for semi-aquatic mammals. The cross-section is rather similar in all the individuals examined, but is slightly more elongated in the Sea otter and slightly less elongated in the Giant otter. This could have an adaptive value looking at the biology of these two species. However, the difference is subtle and exact measurements of the height and width of the cross-section of hairs embedded in a mounting medium, like cellulose acetate (TEERINK 1991), should be done to clear that point.

The length of the PHs is maximal in the otter species that occur in cold waters; those species also belong to the most aquatic otters. All the otter species living in warmer climates have shorter PHs. PH length does not vary much within this group, except for *L. perspicillata* and *P. brasiliensis*, which have the shortest hairs, and no influence of an association with water on PH length can be recognized. The Sea otter has longer PHs than any other otter species. This animal is the most exposed to cooling in water and also in air, because unlike the other Lutrinae, it is permanently exposed to external temperatures and has no rest in holts or other sheltered areas. The length of the SHs follows the same tendency. Moreover, in the species living in cold climates, the ratio of SH/PH length is higher, particularly in *E. lutris* (0.67) and in *L. canadensis* (0.64). All the species living in warmer climates have a SH/PH length ratio between 0.44 and 0.51, except *A. cinereus*, which has a ratio of 0.55. As already said, longer SHs allow more air to be trapped in the hair coat, and thus a better insulation is provided. However, PH length is limited because too long PHs would decrease the insulative capacity of the fur in water. Thus in species, which need very effective insulation against cold, the difference between SH and PH length may decrease. The maximal ratio observed in the Sea otter is 0.67, and so we would expect a ratio higher than 0.7 to be disadvantageous, because a minimal difference between PH and SH length may be necessary to allow the PHs to protect the SHs from getting wet; we also expect a minimal ratio of 0.4 to be necessary. In terrestrial mammals, higher and lower values than those measured in otters are found. It is interesting to observe that in *L. provocax*, the two individuals living in coastal areas, and probably feeding in seawater, have significantly longer SHs than the individuals living in inland areas, and thus have a better insulated hair coat. We noticed that within an otter species, environmental conditions have only a limited value on PH length (chap. 1); this is apparently not the case for the SHs. Further studies to elucidate this problem would be interesting, particularly comparing SH length between populations living in continental and those of the same species living in coastal waters, for example, in *Lutra lutra*.

The adaptive value of PH width is less clear. The highest values are still observed in the species living in cold climates and the lowest in those living in warm climates, if you exclude the Sea otter. However, *L. provocax* has a similar width than the South Asian *L. lutra* and *L.*

longicaudis, which live in warmer climates. The species with the highest values all belong to the *Lutra* and *Lontra* genera and constitute the group of most aquatic otter species. The Sea otter has relatively thin hairs, thinner than in some tropical species and very thin compared to their length. Actually, thinner hairs may be advantageous, because air circulates better through a coat made of thicker hairs. On the other hand, the hair width was measured on the thickest part of the hair shaft and thus represents the shield width. As reported earlier, otters have a flattened shield, which allows the PHs to lie flat against the body, and a flat but wide shield may allow the PHs to build a better protective layer for the underfur in water, creating additionally non-turbulent water flow over the body. The fact that all of the most aquatic otter species have a wider shield (even if some differences are not significant) may corroborate this theory. The Sea otter, which relies on longer PHs and SHs and on a denser hair coat (see chap. 3), may not need PHs with a wider shield. In Sea otters, the shield is not only relatively thinner but also shorter than in other otter species. This can be easily explained by the fact that in Sea otters, the difference in length between SHs and PHs is less important than in other otter species, and so the shield, which is always above the underfur layer (otherwise this would result in disadvantageous openings of the underfur layer), is shorter. In the Sea otter, longer SHs may be more important than a protective shield. *P. brasiliensis* and *L. perspicillata* have the thinnest shields, despite of being more aquatic than *Aonyx* and *Amblonyx*, but the hair width show some proportionality to hair length and these two species can apparently not rely on longer PHs or thicker shield for their thermo-insulation.

Medulla width is the most puzzling parameter. Indeed, the thickest hairs have the thickest medulla but the opposite is true only for *L. perspicillata*. Medulla width is not proportional to hair width, and the medullary index shows no correlation with climate, systematical grouping or relation to the aquatic environment. The Sea otter is distinguished strikingly from the other species by its very thin, often fragmented medulla. Medulla width and medullary index are probably the hair parameters for which the difference between the Sea otter and the other species is the most obvious. Less important interruptions of the medulla at the shield or at the pars intermedia were observed in a few other species, but they are not correlated with climate, systematical grouping or relation to the aquatic environment, except perhaps in *L. felina*, the curious longitudinally split medulla of which could have a relation to the marine habitat of this species. We could demonstrate that a lower medullary index makes the hair shaft less breakable. The otter species have values, which correspond to those found in other semi-aquatics. The difference between the medullary indices of the different otter species has apparently not a relevant significance (again if you exclude the Sea otter). The Sea otter needs particularly solid hairs because of its high dependence on an intact fur, the damaging effect of sea water, and because of its intense grooming activity, which is actually aimed to keep the insulative quality of the fur, but whose mechanical action also may, after a

while, damage the fur. The structure of the medulla in otters differs to that observed in other mustelids and we have already discussed that the way the medullar cells and the gas spaces are organised may have an adaptive value. However, further studies on the medulla structure, medulla width, cortex width and properties of the hair shaft (mechanical and others) should be done in different species to get more information on the function of the medulla.

The cuticle structure of all the *Lontra* and *Lutra* species and of *E. lutris* shows great similarities: they all display a narrow diamond petal pattern over almost the entire lower hair shaft. Petal like scales appear in Mustelinae and in many other zoological groups, for example in marsupials (LYNE & MCMAHON 1950), in some rodents, or in foxes (TEERINK 1991, MEYER et al. 2002). However, in *Lontra*, *Lutra* and *E. lutris*, the scales have a particular shape. They are more elongated, symmetrical and regularly arranged, particularly in the species, which live in colder water, and this pattern appears over a longer part of the hair shaft. This unique cuticular structure allows an interlocking of adjacent hairs, and thus a better trapping of air bubbles (WILLIAMS et al. 1992, WEISEL et al. 2005). This is particularly advantageous in SHs, as we will see in chap. 4, but also probably in PHs. In *Lontra canadensis*, *Lutra lutra* (Europe) and *Enhydra lutris*, this interlocking system may work particularly well because of the shape of the scales, and moreover the interlocking happens over quite a long distance. For example, in a 3 cm long PH of *L. lutra* the shield of which takes less than half of the hair shaft, that part of the hair shaft that interlocks with other hairs is about 1.5 cm long. This corresponds to the length of the secondary hairs. In *E. lutris* the part of the hair shaft that interlocks can be about 2.5 cm long, again the maximal SH length measured in this species. In the tropical *Lutra* species or subspecies and in *Lontra longicaudis*, the smaller and somewhat stockier scales may provide a less efficient interlocking, and anyway this interlocking would happen over a shorter distance because the PHs are shorter. In *Lontra provocax* and *Lontra felina*, a well-defined narrow diamond pattern appears somewhat later on the hair shaft than in *L. canadensis*, *L. lutra* and *E. lutris*. However, the pattern appears just below the pars intermedia, which occupies a small part of the hair shaft, and thus it is also present over almost the entire lower shaft, which means over quite a long distance (remember the PHs of *L. provocax* and *L. felina* are significantly longer than those of *L. longicaudis*). In the non-*Lutra/Lontra/Enhydra* species an interlocking is more unlikely or happens perhaps over only a short distance. *Lutrogale perspicillata* is the species the cuticle structure of which resembles that of *Lontra* and *Lutra* the most, but its PHs are so short that an interlocking would not occur over more than 4 mm.

Mammals belonging to a zoological group may share some common hair features. However, within a zoological group, the hair characters do not necessarily correspond to evolutionary lineages (HOMAN & GENOWAY 1978). This is also the case in the Lutrinae. Indeed, the hairs of *Lontra*, *Lutra* and *E. lutris* share many common features despite of the genetic dis-

tance between them. The PHs of *Lutra lutra* are, for example, very similar to that ones of the distantly related *L. canadensis*, but very different from those of the closer related *Aonyx* species. The two *Aonyx* species have PHs quite different from those of *Amblonyx*, despite of the very small genetic distance between these two genus, and the ecological similarities.

PERRIN & CAMPBELL (1980) remarked that it might be difficult to differentiate between the hair characteristics which reflect phylogenetic relationships, and those which reflect ecological relationships. The Lutrinae share characteristics with other semi-aquatic species, like flattened cross-section, PH length or medullary index. The medulla structure does not appear in other mustelids and, according to our knowledge, also not in other semi-aquatic mammals, but could have an adaptive value. The cuticle structure shows features common to all Mustelinae and Lutrinea, i.e. the petal like scales, which are not found in other mustelid subfamilies. The Mustelinae are the closest relatives to otters and their ancestor branch (KOEPFLI & WAYNE 1998). So, the petal like scales have a phylogenetic origin, also because this pattern has not been observed in other semi-aquatic mammals. However, in the most aquatic otter species, particularly in those living in cold climates, the scales evolved to a shape and to an arrangement which improves the insulative property of the fur. Thus, hair features of the Lutrinae reflect both, phylogenetic and adaptive relationships. Within the Mustelinae, the species the PHs of which resemble those of otters the most are the semi-aquatic minks (*Mustela vison* and *Mustela lutreola*), despite of not being closer related to otters than the other members of their subfamily (KOEPFLI & WAYNE 2003, FLYNN et al. 2005).

Otter species which are very closely related, like *L. felina* and *L. provocax*, *L. lutra* and *L. sumatrana* or *Aonyx capensis* and *Aonyx congicus*, have very similar hair features, but they also have a rather similar ecology. So both, the ecological and the taxonomic position may explain the similarity of hair characteristics within these pairs. In *L. felina* and *L. provocax*, hair length and width values are closer to those of *L. canadensis*, whereas cuticle structure has more similarities with that observed in *L. longicaudis*. *L. felina* and *L. provocax* are phylogeneticaly closer to *L. longicaudis* but ecologically closer to *L. canadensis*. *L. provocax* and *L. felina* probably may have derived quite recently from ancestors living in the tropical areas of South America and rapidly evolved longer PHs and SHs, whereas the cuticle structure may have more similarities with that of the tropical ancestor. The inter-subspecies variability observed in the cuticle structure of *Lontra longicaudis* could not be explained by ecological particularities of the two populations. The individuals from the Orinoco and Amazon Basin perhaps live in larger water bodies, but the individuals from Central America can also occur at higher latitudes and thus in colder climates (CASTRO-REVELO & ZAPATA-RIOS 2001). Anyway, the examination of more specimens from exact known locations and also a better knowledge of the species are necessary to clear these aspects. It is very surprising, that *L. felina* does not show particular adaptations of the hair features, despite of its particular ma-

rine life. This could maybe be based on the fact that the separation from non-marine ancestors has been too recent to have permitted the apparition of particular adaptations. The thermo-insulating need of the Marine otter is not comparable to that of the Sea otter because it just feeds in salt water and rests on land, not like the Sea otter, which spends all its time in water. However, its small size is disadvantageous for thermoregulation in cold oceanic water. The Marine otter does not need fresh water to drink or to rinse its fur, unlike the other otter species, which sometimes use the resources of coastal waters (KRUUK 2006). They also do not have the very loose skin of the Sea otters, which allow them to pull the fur from the back like a too big pullover, and thus to lick and to comb every part of the hair coat (KENYON 1969). The only particularity of the fur of the Marine otter noticed is that their PHs are more straight and coarse than in other otter species. This could prevent the hairs from sticking together after a bath in salt water, unlike what happens with the hairs of *Lutra lutra* (KRUUK & BALHARRY 1990). The curious longitudinally split medulla observed in some hairs could perhaps have a relation to the marine way of life of *L. felina*, but much more individuals should be examined to know how often this structure appears. The sebaceous glands could also have undergone a particular adaptation. Anyway, much more research on this small marine mammal, especially on its physiology and adaptation to salt water is needed.

Pteronura and *Enhydra*, which are the most divergent taxa and the only representatives of their respective genus, show the most specific PHs. In the phylogenetic study by VAN ZYLL DE JONG (1987), these two species are also the “outsiders”, each one being at the opposite external position on the trees showing the relationships between otter species. Actually, molecular biology showed that the Sea otter has no outsider position on the family tree of otters; i.e the Sea otter belongs to the *Lutra*, *Aonyx* and *Amblonyx* branch, and is, thus, closer related to *Lutra lutra* than to *Lontra canadensis* (KOEPFLI & WAYNE 1998). However, the Sea otter is an outsider by its way of life, and it shows many morphological adaptations like, for example, enlarged hind feet, larger lungs or broad crushing molars (KENYON 1969). Every PH and SH feature examined in this study appeared to be an adaptation to the challenging ecology of Sea otters. The Giant otter is genetically the most distant from the other Lutrinae. Shorter hairs are typical for otter species living in warmer climates. However, the Giant otter has significantly shorter hairs than every other otter species, except for the Smooth otter (*L. perspicillata*), and to a lesser extent, the Small-clawed otter (*A. cinereus*), and it has also thinner PHs. There is no clear adaptive reason why the Giant otter has shorter and thinner hairs than species which live in similar climates. This could be related to its diurnal activity; moreover, Giant otters are often reported to bask in the sun (DUPLAIX 1982, STAIB 2002). Giant otters are almost exclusively piscivorous and have a strong relation to water, so their hairs should be better adapted to prevent heat loss in water (at least be longer) than those of less aquatic species like *Aonyx* and *Amblonyx*. *Aonyx capensis* and *Amblonyx cinereus* have

a wider distribution range and are thus exposed to a wider range of environmental conditions, also to somewhat colder temperatures, which could explain why their hairs are longer, but *Aonyx congicus* lives in the same type of habitat than *P. brasiliensis* (African rainforest versus South American rainforest) and is rather associated with mud than with water (JACQUES pers. communication). Despite of this, this species has significantly longer PHs and SHs. The hairs of *A. congicus* are apparently more influenced by phylogenetic than by ecological factors.

Several authors suggested that the closest living relative of the Giant otter could be the Smooth otter (*L. perspicillata*), because of morphological and behavioural similarities (see 2.1). Both diverge from the other otter species by their shorter and to a lesser extent thinner hairs. Otherwise, their hair characteristics do not resemble each other in a particular way. Cuticle structure in *L. perspicillata* is somewhat intermediary between the cuticle structure in *Lutra* and in *Amblonyx*. Particularly, the hairs of *Amblonyx* can be confused with those of *L. perspicillata*, which is quite surprising because of the difference in ecology of these two species. However, *L. perspicillata* and *A. cinereus* have produced several litters of healthy hybrids (MELISH & FOSTER-TURLEY 1996), which speaks for a close genetical relationship between them. The short hairs of *L. perspicillata* are also somewhat surprising. Indeed, like the Giant otter, the Smooth otter lacks some of the adaptation to an aquatic mode of life present in the *Lutra* and *Lontra* species, like for example, a modification of the genitalia aimed to improve the hydrodynamic of the body, but despite of this, it is a strongly aquatic species, with large webbed feet. In some areas, it occurs in larger water bodies than *Lutra lutra* (KRUUK et al. 1994a). However, the geographical range of *L. perspicillata* shows that it can also occur in drier areas than *Lutra lutra*. The cuticle structure resembles that of *Lutra* and *Lontra* more than that of *P. brasiliensis* but, as we already remarked, this cannot be a great help to improve the insulative value of the fur. The short and thin hairs of the Giant and Smooth otters allow a better heat loss, compared to those of the other Lutrinae, which is advantageous in hot climates but can be disadvantageous, particularly in water. According to our knowledge, the thermoregulation of those species has not been studied until now. Genetic analyses of the Smooth otter would also be welcome in order to clarify the taxonomic position of this species.

The relationships between different PH parameters, shown in Figs. 94 to 97, corroborate the outsider position of the Sea otter. MEYER et al. (2001) found that the zoosystematical groupings within the Cervidae show parallels with the correlations between some of the PH parameters. In the Lutrinae, the correlations between PH parameters are apparently influenced by both zoosystematical and ecological relationships. The PH length is more or less proportional to the PH width in otter species, when you exclude the Sea otter. A correlation between PH width and scale length can be observed in a group made of the *Lutra* species, *L.*

canadensis, *L. perspicillata*, and *P. brasiliensis*, all being only distantly related but sharing a similar association to water. This group can be recognized for several other PH parameter relations between: PH width-scale perimeter, PH width-scale area, PH length-scale length, PH length-scale perimeter and PH length-scale area. All the Old World otter species (*Lutra, Aonyx, Amblonyx, Lutrogale*) can be grouped together for the following parameter relations: PH width-scale length, PH width-scale perimeter and PH width-scale area.

2.5 Conclusion

The PH features of the Lutrinae tend to follow the morphological variations present within this subfamily. Indeed, the PHs of the different species share many common characteristics, and each can be recognized as being a hair from an otter. However, they show enough variations to allow an identification at species, or at least at genus level. The Sea otter (*Enhydra lutris*), which morphologically and ecologically diverges the most from the other species, and the Giant otter (*Pteronura brasiliensis*), which can also easily be recognized and is genetically the most divergent otter, show the most specific PHs. Otters share common characteristics with other semi-aquatic mammals, and also with other mustelids. The cuticle structure seams to be influenced by phylogenetic relationships. Indeed, petal like scales appear on the PHs of all Lutrinae and Mustelinae, which are their closest relatives and constitute their ancestor branch. However, within the Lutrinae subfamily, adaptive pressures have modified the PH structure. The group of most aquatic otters, made of the *Lontra* and *Lutra* species and *E. lutris,* exhibit a rather similar cuticle, despite of not being closer related to each other than to other otter species and despite of the polyphyly of the *Lutra* genus. Within this group, the species living in cold climates have longer PHs and longer scales on the lower hair shaft, which allows an interlocking of the PHs over this part of the shaft, thus improving the air trapping system of otter coats. This interlocking system will be further discussed in chapter 4, which is aimed to explain the adaptive cuticle structure of the SHs. Generally, the Lutrinae living in cold climates have longer PHs and SHs. Within the species that live in warmer climates, the less aquatic otters (*Aonyx*, *Amblonyx*) show less adapted cuticle structures. The hairs with the less insulative value are those of the Giant otter (*Pteronura brasiliensis*) and the Smooth otter (*Lutrogale perspicillata*), which is not easy to explain because those species are strongly associated to water and are exposed to aquatic cooling during their foraging trips, even in a tropical habitat. More knowledge of the physiology of these animals and generally of all the Lutrinae living in the southern hemisphere will be welcome. Particularly the adaptation of the small Marine otter (*Lontra felina*) to its exclusively marine environment may be a fascinating subject of research.

CHAPTER 3:
HAIR DENSITY IN THE LUTRINAE

3.1 Introduction

Animals living in an aquatic environment need some specific anatomical and physiological adaptations. A particularly high challenge for endotherms like mammals is the conservation of their body temperature. While whales (Cetacea) and seals (Pinnipedia) rely on a thick insulating fat layer, semi-aquatic mammals are protected from colder temperatures by a dense hair coat, in which an air layer is trapped (SOKOLOV 1962, FRISCH et al. 1974, REYNOLDS 1993). Semi-aquatic mammals have hair densities of, for example: 36,000 hairs/cm² for the Water rat (*Hydromis chrysogaster*), between 31,000 and 37,000 for the European beaver (*Castor fiber*), and between 67,000 and 84,000 for the Platypus (*Ornithorhynchus anatinus*) (GRANT & DAWSON 1978, DAWSON & FANNING 1981, SOKOLOV 1982, FISH et al. 2002).

In the Lutrinae subfamily, data on the hair density are available for only three species, the Sea otter (*Enhydra lutris*), the North American River otter (*Lontra canadensis*) and the Eurasian otter (*Lutra lutra*).

The Sea otter (*Enhydra lutris*), which lives in the cold waters of the North Pacific, is considered to be the animal species with the densest hair coat (TARASOFF 1974). However, there is some discrepancy in the figures found in the literature, and often information on the method of study and on the sampling (how many samples, sex, season) is missing. BARABASH-NIKIFOROV et al. (1968 in SOKOLOV 1982) found for the Sea otter a hair density of 32,875 hairs/cm² on the back and 44,979 hairs/cm² on the belly. According to WILLIAMS et al. (1992), the Sea otter has 164,662 hairs/cm² on the fore feet and 77,526 hairs/cm² on the back. Further data on hair density in the Sea otter at the back are: 100,800 hairs/cm² (SCHEFFER in KENYON 1969), 134,052 hairs/cm² (TARASOFF 1972), and between 109,999 and 127,210 hairs/cm² (FISH et al. 2002).

Data available for the North American River otter (*Lontra canadensis*) are 66,937 hairs/cm² (TARASOFF 1972) and 57,833 hairs/cm² on the back (TARASOFF et al. 1972, TARASOFF 1974). FISH et al. (2002) give 80,000 hairs/cm², but they analysed a tanned pelt, which could bias the results (KASZOWSKI et al. 1970).

According to CEREVITINOV (1958 in HEPTNER & NAUMOV 1974), the Eurasian otter (*Lutra lutra)* has 35,000 hairs/cm² on the back and 50,000 hairs/cm² on the belly. DE JONGH (in KRUUK 2006) also counted about 50,000 hairs/cm², but on the dorsum. SOKOLOV (1982) found about 41,000 hairs/cm² but information on the counting method is not available.

It is unclear if and how the hair coat of the Lutrinae varies during the year. According to HARPER & JENKINS (1982), *Lutra lutra* is having a summer and a winter moult. KENYON

(1969) observed that a captive Sea otter was loosing more hairs in summer than in winter. While studying a Sea otter population of the Commander Islands, BARABASH-NIKIFOROV (1935) found more of their own hairs in the scats at the end of spring and in summer. He also studied the hair density and came to a result of 19,224 hairs/cm² in summer and 22,382 hairs/cm² in winter on the back, and 16,942 hairs/cm² in summer and 22,382 hairs/cm² in winter on the abdomen (BARABASCH-NIKIFOROW 1947). However, these results differ so widely from the previously mentioned published data, that they should be considered with care.

The aim of this study was to complete the information available in the literature on the hair density of *Lutra lutra* and *Enhydra lutris* with data obtained by a reliable, appropriately described method. Moreover, hair density was studied at different body regions. Possible variations of the otter hair coat during the year were also analysed. We already observed that the season has no influence on the primary hair characteristics, at least at the dorsal body region (see chapter 1). In order to complete the information on possible seasonal variations of the hair coat, primary (PH) and secondary hairs (SH) from the dorsum and from the ventrum of Eurasian otters which died at different seasons were analysed.

3.2 Material and methods

3.2.1 Samples

Eurasian otter *Lutra lutra*:

The samples all come from individuals which had died accidentally in Eastern Germany *(L. lutra lutra*) in 2005 and 2006. Two of the otters died in summer and 4 in winter; the sex and month of death are indicated in Table 20.

Tab. 20: Date when collected and sex of the studied *Lutra lutra* individuals

Date collected	18-August	29-August	4-October	4-November	4-January	27-January
Sex	Male	Female	Female	Female	Female	Male

Sea otter *Enhydra lutris:*

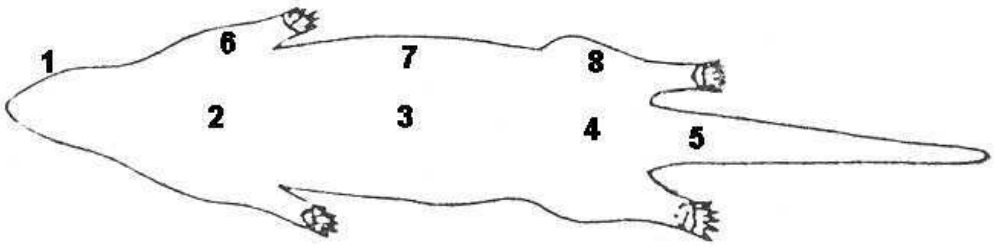
The samples come from animals that had died in Alaska (*E. lutris kenyoni*). Two individuals were studied, an adult male (date when collected: 28-Apr-05) and a juvenile female (date when collected: 20-Oct-05).

Preparation of the samples:

It is imperative to take skin samples from freshly dead or frozen carcasses, because the skin of tanned or air dried pelts are respectively stretched or shrunken, which modifies hair density. Skin samples of about 10 cm² were taken from the following body regions (see Fig. 98):

1. Cheek
2. Dorsum (anterior, withers)
3. Dorsum (medium, back)
4. Dorsum (posterior)
5. Tail
6. Shoulder
7. Flank
8. Buttock
9. Ventrum (anterior, chest)
10. Ventrum (medium, central)
11. Ventrum (posterior, abdomen)

Dorsal



Ventral

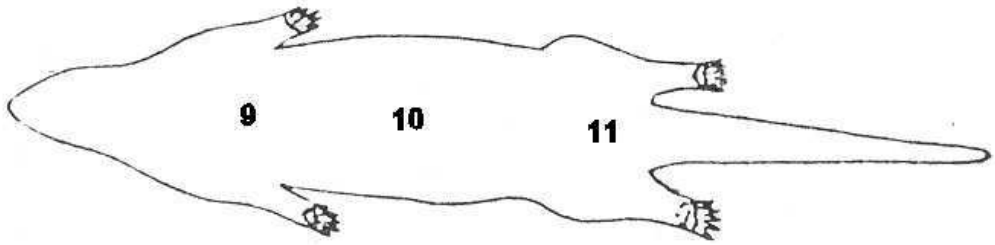


Fig. 98: Regions from where samples were taken

The skin samples were immersed into 4% formalin solution for 3-4 weeks. During this time, it is recommended to change the formalin 2 or 3 times and to turn the samples within the container, in order to facilitate the penetration of the formalin. If the samples are frozen, it is best to let them thaw in the formalin solution at room temperature. If the samples are transported and especially if they are sent, it is recommended to rinse them in ethanol (70 or 80%) for 1-2 days and then to put them in a small plastic or glass container with fresh ethanol, because of the toxicity of formalin.

3.2.2 Histological analysis

3.2.2.1 Embedding in Technovit 7100 (Kulzer GmbH)

The skin samples were carefully shaved and cut into small pieces (max 5 x 10 mm) that were rinsed in following solutions:

Day 1: 70% ethanol (24 hours)

Day 2: 80% ethanol (24 hours)

Day 3: 90% ethanol (4 x 20 min)

96% ethanol (4 x 20 min)

100% ethanol (4 x 20 min

100 ml Technovit 7100 + 1 g Harder I (overnight in the freezer)

Day 4: The samples were put in Teflon forms and completely covered with a solution made of 15 ml Technovit I (Technovit 7100 + 1 g Harder I) + 1 g Harder II (overnight in an oven ± 40°C).

Day 5: The hardened blocs were covered with Technovit 3040 (3 parts per volume powder: 1 part per volume liquid). This yellow resin cures very fast (5-10 minutes), bonds with the embedded blocs, and allows the samples to be mounted on the bloc-holder of the microtome.

3.2.2.2 Sectioning

After being taken out of the forms, the blocs were mounted on an Autocut microtome (Reichert-Jung), and cut into 3 µm sections. The sections should be as parallel as possible to the skin surface and contain the hair follicles. The sections were stretched on Aqua. dest., mounted on microscope slides and dried on a heating plate at 70°C.

3.2.2.3 Toluidine blue staining

The sections were stained for 1 minute with toluidine blue (RICHARDSON et al. 1960), then washed two times in Aqua. dest., cleared in 3 successive baths of 80% ethanol and 2 baths of 96% ethanol, and mounted with coverslips using the medium Depex (Serva, GmbH).

3.2.2.4 Determination of the hair density

The number of hair follicles per cm² was determined light microscopically from the histological cross-sections using a Zeiss microscope equipped with a drawing tube. For each individual and body region, the primary hairs were counted on 3 section areas of 2x2 mm, and the secondary hairs on 3 sections areas of 0.25x0.25 mm. The results were used to evaluate the number of hair follicles per cm².

Additionally, the number of hair follicles in the anagen stage (growing) and in the telogen stage (resting) was determined on the mid-dorsum (3) and mid-ventrum (10) of each individ-

ual (analysis of 10 hair bundles/section). Anagen hairs can be recognized in the histological preparations by their darker blue stained hair follicle wall.

3.2.2.5 Longitudinal sections

Longitudinal sections of a few skin samples were made, using the same histological method previously described, in order to observe the typical structure of the hair follicle types present.

3.2.3 Analysis of primary and secondary hair length and width

In order to complete our information about the seasonal variation of the Eurasian otter hair coat, PH and SH length and width (10 hairs/ind.) were measured at the medium part of the dorsum and ventrum of following individuals:

- 6 individuals died in winter (3 males and 3 females)
- 6 individuals died in summer (3 males and 3 females)

3.2.4 Statistical analyses

Statistical analyses (ANOVA, Student's t-test) are based on mean values per individual. Differences between body regions were determined using subsequent Post-hoc tests (Tukey).

3.3 Results

3.3.1 Hair coat of the Eurasian otter *Lutra lutra*

3.3.1.1 Hair density

The secondary hairs of the examined Eurasian otters were arranged in bundles of 20 to 30 hair follicles, sometimes up to 40 hair follicles (Fig. 99). Groups of 3 to 10 bundles were formed around at least 1 primary hair follicle. Groups of 3 or 4 bundles tended to have only 1 PH, whereas 3 to 5 PHs were observed in groups of 9 or 10 bundles. The bundles sometimes formed larger groups, where some subdivisions could be recognized (Fig. 100). For example, a group of 24 bundles with 6 PHs (2 central PHs and 4 lateral PHs) was observed. Typical and rather large sebaceous glands were seen between the bundles and principally around the PHs (Fig. 101).

The Eurasian otter had a mean hair density of about 70,000 hairs/cm² (see Tab. 21). Less than 1000 of them were primary hairs, which represented 1.26%. Table 22 indicates the PH, SH and total hair density of each individual (mean of all the studied body regions).

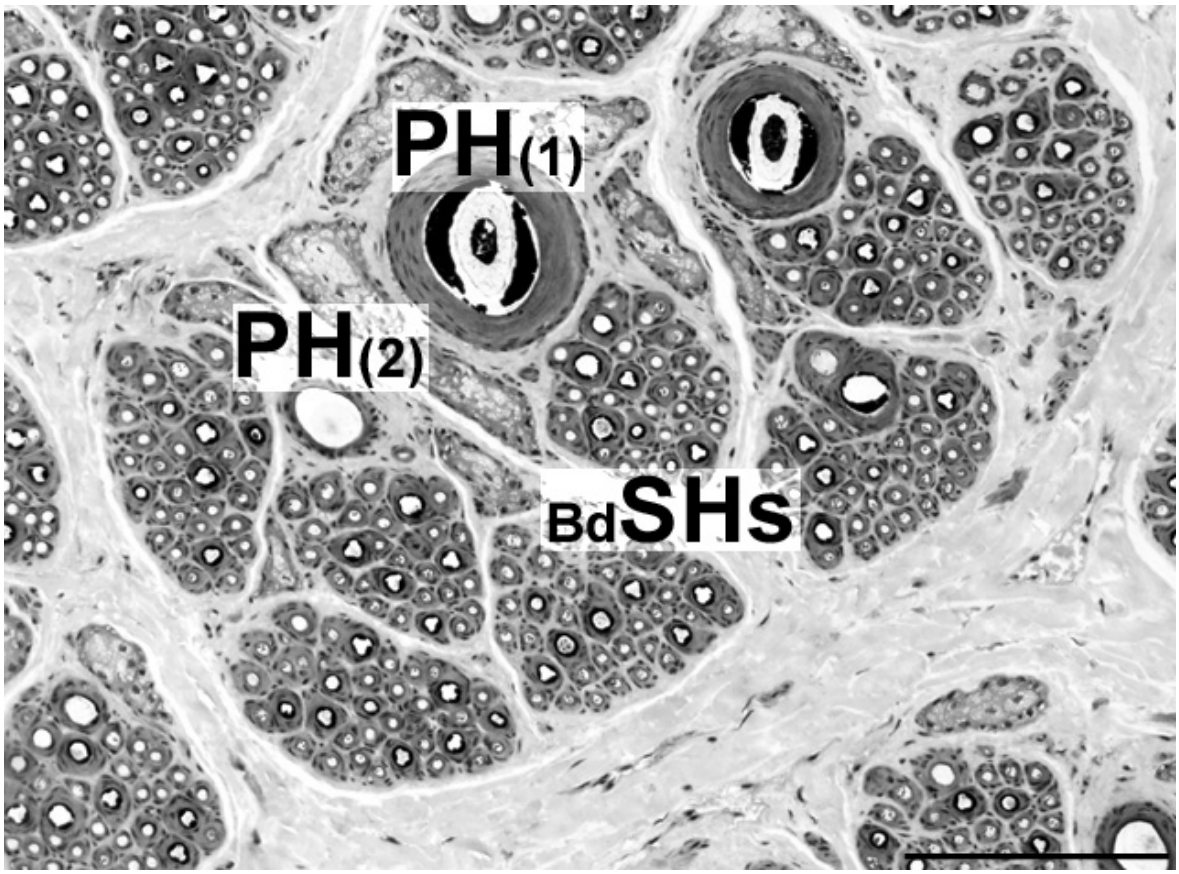


Fig. 99: Horizontal section, shoulder of *Lutra lutra*; PH(1): central primary hair; PH(2): lateral primary hair; Bd SHs: bundles of secondary hairs (magnification bar: 200 µm)

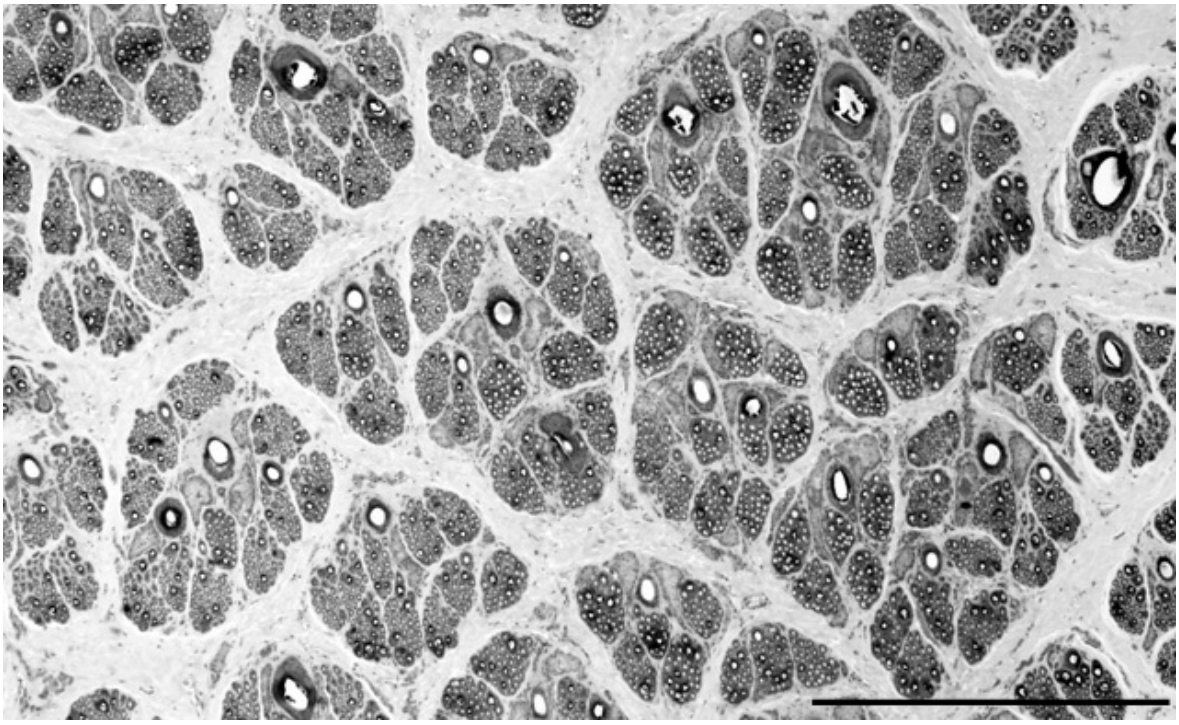


Fig. 100: Horizontal section, tail of *Lutra lutra* (magnification bar: 1000 µm)

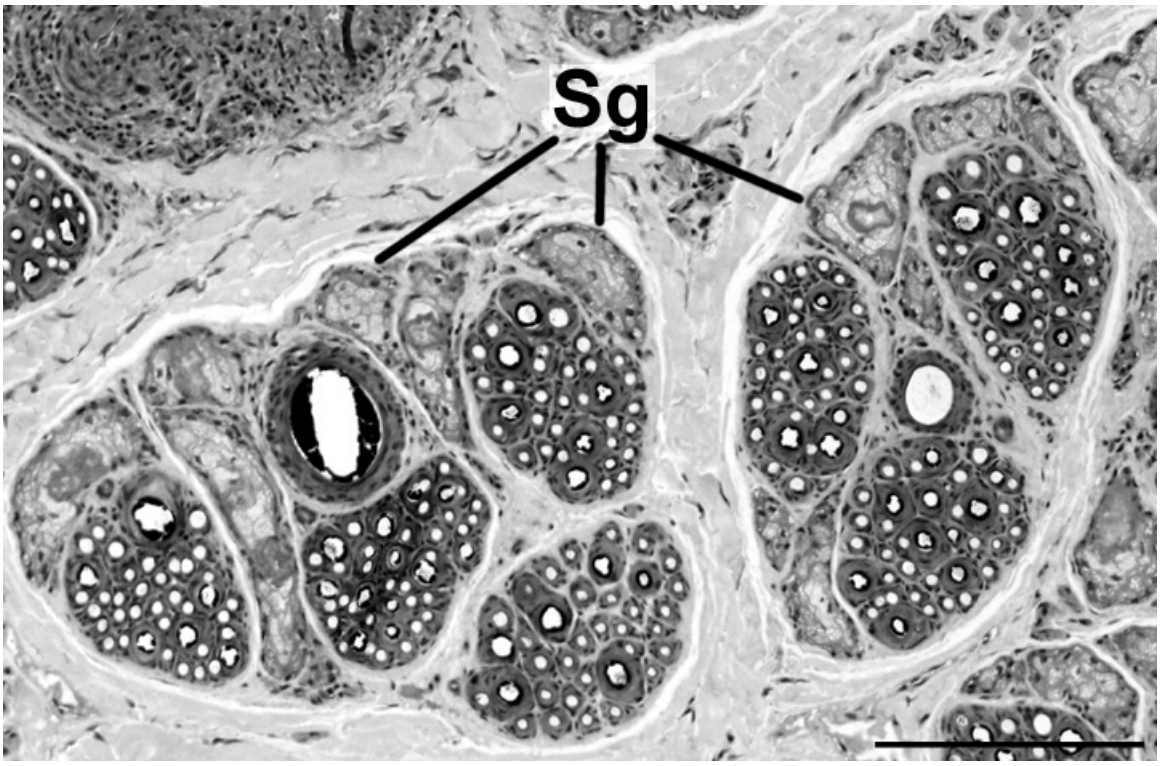


Fig. 101: Horizontal section, shoulder of *Lutra lutra* showing the numerous sebaceous glands (Sg) (magnification bar: 200 μm)

Tab. 21: Mean hair density of *Lutra lutra* for the whole body (n=6 individuals)

	Lutra lutra	
	Mean	SD
PHs/cm²	882	216
SHs/cm²	69015	12037
TOTAL	69897	12105

Tab. 22: Mean hair density of each individual studied (whole body)

	F-Oct		F-Nov		M-Jan		M-Jan		M-Aug		F-Aug	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
PHs/cm²	885	230	878	164	870	215	861	279	825	198	973	149
SHs/cm²	76279	16065	64347	5440	79227	8146	59042	8674	68036	7957	67661	67661
TOTAL	77164	16154	65224	5322	80097	8248	59903	8847	68861	8043	68634	9551

The sex of the animals had no influence on the hair density, neither for the PHs, nor for the SHs (Student's t-test, two tailed, p=0.15 and p=0.81)

The SH density remained constant from one body region to another (ANOVA, F=1.37, p=0.22, n=64, R^2=0.21). That was not the case for PH density (see Fig. 102). The PH density was higher at the cheek and at the chest (1,9) than at the other body regions, except the ventral regions 10 and 11 (ANOVA, F=10.72, p<0.001, n=64, R^2=0.67). All the regions situated at the dorsal and lateral side (2-8) did not differ significantly from each other.

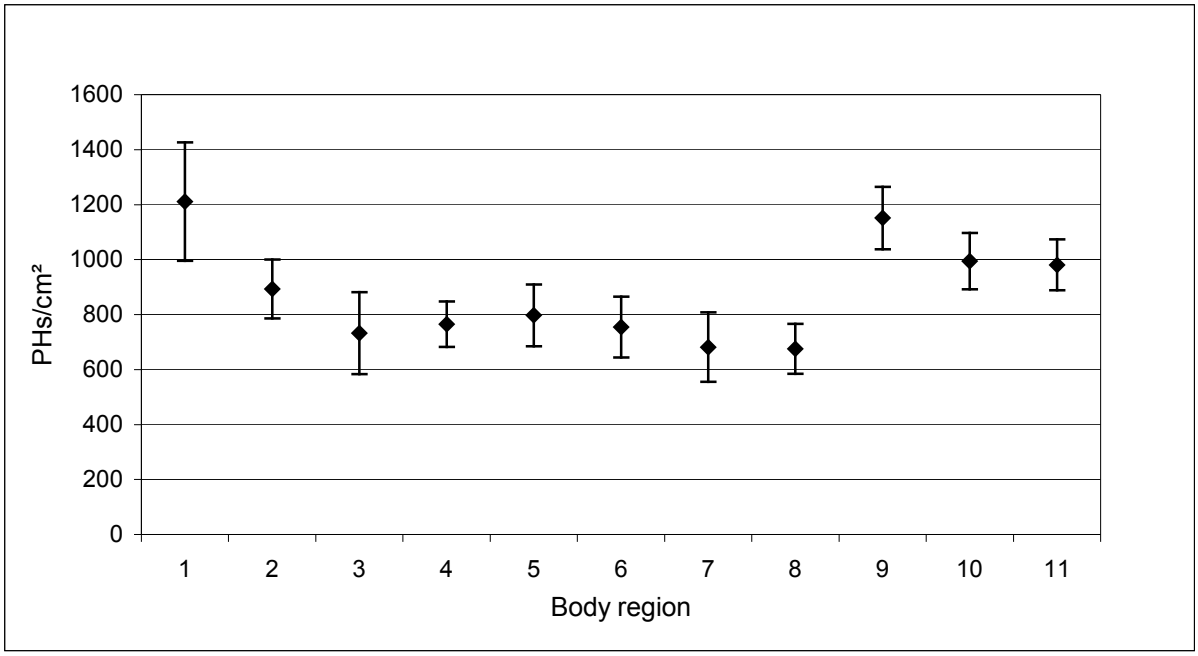


Fig. 102: PH density (mean and SD) of the Eurasian otter *Lutra lutra* (n=6 individuals) at each body region (1.cheek, 2.dorsum ant., 3.dorsum med., 4.dorsum post., 5.tail, 6.shoulder, 7. flank, 8.buttock, 9. ventrum ant., 10.ventrum med.,11.ventrum post.)

3.3.1.2 Possible seasonal variations

The density of hair follicles remained constant from one season to the other (Student's t-test, two-tailed, p=0.6 for the PH density, p=0.81 for the SH density).

Season had also no influence on PH length and width, neither at the dorsum, which confirms the findings of chapter 1, nor at the ventrum (Student's t-test, two-tailed, p=0.95 for the PH length at the dorsum, p=0.43 for the PH width at the dorsum, p=0.83 for the PH length at the ventrum, p=0.84 for the PH width at the ventrum).

The SHs also kept a constant length and width during the year (Student's t-test, two-tailed, p=0.91 for the SH length at the dorsum, p=0.77 for the SH width at the dorsum, p=0.23 for the SH length at the ventrum, p=0.58 for the SH width at the ventrum).

Table 23 shows the percentage of the hairs being in the anagen stage (growing) at the middle of the dorsum and of the ventrum, at different times of the year.

Tab. 23: Percentage of hairs being in an anagen stage at different times of the year

	Lutra lutra					
	04.10.05 (F)	04.11.05 (F)	04.01.06 (M)	27.01.06 (M)	18.08.06 (M)	29.08.06 (F)
Dorsum	7%	40%	14%	15%	27%	14%
Ventrum	6%	34%	19%	25%	28%	21%
Mean	7%	37%	17%	20%	27%	18%

3.3.2 Hair coat of the Sea otter *Enhydra lutris*

The Sea otter had bundles of 50 to 80 secondary hairs (Fig. 103). Groups of 5 bundles (with 1 PH) to 10 bundles (with 3 PHs) were observed (Fig. 104). The hair bundle groups were closer together than in the Eurasian otter. Distinct sebaceous glands could also be observed (Fig. 105).

The adult male studied had a mean hair density of about 130,000 hairs/cm² (mean for the whole body, see Tabs. 24 and 25). The juvenile female had a hair density of about 120,000 hairs/cm². In both individuals, the number of PHs represented less than 1% (0.91% in the adult and 0.76% in the juvenile).

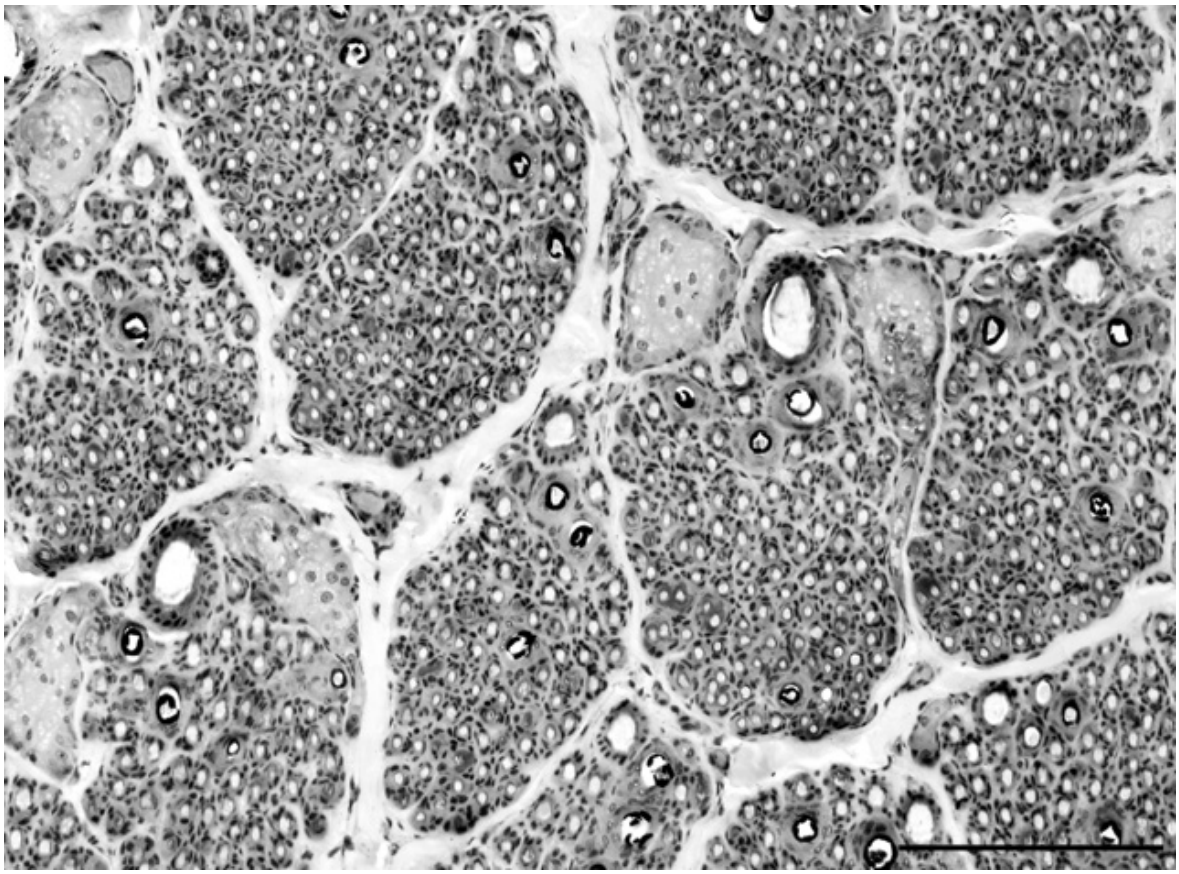


Fig. 103: Horizontal section, dorsum of *Enhydra lutris* (magnification bar: 200 μm)

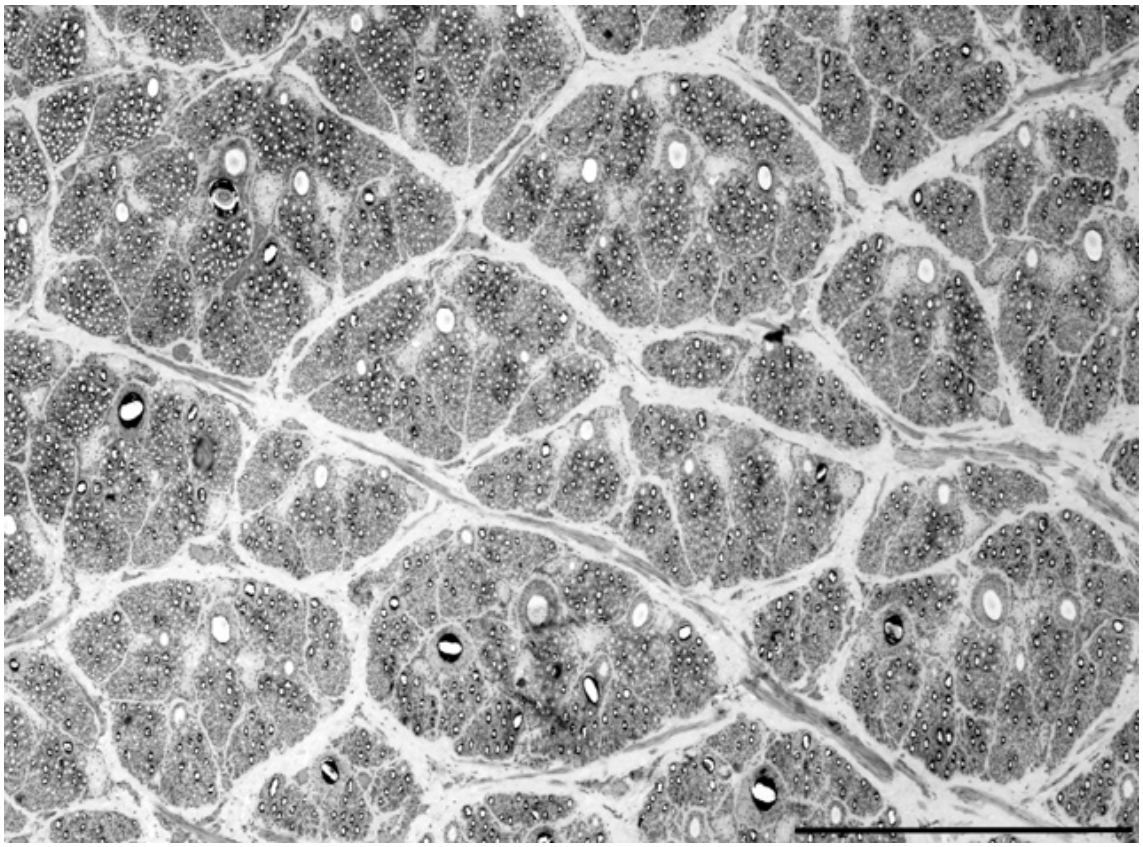


Fig. 104: Horizontal section, ventrum of *Enhydra lutris* (magnification bar: 1000 µm)

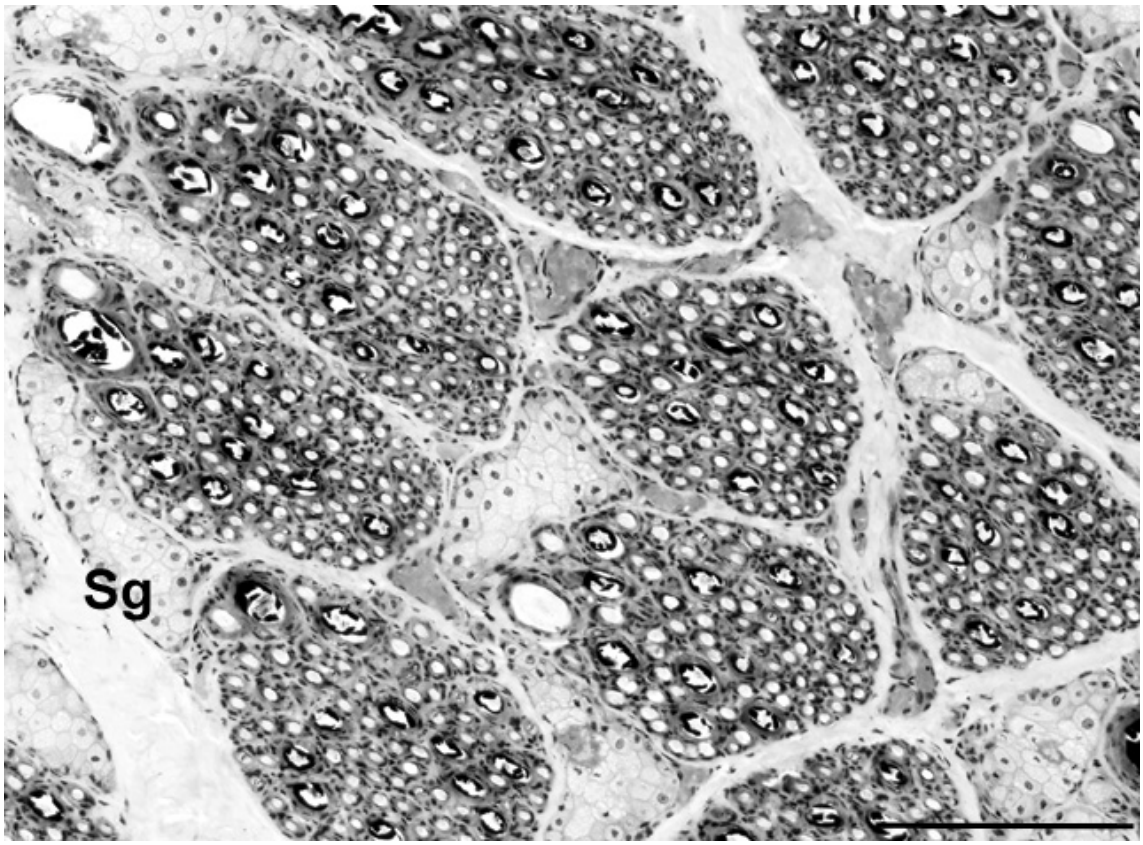


Fig. 105: Horizontal section, dorsum of *Enhydra lutris* with numerous sebaceous glands (Sg) (magnification bar: 200 µm)

The PH density of the adult animal ranged from about 1000 hairs/cm² at the mid- and posterior dorsum (3,4) to about 1400 hairs/cm² at the tail and chest (5,9) (see Tab. 24 and Fig. 106). The small number of individuals did not allow any statistical analyses. The SH density was quite constant all over the body, except at the cheek, where it was much lower than at the other regions (see Tab. 24 and Fig. 107). The mean hair density of the adult Sea otter, when the cheek was excluded (mean calculated with the regions 2-11), was of about 137,000 hairs/cm² (see Tab. 25). No differences between the hair density at the dorsal side and at the ventral side of the body could be observed. Only 4% of the hairs of the adult individual, which had died at the end of October, were in a growing stage (Tab. 26).

Tab. 24: SH, PH and total hair density of the two Sea otters studied at different body regions

	Male				Female (Juv.)		
	PHs/cm²	SHs/cm²	Total		PHs/cm²	SHs/cm²	Total
1. Cheek	1283	61733	63017	1	1050	56533	57583
2. Dorsum (anterior)	1150	131067	132217	2	933	124000	124933
3. Dorsum (medium)	1017	124933	125950	3	950	140533	141483
4. Dorsum (posterior)	975	132933	133908	4	775	147067	147842
5. Tail	1392	153067	154458	5	858	130533	131392
6. Shoulder	1208	130933	132142	6	975	120133	121108
7. Flank	1158	144133	145292	7	808	137333	138142
8. Buttock	1058	142400	143458	8	750	155467	156217
9. Ventrum (anterior)	1367	118933	120300	9	1325	94400	95725
10. Ventrum (medium)	1142	137733	138875	10	717	118800	119517
11. Ventrum (posterior)	1342	143467	144808	11	842	81733	82575
Mean	1190	129212	130402	Mean	908	118776	119683

Tab. 25: Hair density of an adult Sea otter *Enhydra lutris*

	Enhydra lutris	
	Mean	SD
Total	130402	23221
Total without cheek	137141	9678
Dorsum (2,3,4,5)	136633	10710
Ventrum (9,10,11)	134661	10440

Tab. 26: Percentage of hairs in the anagen stage in the two studied Sea otters

	Enhydra lutris	
	20.10.05 (M)	28.04.05 (F-juv)
Dorsum	4%	16%
Ventrum	5%	15%
Mean	4%	15%

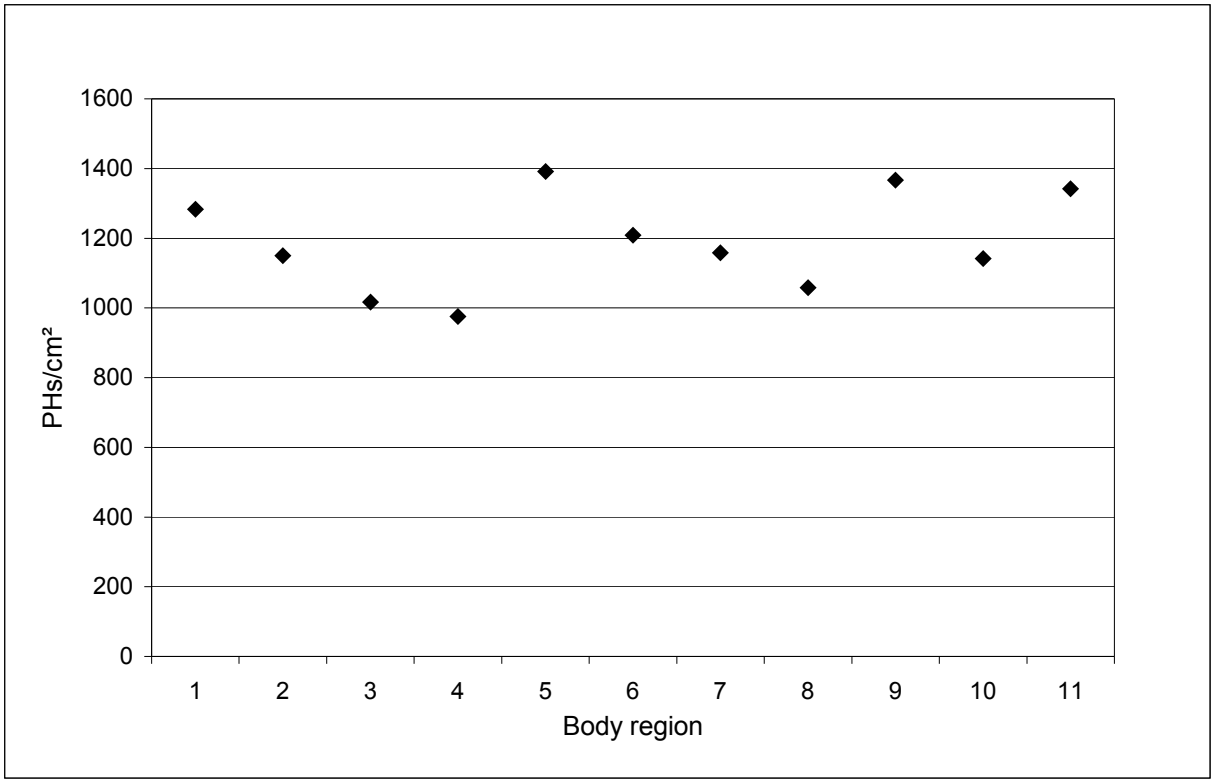


Fig. 106: PH density of an adult Sea otter at different body regions

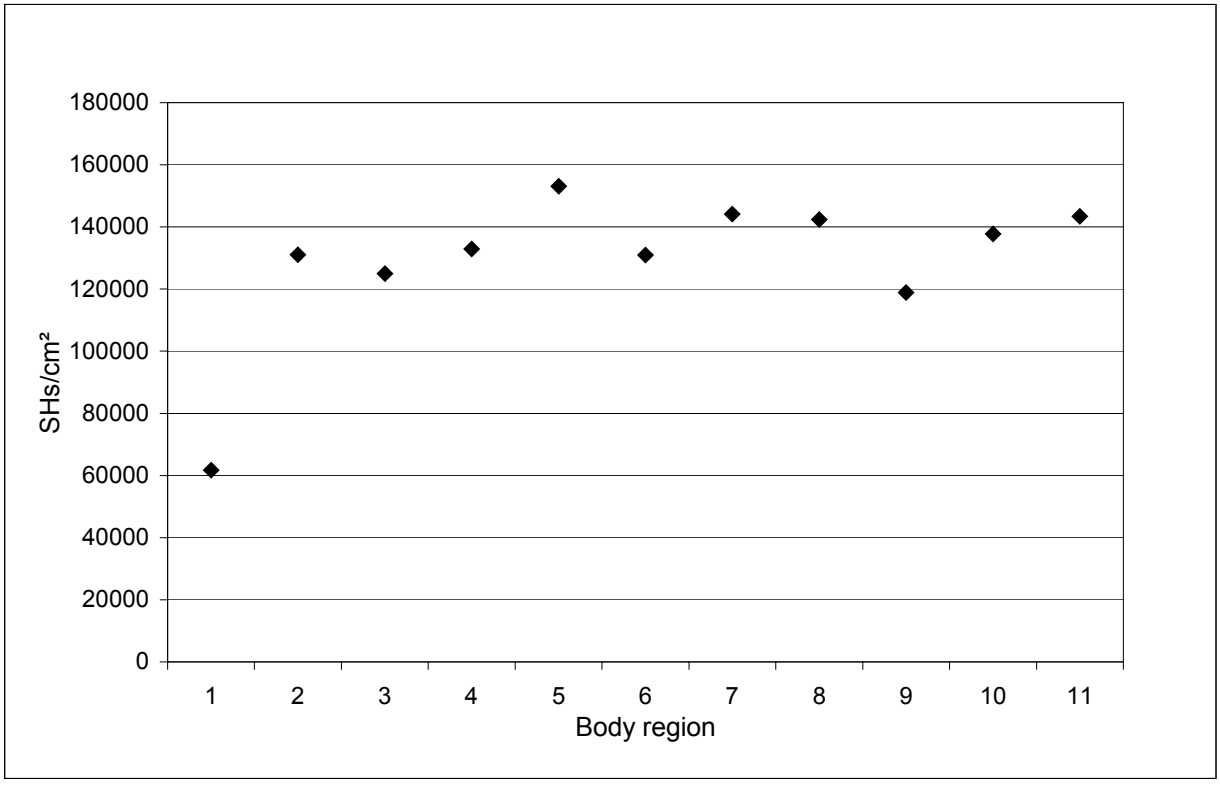


Fig. 107: SH density of an adult Sea otter at different body regions

3.3.3 Longitudinal sections

In both species, the secondary hairs from at least one bundle emerged from the skin through one common hair canal opening (Figs. 108 & 109). The primary hairs shared their canal with a bundle of secondary hairs. The hair canals from which one PH and several SHs emerged were somewhat deeper than the hair canals from which only SHs emerged (see Fig. 108).

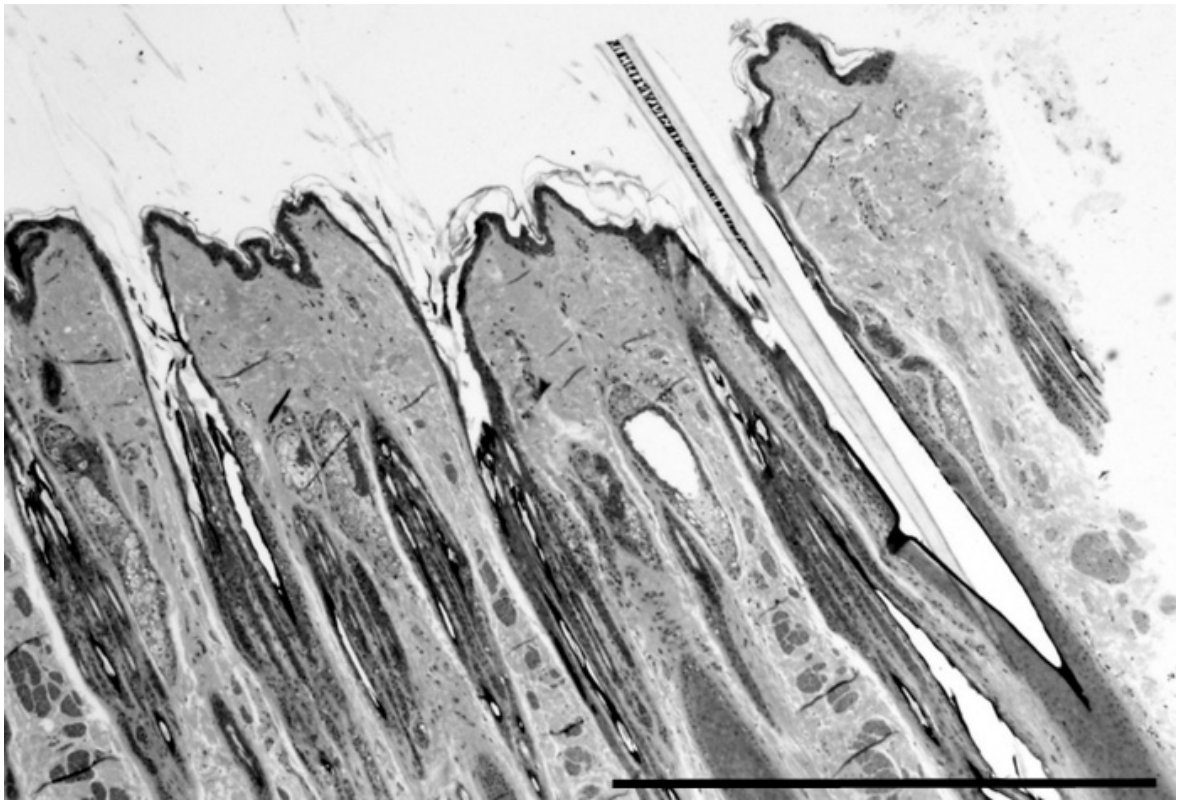


Fig. 108: Longitudinal section, dorsum of *Lutra lutra* showing the hair canals (magnification bar: 1000 μm)

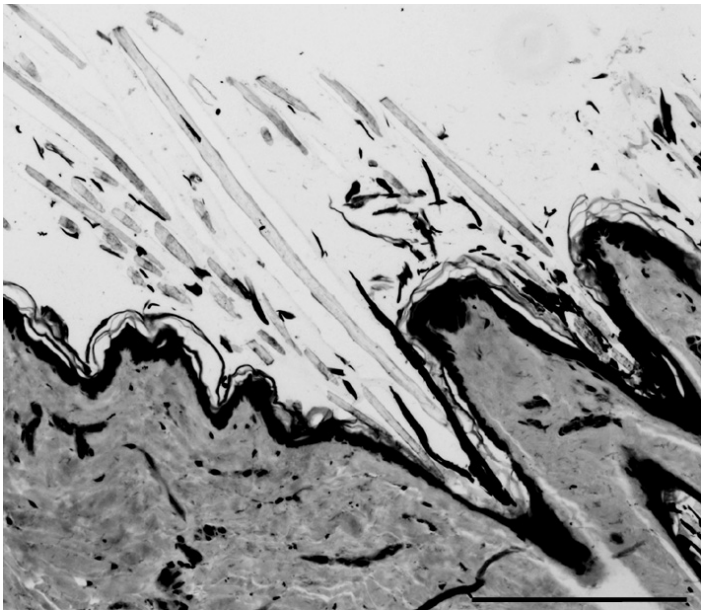


Fig. 109: Longitudinal section, dorsum of *Lutra lutra* showing a bundle of SHs emerging from a hair canal (the SHs were partly cut during the sectioning (magnification bar: 200 μm)

3.4 Discussion

3.4.1 Organisation of the hair follicles

Many mammals have their primary hair follicles forming a “trio group”, one central PH and two lateral PHs on each side, associated with more numerous secondary follicles, which together form follicle groups (RYDER 1973). However, many species do not follow this rule. SCHEFFER (1964b), for example, has not observed “basic trios” in pinnipeds. In the Wild cat (*Felis silvestris*), 1 central PH was observed together with up to six hair bundles, each containing 1 lateral PH and 3 to 20 SHs (MEYER et al. 1982). Groups of hair bundles without a central PH were also present.

The hair follicles of *Lutra lutra* and *Enhydra lutris* were not forming basic trios. However, it could happen that 3 PH follicles were found in a row. There was some irregularity in the way follicles were distributed: on one skin section, groups of 3 bundles were observed next to groups of more than 20 SH bundles (see Figs. 100 & 104). Central and lateral PHs were also not homogenously distributed. Some bundle groups were associated with only one central PH, other with only one lateral PH, some, particularly the larger groups, with several lateral and also several central PHs. One PH could be quite isolated, whereas the next ones were close to each other, even on a single section. This irregularity of hair follicle type distribution was observed at every body region and in both species. The most obvious differences between the two species were the larger size and higher density of the bundles, and also the smaller size of the PH follicles in *Enhydra lutris*.

DE JONGH (1986) observed bundles of about 20-22 underhairs at the base of one, or sometimes more PHs on shaved skin samples from the dorsal side of the Eurasian otter. We observed bundles of 20 to 30, sometimes up to 40 SHs, and much less PHs than SH bundles. So one SH bundle was not obligatory associated with one PH.

SCHEFFER (in KENYON 1969) counted 60 to 110 SHs per bundle in two adult Sea otters. WILLIAMS et al. (1992) observed on shaved skin samples between about 40 and 100 hairs per bundle, except at the foot and leg, where the mean number of hairs per bundle was of about 20. Each bundle had generally only one PH, sometimes 2 or 3. Our findings concerning the number of SHs per bundle correspond to those of these two authors. Indeed, we also observed between about 50 and 80 SHs per bundle, sometimes more. Again, we cannot confirm that each bundle was associated with one PH. If that was the case, the ratio of PHs would be more than 1% of the total number of hairs.

Very often, several hairs emerged from the skin passing one hair canal opening. In the mink, a large number of secondary hair follicles shared one common opening with one primary hair (RYDER 1973). In seals, primary and secondary hairs also emerged from one common funnel (SCHEFFER 1964b). In chinchillas, about 80 hairs, consisting of one PH and two groups of SHs, were emerging from a single hair canal of the skin (WILCOX 1950). We could also

observe numerous SHs emerging from a common funnel, together with one PH. Apparently some hair canal openings are used only by SHs. The hair canals from which one PH and several SHs emerged appeared to be deeper than the hair canals from which only SHs emerged. Further histological work is necessary to see how many SHs are really sharing one common funnel.

The exact depth of hair follicles insertion was not measured, but during sectioning, we could observe that the skin of *E. lutris* was thicker, and that the hair follicles were deeper inserted than in *L. lutra*.

3.4.2 Suitability of the method

A high discrepancy can be observed between some of the published data on the hair density of otters and other species. For example, the Australian Water rat (*Hydromys chrysogaster*) is supposed to have a hair density at the mid-dorsum between 16,900 and 19,300 hairs/cm² according to DAWSON & FANNING (1981), and between 40,961 and 48,845 hairs/cm² according to FISH et al. (2002). The data on the hair density of the Sea otter range between 16,943 hairs/cm² (BARABASCH-NIKIFOROW 1947) and 164,662 hairs/cm² (WILLIAMS et al. 1992).

Important variations can be observed even within the data published by the same author. TARASOFF (1972) gave for the tarsus of *L. canadensis* a hair density of 29,586 hairs/cm² in one publication, and 58,667 in another article published in the same year (TARASOFF et al. 1972). The data for *E. lutris* also differ: 75,200 hairs/cm² at the tarsus and 134,052 hairs/cm² at the midback versus 107,301 at the tarsus and 125,333 at the midback. Data on hair density published by the same author in 1974, showed an inter-individual variability of up to 20,000 hairs/cm².

Those differences may be surprising, but hypotheses to explain them can be submitted. First, the method used can bias the results. Some authors counted cut hair fibers emerging from shaved skin (DE JONGH 1986, WILLIAMS et al. 1992). Another method relied on counting the hair follicles (TARASOFF 1972, 1974 and in this study). However, techniques used to measure fur density are often not reported and information on the origin of the samples (individuals, body regions) is often missing.

If possible, the skin samples should be taken from fresh or frozen carcasses because pelts undergo changes in size and shape during the tanning process. KASZOWSKI (1970) found a mean SH density for the mink (*Mustela vison*) of 20,803 hairs/cm² when analysing samples from tanned pelts, and of 14,712 hairs/cm² in samples from living animals (mean for several body regions and several individuals in the winter pelage). To obtain these results, the hairs were removed from a delineated area of the skin (less than 1 cm²), embedded in balsam and cross-sectioned. The PHs and SHs were then counted on a small part of the section under

light microscope. When the samples are taken from carcasses, it is important to make sure that they conserve their size and structure by choosing on appropriate conserving and embedding process. Some samples were taken on carcasses but then dried, which can also alter the skin. It is also recommended to avoid the storing of the skin samples in the freezer or in formalin for years. Even if every precaution is taken, slight modifications of the skin of the freshly dead animals due to environmental conditions, like desiccation by the sun, cannot be excluded.

Another explanation for the observed variability is that the data are not the result of an exact count of the number of hairs per cm², but an evaluation using counts made on very small surfaces or calculations made with the number of hairs per bundle and the number of bundles on a mostly small surface area (few mm² to max 1 cm²). For species having high densities, the biases caused by this counting mode can be rapidly important.

Differences of several thousands hairs/cm² are not relevant for species having a very dense fur. Indeed, the standard deviations calculated in this study were high. Moreover, the intraspecific variation is apparently important in densely haired species. In our study, the Eurasian otter with the less dense fur had about 60,000 hairs/cm² and the one with the densest fur had about 80,000 hairs/cm² (mean for the whole body). TARASOFF (1974) determined the hair density of 3 North American River otters and 12 Sea otters and found an inter-individual variability of up to 13,000 hairs/cm² in the River otter and up to 20,000 hairs/cm² in the Sea otter. DE JONGH (1986) found a difference of 20,000 hairs/cm² between the hair densities of two Eurasian otters at a given body region. Moreover, bad health conditions of an individual studied, which could induce abnormal hair loss, cannot be excluded. Thus, one should be cautious with the results of hair density evaluations made using a single animal.

3.4.3 Hair density of *Lutra lutra* and *Enhydra lutris*

The claim “the Eurasian otter has up to 50,000 hairs/cm²” became “popular” and is widely used in educational activities, always giving rise to comments like “no, really? Amazing”. Actually, very few studies have been done on the hair density of *Lutra lutra*. CEREVITINOV (1958 in HEPTNER & NAUMOV 1974) found a hair density of 34,972 hairs/cm² on the back and 50,668 hairs/cm² on the belly of *L. lutra*. SOKOLOV (1982) gave a hair density of about 41,000 hairs/cm². Two individuals analysed by DE JONGH (1986) had a hair density of 52,857 and 48,906 hairs/cm² respectively, on the dorso-rostral region, and 62,876 and 42,416 hairs/cm² on the dorso-caudal region of the trunk. To obtain these data, the number of hair bundles and the number of hairs per bundle were counted on shaved skin samples using a SEM.

More data are available on the hair density of *Enhydra lutris*, the so-called “owner of the densest fur among all mammals”.

BARABASCH-NIKIFOROW (1947) found between about 17,000 and 19,000 hairs/cm² on the back and between 16,000 hairs/cm² and 22,000 hairs/cm² on the belly. About 1% were guard hairs. Those observations were made on tanned pelts (two winter and two summer) but no further details on sample preparation and counting method were given. Further found by the same author (1968 in SOKOLOV 1982) are about 33,000 hairs/cm² on the back and 45,000 hairs/cm² on the belly.

SCHEFFER (in KENYON 1969) calculated a hair density on the midback of an adult male Sea otter (sample preserved in formalin) of 100,800 hairs/cm² on the basis of 1400 hair bundles/cm² with 71 underhairs and one guard hair per bundle.

TARASOFF (1972, 1974) took skin samples from the midback, tarsus and interdigital web of the foot of three adult *Lontra canadensis* and twelve *Enhydra lutris*. The skin samples were prepared for histological examination and sectioned parallel to the skin surface. Hair follicles were counted in 75 to 100 hair bundles per body region, and the number of hairs per cm² calculated. The mean numbers of hairs per cm² for *Lontra* canadensis were the followings: tail 62,144 > tarsus 58,667 > midback 57,833 > foot 1,648. The hair densities of *Enhydra lutris* were: tail 131,094 > midback 125,333> tarsus 107,301 > foot 3,375 (1974).

WILLIAMS et al. (1992) examined skin samples taken from different regions from a fresh adult Sea otter. They counted the number of hairs per bundle and the number of hair bundles per cm² on dried, shaved skin samples. Thus, they obtained following hair densities (per cm²): forearm 164,662 > side 157,264 > rump 118,691 > stomach 82,251 > back 77,526 > chest 34,639 > leg 30,761 > foot 26,413. He counted between about 40 and 100 hairs per bundle, except at the foot and leg, where the mean number of hairs per bundle was of about 20. Each bundle had generally only one guard hair, sometimes 2 or 3.

FISH et al. (2002) gave a density on the mid-dorsum of 118,877 hairs/cm² for *E. lutris* and 80,312 hairs/cm² for *L. canadensis*. However, the samples were taken from tanned pelts.

We found for *Lutra lutra* a higher hair density than the previously published data. However, DE JONGH (1986) found a hair density of about 63,000 hairs/cm² on the dorso-caudal region of one individual, which is within the range of our values (see Tab. 22). The “classical” hair density of 50,000 hairs/cm² for *L. lutra* is an acceptable value if you consider the error range due to the counts and the inter-individual variability. However, we consider this value as being the lower limit, because we found a mean individual density ranging between about 60,000 hairs/cm² and 80,000 hairs/cm². These values were calculated using the values found on 11 body regions (remember the hair density did not differ significantly from one body region to another) and can be considered as reliable. Moreover, we found a mean hair density of 70,000 hairs/cm². This value was calculated using the hair density measured on 11 regions chosen all over the complete body (appendices excluded) in 6 individuals from both

sexes, which had died at different times of the year, and can thus be considered as being a reliable mean value for the species.

However, *Lutra lutra* has a very large distribution range, and we cannot exclude that individuals living in other climatic regions could have different hair densities, particularly the subspecies living in tropical climates. The hair density of 34,972 hairs/cm² that CEREVITINOV (1958 in HEPTNER & NAUMOV 1974) found on the back of *L. lutra* is apparently an underestimated value. The same author found about 50,000 hairs/cm² on the belly, which is closer to our findings. We will discuss the variability between body regions later.

The values for the hair density of *L. canadensis* published by TARASOFF (1972, 1974) and FISH et al. (2002) correspond to our results, so this contradicts the belief that North American River otters have a denser fur than Eurasian otters (SCHREBER 1776, in HARRIS 1968). This would also be surprising because both species live in similar climates and have the same food habits and thus, association to water.

We could analyse only two Sea otters, only one being an adult, but our findings are within the previously published data, if you exclude the values found by BARABASCH-NIKIFOROW. We found a mean hair density for the whole body of the adult animal of 130,000 hairs/cm². Total hair density equaled 137,000 hairs/cm² if the cheek is excluded, the only region which can be considerably distinguished from the others. The hair density of the juvenile female was lower but close to the density of the adult male (about 120,000 hairs/cm², mean for the whole body). The animal was estimated being near 1 year old and weighed 17.7 kg, which is close to the mean weight of an adult female. The adult male weighed 31 kg, i.e. also about the mean weight of adult males. The mean hair density for both individuals was about 125,000 hairs/cm² for the whole body, and 131,000 hairs/cm² for the whole body without the cheek.

The results of TARASOFF's work (1974) are the closest to ours, and were also obtained using a similar method (count of the hair follicles). The hair density found by FISH et al. (2002) on the mid-dorsum is also quite close to our values, despite having been investigated on tanned pelts. WILLIAMS (1992) found densities quite close to ours on the flank and rump, but the values for the stomach, back and, particularly, the chest were much lower than ours, which is quite puzzling. A density of 34,000 hairs/cm² on the chest would be quite low if we consider the habitat and behaviour of the Sea otter. We cannot comment the 164,662 hairs/cm² found on the forearm, because we did not analyse this part of the body. However, this figure should be considered with caution. To sum up, a mean hair density for *E. lutris* between 120,000 and 140,000 hairs/cm² may be a good estimation, if we consider our findings and some of the previously published data.

One is sure, the hair density of the Eurasian, North American and, especially, the Sea otter is really high, even if compared to the hair density of other semi-aquatic mammals. The Euro-

pean beaver (*Castor fiber*) for example, has a hair density between about 30,000 and 38,000 hairs/cm² (SOKOLOV 1982, FISH et al. 2002), and the Muskrat (*Ondatra zibethicus*) between about 11,000 and 49,000 hairs/cm² (SOKOLOV 1962, FISH et al. 2002). Only the Platypus (*Ornithorynchus anatinus*) has densities similar to river otters, with between about 67,000 hairs/cm² and 84,000 hairs/cm² (GRANT & DAWSON 1978, FISH et al. 2002). The Mink (*Mustela vison*), another semi-aquatic mustelid famous for its fur, has between about 15,000 and 34,000 hairs/cm² (KASZOWSKI et al. 1970, FISH et al. 2002). Beavers and muskrats are wetland inhabitants, but they feed mostly on plants on land. Minks are also less related to the aquatic environment then otters. Platypuses feed while swimming in water between 5 and 25°C (GRANT & DAWSON 1978), and their small size is disadvantageous for thermoregulation, so a very dense fur may be useful. A well-furred otarid, the Northern fur seal (*Callorhinus ursinus*) has about 50,000 hairs/cm² (SCHEFFER 1964a), whereas phocids like the Harp seal (*Pagophilus groenlandicus*) or the Common seal (*Phoca vitulina*), which rely on blubber to protect themselves against cold, have only about 1100 to 1800 hairs/cm² at the dorsum (TARASOFF 1974, BOLLHORN 1999). A good insulated terrestrial small carnivore like the Wild cat (*Felis silvestris*) has up to 30,000 hairs/cm² (MEYER et al. 2002). The Arctic fox (*Alopex lagopus*) has up to 40,000 hairs/cm² (KORHONEN & HARRI 1986).

A high hair density allows better thermal insulation of the fur (TREGEAR 1965). This is particularly important for the Sea otter, which lives in the cold oceanic waters of the North Pacific. This species feeds, rests and mates in the water, often even gives birth in water and never comes ashore. Thus, Sea otters are permanently exposed to cold water and cold air, and their survival relies on a fur, which provides excellent insulation in air and water. The insulation capacity of a hair coat also depends on the length of the hairs (SCHOLANDER et al. 1950). The Arctic fox (*Alopex lagopus*), for example, has SHs longer than 3 cm and PHs up to 6 cm long (KORHONEN & HARRI 1986). Semi-aquatics tend to have shorter hairs than terrestrial mammals. The Sea otters has the longest hairs among the Lutrinae, but this length does not exceed 4 cm (see chap. 2). Longer hairs would probably stick together in water and induce conspicuous openings of the fur, which would be disadvantageous for the thermoinsulation in water. The fur of Polar bears (*Ursus maritimus*), whose PHs are up to 15 cm long, has a high insulation capacity in air but not in water (SCHOLANDER et al. 1950). Thus, the insulation capacity of the fur of semi-aquatic species cannot be improved by longer hairs, and so relies principally on hair density.

Another advantage of high hair density is the fact that it improves the buoyancy. In the muskrat, for example, the layer of air trapped in the fur amounts to 21.5% of the animal's total dry volume, and reduces its specific gravity to 0.79 (JOHANSEN 1962a). Air gives the Sea otter a buoyant force of 0.94 N, and thus, decreases the effort needed to float (FISH et al. 2002).

Sea otters rest and feed while floating on their back, so an enhanced ability to float reduces energy costs.

3.4.4 Ratio of primary to secondary hairs

Another particularity of the otters fur is the ratio of PHs to SHs. We found that in the Eurasian otter only 1.26% of the hairs were PHs. This represents about 900 PHs for about 69,000 SHs/cm². The ratio of PHs is even smaller in the Sea otter. The total number of PHs/cm² was only slightly higher in the Sea otter (1190 for the adult male and 908 for the juvenile female), whereas the mean SHs density was of about 129,000 for the adult male and 119,000 for the juvenile female. Thus, respectively 0.91 and 0.76% of the total hairs were PHs. DE JONGH (1986) observed in *L. lutra* one or sometimes more guard hairs for 20-22 underhairs, which represents between 4 and 5% of the total number of hairs. BARABASCH-NIKIFOROW (1947) found 1% of PHs in *E. lutris*. SHs of semi-aquatic species are finer and denser than those of terrestrial species (FISH et al. 2002). Indeed, the insulating air layer is entrapped by the SHs, which are not wetted and prevent the skin surface from getting wet, whereas the wet PHs lie flat against the body and build a protective layer for the SHs. In the mink (*Mustela vison*), about 1.5% of the hairs are PHs (KASZOWSKI et al. 1970). To compare with terrestrial mammals, the fox has about 5% of PHs, the domestic dogs and cats between 7 and 14%, whereas, domestic cows, horses and pigs have no SHs, less SHs than PHs or about the same amount (RYDERS 1973, MEYER 1986). In the White-tailed deer (*Odocoileus virginiatus*), about 33% of the hairs are PHs (BUBENIK 1996). In the Fur seal (*Callorhinus ursinus*), PHs represent 3 to 4% of the hairs, whereas SHs are vestigial in the Walrus (*Odobenus rosmarus*) and have practically disappeared in Monk (*Monachus schauinslandi*) and Elephant seals (*Mirounga angustirostris*) (SCHEFFER 1964ab).

3.4.5 Variation according to body region

Few studies have looked at the variations of hair density according to body regions, in otters and in mammals in general. Semi-aquatic mammals tend to have a higher hair density on the belly than on the back. So the Australian Water rat (*Hydromys chrysogaster*) has a hair density of 30,500 to 36,300 hairs/cm² on the ventral side and 16,900 to 19,300 hairs/cm² on the dorsal side (DAWSON & FANNING 1981). The Nutria (*Myocastor coypus*) is also supposed to have a higher hair density on the ventrum (14,000 hairs/cm²) than on the dorsum (6,000 hairs/cm²), whereas the hair coat of the Muskrat (*Ondatra zibethicus*) is rather uniformly dense with about 11,000 hairs/cm² on the back and 12,000 hairs/cm² on the abdomen (SOKOLOV 1962).

CEREVITINOV (1958 in HEPTNER & NAUMOV 1974) found that the Eurasian otter also had a higher density on the belly (50,000 hairs/cm² versus 35,000 hairs/cm²). WILLIAMS et al.

(1992) observed quite similar densities on the back and on the stomach of the Sea otter, however, he found a relatively low density on the chest. Our results do not confirm these findings. Indeed, there was no significant difference between the hair densities of the 11 studied body regions in *L. lutra* (n=6 individuals). Even the cheek had the same hair density than the regions of the trunk. So we refute the belief that Eurasian otters have a denser hair coat on the belly. In the Sea otter, the hair density also appears to be quite constant over the body (without the appendices) except at the cheek. There, the hair density represented about half the density observed on the trunk, but was still very high (about 60,000 hairs/cm²). The mean hair density on the dorsum of the adult male (mean for the 4 regions situated at the dorsal side) was similar to the mean density of the ventrum (mean for the 3 regions situated at the ventral side, see Tab. 25). In the juvenile, hair density was somewhat lower on the ventral regions.

The ventral side of an animal which swims mostly horizontally, like Eurasian otters do, may be more submitted to heat loss. This would explain why some semi-aquatics have a higher hair density on the ventral side. However, in an aquatic environment, the hair coat should provide a good insulation for the whole body, at least for the trunk. This may be particularly true of otters, because when they hunt in the water, they often have to make rapid direction changes or swim vertically. They also play a lot in the water, they are very flexible and often turn and twizzle. Thus, their moves in the water may be more tridimentional then those of other semi-aquatics, which could explain a higher necessity of an uniform hair coat. The dorsal side of the Sea otters is longer immersed in water because these mammals rest and feed while floating on their back. However, when diving more or less vertically, the whole body is almost equally exposed to water pressure. Moreover, Sea otters also travel swimming on their belly at the surface of the water, and the belly can be exposed to very low air temperatures when the animal is floating. So it would not be really advantageous for the Sea otter, to have a less dense hair coat on one side of the body. The somewhat lower densities observed on the ventral regions of the juvenile is perhaps due to the fact that juveniles dive less and do not swim at the surface over longer distances. However, more individuals (adults and juveniles) should be analysed to make some reliable conclusions on the variations of the hair density over the body of the Sea otter.

When looking at terrestrial small carnivores, the Raccoon dog (*Nyctereutes procyonoides*) and Arctic fox (*Alopex lagopus*) have a higher hair density on the back than on the abdomen (KORHONEN & HARRI 1986), whereas the Wild cat (*Felis silvestris*) has more hairs at the ventrum than at the dorsum (MEYER et al. 1982). An animal can have its back more exposed to external temperatures then the rest of the body, especially when it is resting in a curled position. This is particularly true for the Arctic fox, which is able to rest even when ex-

posed to extreme climatic conditions. Terrestrial mustelids also tend to have a higher hair density on the back (SOKOLOV 1982).

Unlike secondary hairs, the primary hair density varies with the body region. In *Lutra lutra*, the PH density was higher on the cheek and on the ventral regions, particularly on the chest, than on the regions situated on the dorsal side. On the dorsum, the PH density was slightly higher on the anterior part. Those regions mostly experience drag when the otter swims, and so a higher density of PHs, which constitute a protective layer for the SHs, would make sense. We already observed some differences in the PH features between the mid-ventrum and the dorsum (see chap. 1); the PHs on the ventrum were slightly slimmer and had a thinner medulla and smaller cuticle scales. The PHs not only play a role in thermo-insulation of the coat, they also give a mechanical protection to the animal. This could also explain why the Eurasian otter had more PHs at the cheek and chest, and generally at the anterior and ventral parts of the body, because these parts are more exposed to physical hazards when the otter is moving on land, for example in burrows or dense vegetation. The higher PH densities could also be related to the sensory function of primary hair follicles. Particularly at the cheek, the additional PHs could complete the function of the vibrissae. The adult Sea otter male studied had a higher PH density on the cheek, tail, chest and posterior ventrum. The juvenile had a higher PH density at the tail, and to a lesser extent at the cheek. Further studies are needed to make some conclusions on the variability of the PH density over the body of the Sea otter.

3.4.6 Variation according to sex

Data on the variation of hair density according to sex are rare. BUBENIK (1996) found no differences between hair density of males and females of the White-tailed deer (*Odocoileus virginiatus*). In this study, we analysed the hair density of 3 males and 3 females of *Lutra lutra* and did not find any significant differences between the sexes. The highest hair density was observed in a male, but the lowest also (see Tab. 22). A difference between the hair density of males and females would have been surprising because the species do not show a clear sexual dimorphism, and both sexes have the same thermoregulatory needs. A correlation between hair density and the weight of the otters was not observed; the male having a hair density of about 80,000 hairs/cm² weighed about 9 kg, another male weighing 10.5 kg and a female weighing 5 kg both had a hair density of about 69,000 hairs/cm². The male Sea otter had a denser hair coat than the female, but the difference was small, and the female was close to the adult size but still juvenile.

3.4.7 Seasonal variation

The available information on possible variations of the otter hair coat throughout the year is somewhat contradictory. According to MATTHEWS (1952, in LING 1970), the Eurasian otter molts in autumn and probably does not undergo a spring molt. HARPER & JENKINS (1982) observed a spring and autumn moult in two captive Eurasian otters. According to their descriptions, the animals showed a heavy hair loss in spring (from March to June) and a slight hair loss, together with the growth of a winter coat in autumn (from August to October). However, they refer to the fact that otter hunting occurred all over the year. SOKOLOV (1982) considers that *L. lutra* has longer hairs in winter. According to OBBARD (1987 in POLECHLA 1991), the hair density of *L. canadensis* is greater in winter than in summer. HARRIS (1968) observed a rapid and almost imperceptible moult in September and a more important spring molt in *L. canadensis*. He also quoted NOVIKOV (1956) as writing about the Eurasian otter "molt lasts a long time and proceeds almost imperceptibly", and meant that further investigation of the coat changes of *L. lutra* was needed.

BARABASCH-NIKIFOROW (1947) counted on the back of the Sea otter 19,246 hairs/cm² in winter and 17,379 hairs/cm² in summer, and on the belly 22,389 hairs/cm² in winter and 16,943 hairs/cm² in summer. We do not consider these variations as being relevant and the figures presented differ too much from the previously mentioned published data. The author also observed in a male captive Sea otter that the fur was "dark-cinnamon-brown, approaching black" in winter and with "bright-brown tones" in summer. However, he observed that this captive animal had a molt occurring throughout the year, with the tendency of being more evident in summer, and concluded that the Sea otter molt was of a "gradual nature and low intensity", which was an "adaptation to the conditions of an aquatic environment with temperature limits between –1 and 15°C". In a previous publication (1935), he reported that the greatest quantity of hairs was found in late spring and in summer, particularly in the droppings – Sea otters swallow some of their own hairs when licking their coat – and already concluded that Sea otters shed their fur very gradually throughout the year, with an apparently most rapid shedding in summer.

It is traditionally admitted, that semi-aquatic mammals like otters do not undergo a spring and autumn molt, but molt throughout the year (TÄNZER 1932, TOLDT 1933, LING 1970). TOLDT mentioned a slight summer molt in the Sea otter. KENYON (1969) and YOCHEN & STEWART (2002) also consider that Sea otters may molt year around, but that the hairs are faster replaced in summer than in winter. Muskrats molt over almost the whole year (LAKROV 1957 in LING 1970). However, HART (1956) observed slight changes in the insulation capacity of the fur of the muskrat between summer and winter. The beaver has a prolonged molt during the late northern summer and autumn by which time many of the old guard hairs are worn or missing (THOMAS 1954 in LING 1970). The mink growth two coats a year, and

the progress of the molt can be easily observed in captivity (BASSET & LLEWELLYN 1949, ALLAIN & ROUGEOT 1980).

Actually, which parameters change between a summer and a winter coat? It could be the length, particularly the length of the secondary hairs. In the mouflon, the wool fibres continue to growth in autumn and became as long as the outer-coat. This is the way in which the coat becomes woollier in winter (RYDER 1962). In the Red deer (*Cervus elaphus*), the outer coat of the males reaches 50 mm in summer and 60 mm in winter; the underhairs are only a few mm long in summer, whereas they can reach 20 mm in winter (RYDER 1977). In the Racoon dog (*Nyctereutes procyonoides*), the PH length in summer equals 80% of the PH length in winter, and the SHs are twice as long in winter than in summer (KORHONEN et al. 1984). The Wild cat (*Felis silvestris*) has longer and thinner hairs in winter (MEYER et al. 1982).

The hair coat can also become denser. In the Short-tailed field vole (*Microtus agrestis*), more follicles become active in spring and particularly in autumn, and more hairs are lost in spring and summer. Thus, the winter coat has more hairs, particularly SHs. Moreover, thicker guard hairs are produced in summer, allowing the air to better circulate through the sparse coat with coarse hairs in summer (JOHNSON 1970). Some may consider that it does not make sense to compare the hair follicle density between summer and winter, because it used to be admitted that once the adult coat is established, no further development of hair follicles occurs (JOHNSON 1970). However, MOREJOHN & HOWARD (1956) suggested that new follicles are developed for each hair generation in the Pocket gopher (*Thomomys bottae*), and LYNE & BROOK (1964) observed how newly formed follicles developed from the outer root sheath of existing wool follicles (in LING 1970). Indeed, the Wild cat (*Felis sylvestris*) and the American mink (*Mustela vison*) have a higher density of SH follicles in winter (ALLAIN & ROUGEOT 1980, MEYER et al. 1982). In the mink, the number of hair follicles per bundle increases by 47% from summer to winter.

Our study shows that the density of the PH and SH follicles does not change from summer to winter in *Lutra lutra*. The PH and SH length and width also remain constant during the year. Structural features measured of the PH shaft (medulla width, medullary index and cuticle scale parameters) also do not show seasonal variations (see chap.1). Our findings confirm the belief that a semi-aquatic animal like the otter molts throughout the year. This is rather expected for an animal evolving in a medium, which imposes only limited seasonal variations in temperature. The distinct longer and denser winter coat of the mink does not coincide with the moulting pattern observed in the otter and other semi-aquatics. However, minks are less associated with water than otters.

The observations made on the skin sections let us conclude that *L. lutra* undergoes a continuous molt following a mosaic pattern, which means that a hair follicle can be in a different growing stage than the surrounding ones, and thus hair follicles being in a growing (anagen)

stage can be seen next to follicles being in a resting (telogen) stage all over the body. A mosaic of follicles being in both growing and resting stages was observed on every skin section (in each individual and all over the body). The percentage of hairs being in a growing stage was determined for each individual at the dorsum and ventrum (see Tab. 23). This coat was never in a resting stage. The resting stage is the condition in winter of the coat of an animal, which undergoes seasonal molts (JOHNSON 1970, MEYER et al. 1982). Here the percentage of hairs being in a growing stage was as high as 17% at the beginning of January, a time of the year where the coat of an animal molting seasonally is in its prime condition, which means in a resting stage. The ratio of anagen follicles was quite similar for the two animals found in January and for the two found in August. These results contribute to prove that Eurasian otters are molting gradually throughout the year. However, we have no data for the spring and more hairs were in a growing stage at the beginning of November, so we cannot exclude that the hairs are replaced more rapidly at certain times of the year. Further investigations are necessary to clear this feature. The skin sections of the Sea otters also show a gradual replacement of the hair coat which progresses mosaically, but again more samples should be examined to determine if the hair coat is renewed more rapidly at certain periods of the year.

3.5 Conclusion

The Eurasian otter (*Lutra lutra*) has a mean hair density of about 70,000 hairs/cm² (n=6 individuals). The mean individual density ranges between about 60,000 and 80,000 hairs/cm². About 1.3% are primary hairs (PH). The density of the secondary hairs (SH) remains constant all over the body, whereas PH density is higher on the cheek and on the ventral side, particularly on the chest, and to a lesser extent on the anterior dorsum. The hair density is not influenced by the sex. The Eurasian otter molts mosaically throughout the year. Indeed, the hair follicle density does not change between summer and winter, and the PHs and SHs also keep a constant length and width. Moreover, between 17 and 37% of hairs being in a growing (anagen) functional stage were observed in summer and in winter. However, we cannot exclude that hair replacement is perhaps more rapid at some periods of the year.

The Sea otter (*Enhydra lutris*) has a mean hair density between 120,000 and 140,000 hairs/cm². The PHs represent less than 1% of the total hairs. Hair density is lower on the cheek (around 60,000 hairs/cm²) but remains quite constant over the regions of the trunk.

A very interesting further study would be of course to investigate hair density of the other Lutrinae, and to get information about the hair density of species living in tropical climates, of those being less associated with water like the Congo otter (*Aonyx congicus*) and of the Marine otter (*Lontra felina*), which also lives in cold oceanic waters like the Sea otter. Many pelts were examined to get the samples necessary for hair structure analysis (see chap. 2). As we

already said, tanned pelts are not suited for hair density investigation. However, they can give an approximate idea of how dense a coat may be, by comparing different pelts. Every otter species has a dense coat, indeed they all have been, or are still, hunted for their fur, but one of the densest coat examined was curiously the coat of the Giant otter (*Pteronura brasiliensis*) living in the tropical rainforest. On the five pelts examined, the coat was so dense that the skin surface was not visible, and it was very difficult to cut the hairs at their base. The only other coat that looked so dense was the coat of the Sea otter. Giant otters have short and thin hairs, which is a characteristic of otter species living in warmer climates, but their hair density is apparently higher than in species living in colder climates. This could be a compromise between avoiding overheating on land and avoiding aquatic cooling in the water. Moreover, a dense coat also provides protection against environmental heat due to solar energy. This point should be cleared, by getting precise data on the hair density of the Giant otter. Unfortunately, for the majority of the otter species, suitable samples, which means fresh skin samples, are very difficult to obtain.

CHAPTER 4:
SECONDARY HAIR CUTICLE STRUCTURE IN THE LUTRINAE EXAMINED BY SEM

4.1 Introduction

The hair coat of otters is a very good insulator in air and water, because of a combination of specific characteristics: high hair density, appropriate primary (PH) and secondary hair (SH) length, very wavy SHs, PHs with a flattened shield (see previous chapters). The cuticle structure of PHs, additionally, plays a role in the insulating function of the hair coat because in some species, particularly the species living in colder climates, the shape and arrangement of cuticle scales at the lower (proximal) hair shaft facilitate the interlocking of hairs, and thus allow a better trapping of air bubbles, which increases the thermal insulation abilities of the coat. WEISEL et al. (2005) examined the hair cuticle structure of the North American River otter (*Lontra canadensis*) by scanning electron microscopy (SEM) and by light microscopy, analysing especially the interlocking system of the hairs. SHs are particularly important for the retention of the insulating air layer. They exhibit petal-shaped scales, which are arranged in a particular way; usually there are four scales at each level, rotated 45° with respect to those at an adjacent level (Fig. 110). The petal-like scales form sharply sculpted fins with deep grooves between them, which entrap air bubbles. This structure allows the hairs to interlock loosely with each other and to better trap air bubbles, which makes the otter coat a better insulator.

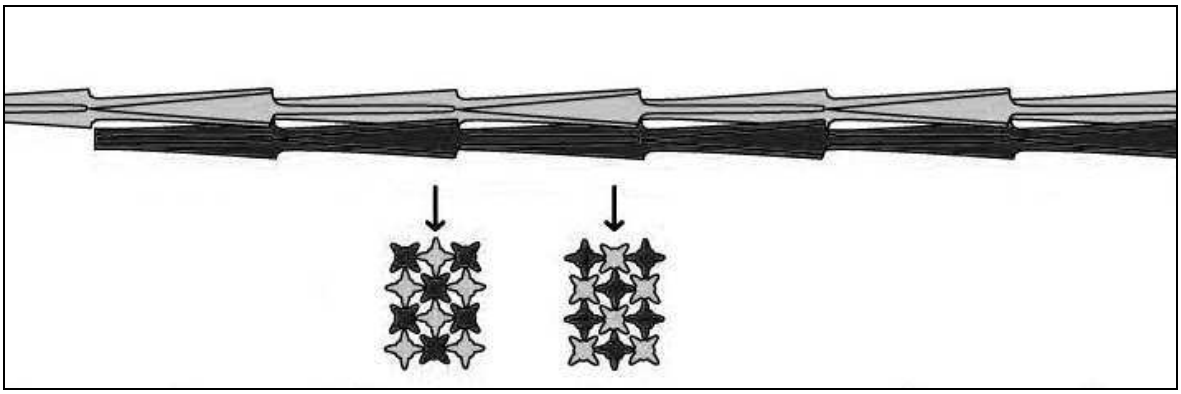


Fig. 110: Interlocking of SHs in *Lontra canadensis* (modified from WEISEL et al. 2005)

In this study, we looked at the cuticle structure of secondary hairs (SHs) of further otter species, and compared it with the SH cuticle structure of *L. canadensis* described by WEISEL et al. (2005). Thus, the SH cuticle structure of the Sea otter (*Enhydra lutris*), the Spotted-necked otter (*Lutra maculicollis*), the Cape clawless otter (*Aonyx capensis*), the Small-clawed otter (*Amblonyx cinereus*), the Giant otter (*Pteronura brasiliensis*) and the Marine otter (*Lontra felina*) was examined by scanning electron microscopy. These species were chosen so that the different climatic regions, different genera and the different degrees of adaptation to water of the Lutrinae are represented. Bundles of SHs were also observed by light microscopy to see how the SHs are intertwined.

4.2 Material and methods

About 5-10 SHs were taken from the dorsal body region of the Sea otter (*Enhydra lutris*), the Spotted-necked otter (*Lutra maculicollis*), the Cape clawless otter (*Aonyx capensis*), the Small-clawed otter (*Amblonyx cinereus*), the Giant otter (*Pteronura brasiliensis*) and the Marine otter (*Lontra felina*). The samples were taken from 2 individuals from each species.

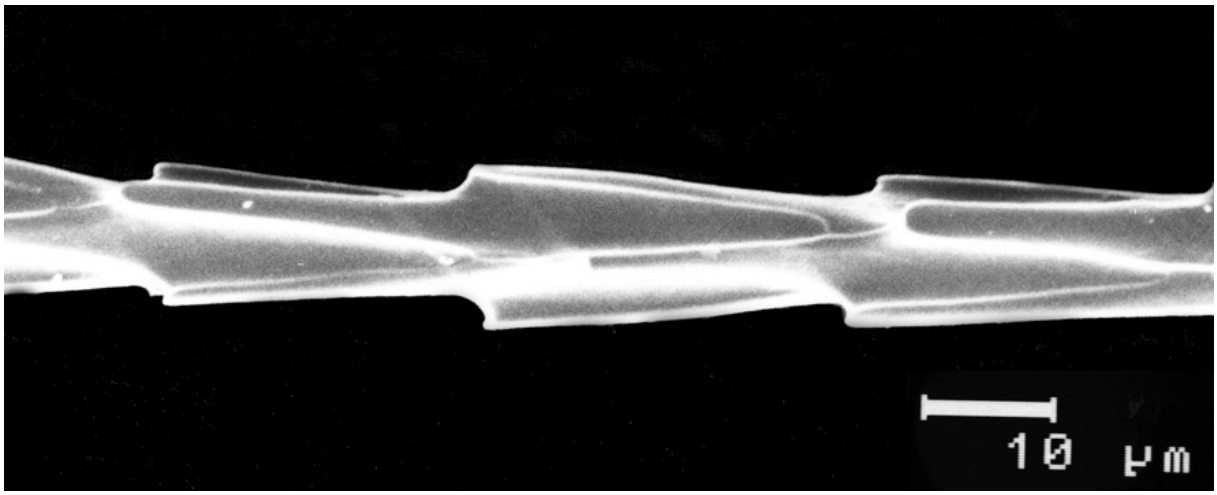
The SHs were thoroughly cleaned for several hours by immersion in a 50/50 (v/v) solution of ethanol/ether (abs.). After cleaning, the hairs were mounted with double adhesive labels (Leit-Tabs, Planert) on specimen holders, sputtered with gold in a sputter coater (Balzers SCD 040), and viewed in a Zeiss DSM 940 scanning electron microscope at 20 kV. The intertwining of the SHs within a bundle was observed by light microscopy.

4.3 Results

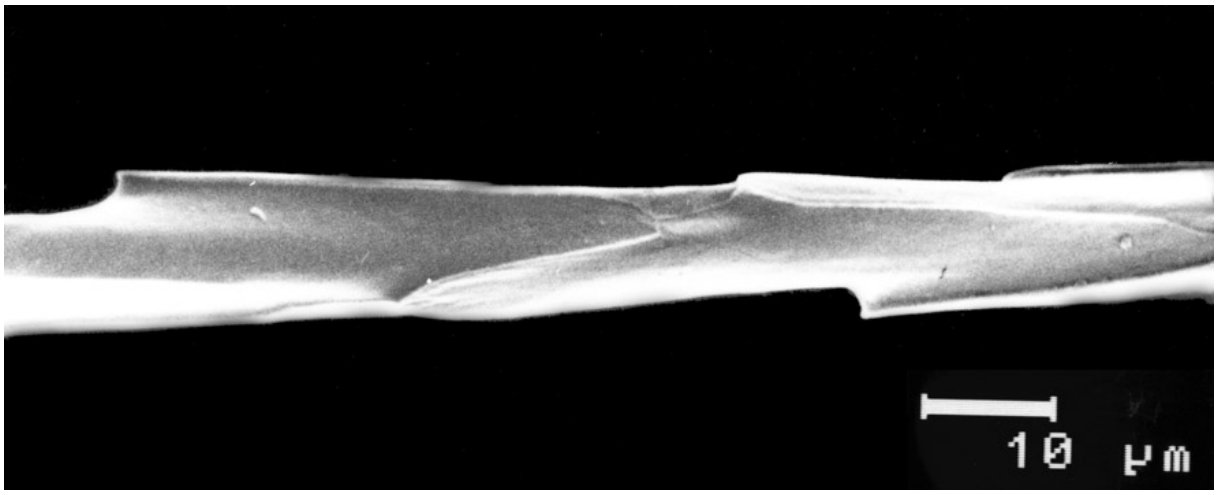
A structure similar to that observed in *L. canadensis* was observed in the other otter species studied. Every examined otter exhibited long and thin petal-like SH cuticle scales almost all along the hair shaft (Fig. 111). Distinctive fins alternated with deep groves, and the point of the thin was directed toward the distal end of the hair. Most often, series of 4 scales lying next to each other enwrapped the hair shaft. The scales were rotated 45° with respect to those situated at an adjacent level, and each “scales collar” was well imbricated in the one situated above and in the one beneath. Slight deviations from this standard pattern were seen on some parts of the hair shaft, the scales being sometimes less symmetrical, and thus the pattern appeared less ordered. No noticeable differences were observed between the SH cuticle of the different species.

In all the species, the SHs were strongly interacting with each other. The SHs of the Sea otter were particularly difficult to untangle, which may be due to their length and density (see chap. 2 and 3).

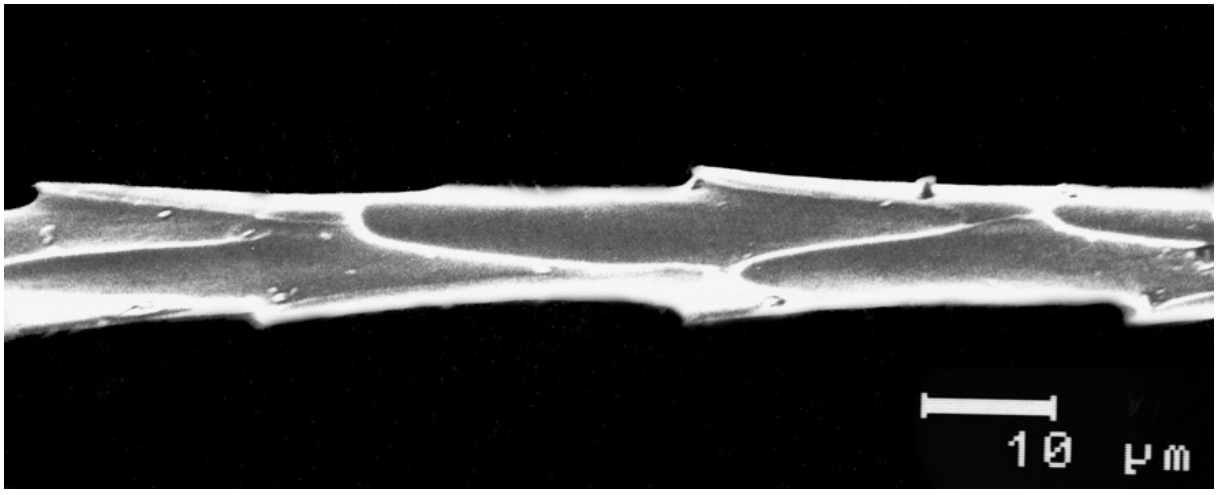
Fig. 111 Scanning electron micrographs showing the cuticle pattern of SHs of the:



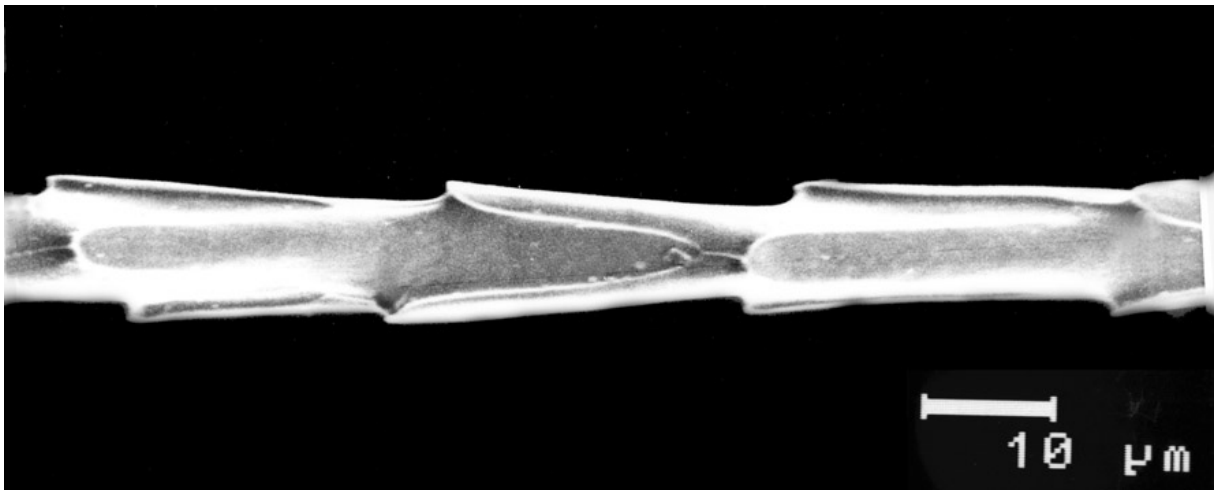
A: Sea otter (*Enhydra lutris*)



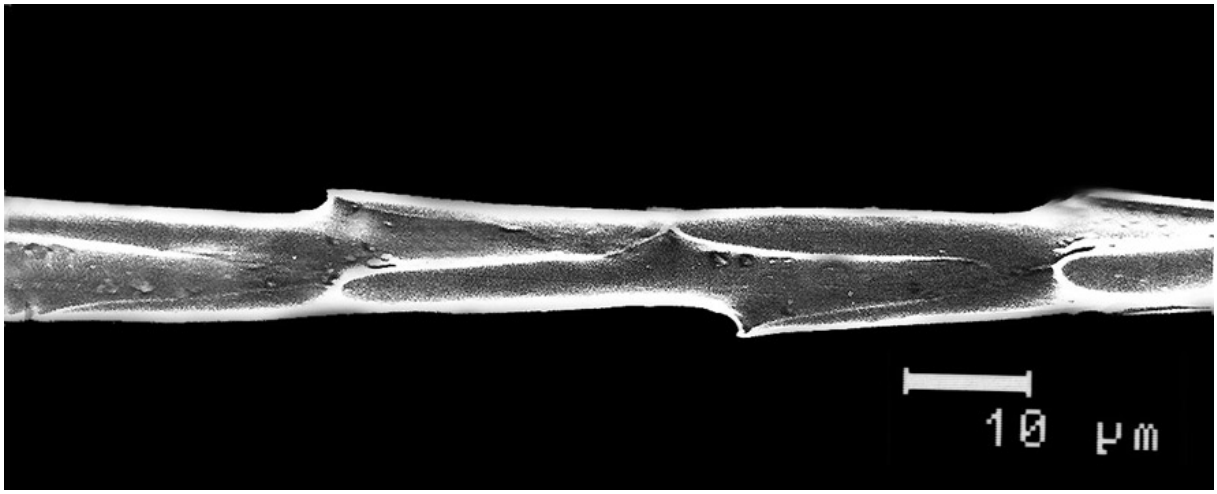
B: Spotted-necked otter (*Lutra maculicollis*)



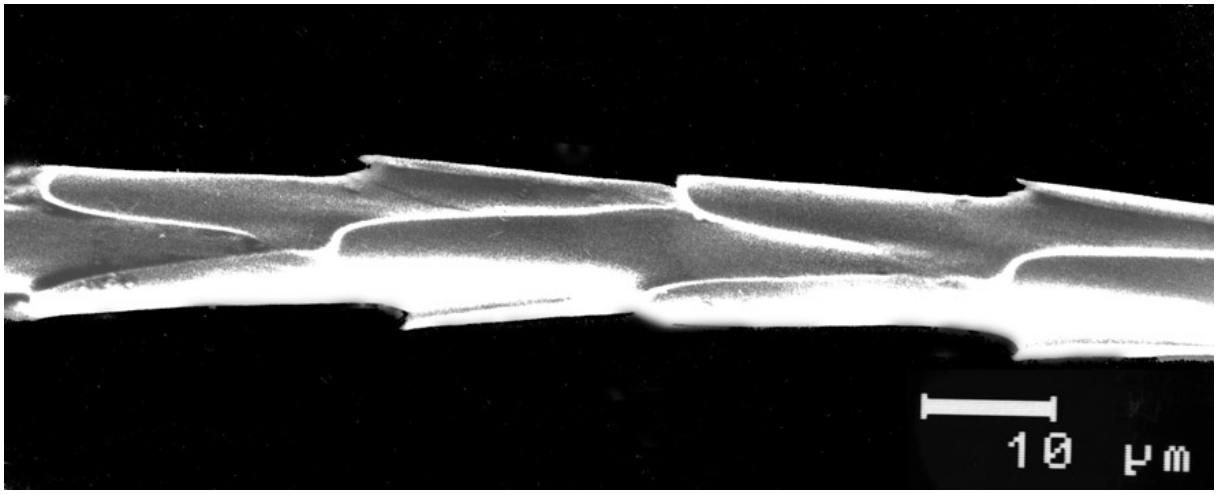
C: Cape clawless otter (*Aonyx capensis*)



D: Small-clawed otter (*Amblonyx cinereus*)



E: Giant otter (*Pteronura brasiliensis*)



F: Marine otter (*Lontra felina*)

4.4 Discussion

The observations made on the SEM micrographs of the SHs of *E. lutris, L. maculicollis, A. capensis, A. cinereus, P. brasiliensis* and *L. felina* suits to the findings of WEISEL et al. (2005) for *L. canadensis*. The SHs of the otter species showed long and narrow petal-like cuticle scales, like those observed on the lower shaft of the PHs of several species. Previous authors observed this cuticle structure in the SHs of *E. lutris* and *L. canadensis* (BROWN 1942, TARASOFF 1974, WILLIAMS et al. 1992). The waved SHs were partly intertwined with each other. Additionally, the cuticle structure also facilitated the interlocking of the SHs, because the fins of one hair can insert into the grooves between the fins of an adjacent hair. WILLIAMS (1992) also observed that the SHs interlock due to the wavy morphology and additionally to the structure of the scales. The interlocked hairs form a web that retains air, prevents water to come in contact with the skin, and thus heat loss is reduced. Air bubbles are not only trapped between the hairs, but also along the hairs in the grooves situated between the fins formed by the petal-shaped scales.

Unlike the cuticle structure of the PHs, the cuticle structure of the SHs seems to be quite similar for all the otter species. The SH cuticle structure has not been described for every otter species, but the species studied here are representative for the different genera, climatic regions and degrees of relation to water encountered in the Lutrinae, and thus we expect the basic SH cuticle structure to be the same in all the members of this subfamily. So, every otter species has SH cuticle scales that facilitate interlocking to improve the building of an air layer, and thus the insulative quality of the fur, independently of its taxonomic position, climatic region or adaptation to the aquatic environment. However, the length of the hairs, and thus the thickness of this insulative air layer varies between species, being higher in the species living in cold climates (maximal in the Sea otter *E. lutris*) and lower in those living in warm climates (minimal in the Giant otter *P. brasiliensis*). Air bubbles coming out of the fur when the animal is swimming are also observed in the Giant otter (pers. observation), but this air layer cannot be more than 5 mm thick (mean length of SHs in *P. brasiliensis*, see chap. 2). Moreover, a higher hair density, particularly SH density, as observed in the Sea otter (see chap. 3), allows a better retention of the air. A cuticle structure similar to that of the SHs is observed on the lower shaft of the PHs of the most aquatic otter species, particularly those living in cold climates, so in those species an interlocking between PHs and SHs may be facilitated, which is an additional improvement of the thermal quality of the fur.

Comparison possibilities with other species are limited. Only few studies looked at the cuticle structure of SHs, because these have no identification value, which is usually the aim of hair cuticle analysis. Petal-like scales appear on the SHs of non-lutrine species, for example in some procyonids and other mustelids like *Martes* (BROWN 1942). However, those data are

not accurate enough to allow a comparison with the interlocking system present in the Lutrinae.

4.5 Conclusion

An additional adaptive feature, which improves the thermal insulation of the hair coat of otters, is found at the cuticle of the secondary hairs. The shape and arrangement of the scales allow a flexible interlocking of adjacent hairs, which facilitates the trapping of an insulating air layer, and thus increases the thermal quality of the coat. Unlike other adaptive features of the hair coat, this characteristic is quite similar in every otter species, and is apparently not or only slightly influenced by factors like climate or relation to the aquatic environment. We do not exclude, however, that the interlocking system could be somewhat more effective in species that need a better insulation, but further studies, including measurements of the fins and grooves formed by the cuticle scales, would be necessary to answer this question. Anyway, the difference between the insulative properties of the hair coats of the different species may be principally due to the difference in hair length and density.

CHAPTER 5:
INFRARED THERMOGRAPHIC STUDY OF THE BODY SURFACE TEMPERATURE IN THE EURASIAN OTTER (*LUTRA LUTRA*), INCLUDING A COMPARISON WITH THE GIANT OTTER (*PTERONURA BRASILIENSIS*) AND THE SMALL-CLAWED OTTER (*AMBLONYX CINEREUS*)

5.1 Introduction

All objects with a surface temperature above absolute zero (0 K, -273.15°C) emit electromagnetic radiation (SPEAKMAN & WARD 1998). Using physical principles, it is possible to calculate the surface temperature of objects from the wavelength and intensity of electromagnetic radiation emitted in the infrared region of the spectrum. This feature, which allows to measure the surface temperature of an object without physical contact with it, is called infrared thermography (IRT).

Infrared technology is currently used in astronomy, building trade, industrial and research settings, and also has a great number of biological applications. In human (RING 1990, SHURAN & NELSON 1991) and veterinary medicine (STEPHAN & GÖRLACH 1971, HILSBERG 1998, EDDY et al. 2001), IR thermography is increasingly used in the evaluation of disease, injury, and inflammation, and allows pregnancy diagnosis by animals. Thermal imaging is also more and more of interest as a research tool in investigating animal physiology, ecology and behaviour (BARBER et al. 1991, STABENTHEINER et al. 1995, TATTERSALL & MILSON 2003, PALMER et al. 2004, TATTERSALL et al. 2004). This method is particularly helpful for work on animal thermoregulation. Measurements of body surface temperature using IRT, combined with measurements of body core temperature and metabolic heat production allow quantification of dissipated heat (WEBB et al. 1992, WARD et al. 2004). Infrared thermography contributes to understand how animals are adapted to their environment because it demonstrates mechanisms for heat dissipation or heat conservation (KLIR & HEATH 1992, PHILLIPS & SANBORN 1994, MCCAFFERTY et al. 1998). IRT can also demonstrate the absence of special thermoregulatory surface area, and thus the reliance on behavioural and ecological strategies to regulate heat loss (KLIR et al. 1990).

Considering mammals living entirely or partly in the aquatic environment, work on thermoregulation using IRT has been done with seals (ØRITSLAND 1968, DEHNHARDT et al. 1998, MAUCK et al. 2003), whales (CUYLER et al. 1992), dolphins (MAUCK et al. 2000, PABST et al. 2002), Polar bears (ØRITSLAND et al. 1974) and beavers (ZAHNER & MÜLLER 2003).

Like other aquatic and semi-aquatic animals, a great challenge for otters (Lutrinae) is to maintain their body temperature in water, the conductivity of which is more than 25 times that

of air. They achieve this based on specific adaptive features, like a highly insulating hair coat and an elevated metabolism (IVERSEN 1972, IVERSEN & KROG 1973, MORRISON et al. 1974, PFEIFFER & CULIK 1998, BORGWARDT & CULIK 1999). However, a thick hair coat reduces the surface available for heat exchange, and thus could lead to overheating. A considerable work has been done on otter metabolism, thermoregulation and the insulative properties of their fur. The most studied species are the Sea otter (*Enhydra lutris*) (KENYON 1969, IVERSEN & KROG 1973, MORRISON et al. 1974, TARASOFF 1974, COSTA & KOOYMAN 1982, 1984, WILLIAMS 1989, WILLIAMS et al. 1992, FISH et al. 2002), the North American River otter (*Lontra canadensis*) (TARASOFF 1974, SPELMAN et al. 1997, STOSKOPF et al. 1997, FISH et al. 2002, WEISEL et al. 2005) and the Eurasian otter (*Lutra lutra*) (IVERSEN 1972, KRUUK & BALHARRY 1990, KRUUK et al. 1994b, KRUUK et al. 1997, PFEIFFER & CULIK 1998). Metabolic rates by Small-clawed otters (*Amblonyx cinereus*) have also been investigated (BORGWART & CULIK 1999).

None of those studies, however, have assessed body surface temperature using IRT. Thus, a study of body surface temperature using thermal imaging was conducted in the Eurasian otter (*Lutra lutra*), in order to document which parts of the body are important thermoregulatory surfaces, and to demonstrate in this way mechanisms for heat conservation or dissipation. The influence of activity and weather conditions on the surface temperature at different body parts was analysed. Moreover, IRT allowed to measure the difference between the temperature at the surface of the body (surface of the fur) and the temperature within the fur close to the epidermis, and thus to illustrate in a new way the insulative properties of the hair coat of otters.

In order to compare the results with data obtained from otter species living in different environmental conditions, measurements were made also in two species originating from tropical regions: the Giant otter (*Pteronura brasiliensis*) and the Small-clawed otter (*Amblonyx cinereus*).

5.2 Material and methods

5.2.1 IRT study in the Eurasian otter (*Lutra lutra*)

An infrared thermographic study of the body surface temperature in the Eurasian otter was conducted at the Otter Centre (OTTER-ZENTRUM) in Hankensbüttel, Germany, from May 2006 until January 2007.

Thermal images of seven Eurasian otters were recorded using a FLIR ThermaCam B20. The measurements were made in May, June and December 2006 and in January 2007. The animals studied were: 3 males kept together in an enclosure in the exhibit area (Tomasz, Kuno and Olli), 2 males kept together in an enclosure also in the exhibit area (Robert and Lukas), one male living alone in an enclosure (Teufel) and one female also kept individually (Naima).

The distance between the otters and the camera ranged from 0.30 to 3 meters. We recorded the activity of the animals during the measurements, and also if the otter was wet or dry. Air (Ta) and water temperature (Tw) were always recorded. The pictures were taken after sunset, on cloudy days, in shadowy places or indoors, in order to avoid radiative heat gain. The pictures were additionally taken on days with little or no ambient wind, in order to avoid important convective heat loss.

The five otters in the exhibit area were always lured with food during the measurements, which lasted 10 to 40 minutes. Thermal images were taken during 13 measurements sessions for Tomasz, Kuno and Olli. Measurements could be made only on 8 sessions in May for Lukas and on 10 sessions in May and June for Robert, because they died in May and in June 2006 respectively. Teufel came to the Otter-Centre at the end of summer and thermal images of this animal were taken during a 15 minutes session in December and a 68 minutes session in January. We focussed our attention on Naima, because this female was very tame and kept alone in an enclosure. Having one individual alone in an enclosure made the measurements easier, because there was no need to identify the photographed animal, and the activity of the animal studied could be recorded for a longer period of time, with minimal risk of error, even in a large enclosure with an important vegetation cover. Thermographic pictures of Naima were made on 26 sessions, which lasted 1 to 2, exceptionally 3 hours. It was possible to take pictures of her while grooming, sleeping outside and even in the sleeping box. On 5 occasions in May, she spent 30 to 40 minutes in an indoor part of the enclosure, usually not accessible for otters (actually the service room), which was the only way to get her active on land for such a long time. On one day in June, she was kept in an enclosure without pool during 5.5 hours.

5.2.2 IRT study in the Giant otter (*Pteronura brasiliensis*) and Small-clawed otter (*Amblonyx cinereus*)

Thermographic pictures from Giant otters and Small-clawed otters were recorded at the Hagenbeck Zoo (Hamburg, Germany) in September 2006.

Thermal pictures of two adult Giant otters (male and female) were taken during 3 sessions. During the first two sessions, which lasted 2 hours and 30 minutes respectively, the animals were in an indoor heated enclosure with pool. During the last session, they spent one hour in a small part of the enclosure, with no access to the water. The distance between thermocamera and animal ranged from 2 to 4 meters, except during the last session, where it was about 1 meter.

Thermal pictures of a group of 6 Small-clawed otters were taken on 3 sessions, which lasted about 2 hours. The otters were kept in a large enclosure with pool, covered with a movable

dome, together with a group of orangutans. Here the distance from observer to otters ranged from 5 to 10 meters and a 12° x 9° lens (FLIR Inc.) was added to the thermocamera.

5.2.3 Thermal imaging and analysis of the thermograms

Measurements of body surface temperatures were made using a FLIR ThermaCam B20. An emissivity of 0.95 was chosen, which is within the range of emissivities for biological material (HAMMEL 1956, PORTER & GATES 1969, SPEAKMAN & WARD 1998). For all pictures, a rainbow colour scheme was chosen. Since electromagnetic radiation travels in straight lines, errors due to angle distortion can occur. However, the effect of viewing angle is negligible for objects with rough surface such as animals, until the angle is less than 10° (CLARK 1976, SPEAKMAN & WARD 1998). Thus, when the angle between a surface and the line of sight of the thermocamera was less than 10°, this surface was excluded from the analysis. The thermal images were analysed using the ThermaCAM QuickView software (FLIR). Mean temperature and SD of a given area of the otter body were calculated using the temperature measured at 5 randomly chosen points situated within this area.

5.3 Results

The studied animals were left in their usual enclosure and were unrestrained during the measurements, with a few exceptions (see material and methods). Thus, they could go into their sleeping box or into the water whenever wanted, and they switched very often between water and land, the tame otter Naima being the only one who stayed longer on land. The otters Robert, Lukas, Olli, Tomasz, Kuno and Teufel were wet on most of the thermograms, and only Naima could be often recorded when dry. The length of the swimming bouts and the time between the swimming bouts varied, as did the body surface temperature of the otters before entering the water. Moreover, the intensity of the activity (walking, running, swimming more or less quickly, animal quite or excited), which influences metabolism and thermoregulation, varied. The way the otter behaved and moved after having left the water (stretching in different directions, shaking, rolling, scratching, grooming) also influenced heat loss. This variability in the experimental conditions, particularly the continuous switches between water and land, the different conditions of the fur (dry, more or less wet), and the fact that both, air and water temperature had to be considered, made the analysis of the thermograms and the presentation of the results difficult. Thus, we will summarise the observations made during this study, and present the heat dissipation and conservation mechanisms using some relevant examples.

5.3.1 Thermographic recordings in *Lutra lutra*

Thermograms could be made at ambient air temperatures (Ta) ranging from 2.8 to 29.4°C and water temperatures (Tw) ranging from 4.5 to 21.4°C. During most of the sessions, Ta was higher than Tw, except when Ta was below 5°C and also during three of the sessions where Ta was between 10 and 15°C. However, the difference did not exceed 3°C. At Ta below 10°C, the difference between Ta and Tw was moderate and did not exceed 1 or 2°C, whereas at higher Ta, the air could be more than 5°C warmer than the water. All the animals studied showed a similar thermoregulatory pattern.

5.3.1.1 General observations

Temperature of feet and legs

The feet appeared to be the most important thermoregulatory surface for the Eurasian otter. Indeed, the feet of all the animals studied were warmer than the rest of the body surface and warmer than Ta, each time they left the sleeping box or after a period of activity on land (Fig. 112). The temperature of the feet decreased while the otter was in the water, and the feet then appeared colder than the rest of the body on the thermograms (Fig. 113). When an otter just left the water, then the surface of the feet was usually at the same temperature as the water (Tw) or slightly above. Afterwards, an increase of Tfeet might depend on Ta, Tw and intensity of activity. At Ta above 25°C, the feet of a wet otter became warmer than Ttrunk and than Ta after a few minutes of activity on land, quite independently of the time spent in the water. At lower temperatures, the increase of Tfeet after a swimming bout was slower (except when the otter was particularly active and excited). Particularly at Ta below 15°C, Tfeet tended to stay constant after a swimming bout for at least 5 minutes, mostly longer. Increase of Tfeet above Ta could be recorded much less frequently, almost only with Naima (see 5.3.1.2&3 for examples of the fluctuations of Tfeet at different Ta and in different situations).

When Tfeet<Ttrunk, which was usually the case after a swimming bout, the temperature difference was relatively low. Indeed, when the otter just left the water, the difference between Tfeet and Ttrunk was usually of about ΔT=1-2°C. Then, during activity on land, the difference increased to ΔT=3-4°C (ΔT_{max}=7°C), when Tfeet remained constant, while Ttrunk increased (see next part). Of course, the difference decreased when the feet began to become warmer until Tfeet=Ttrunk and then Tfeet>Ttrunk. When Tfeet>Trunk, the temperature difference could be considerably more important, and Tfeet up to 20°C warmer than Ttrunk was recorded. Temperatures between 4 and 39°C were measured at the surface of the feet. When a dry otter left the sleeping box after a period of rest, it had “warm feet”, and even if Tfeet in some cases decreased, which depended on Ta and activity, Tfeet usually remained above Ttrunk and Ta, until the otter went into the water, at least at Ta>10°C. Only few data are

available for colder Ta. During one session in December at Ta=2.8°C, the temperature of Naima's feet decreased after she left the sleeping box. After 14 minutes of activity on land, Tfeet was still above Ta, but the tips of the fingers were less than 2°C above Ta. During two other sessions in January at Ta=4.2°C and Ta=7.1°C, the mean Tfeet was still above Ta after respectively 10 and 7 minutes of activity on land, but the tips of the fingers were equal to Ta. However, note that during the winter sessions the floor of the enclosure was mostly wet.

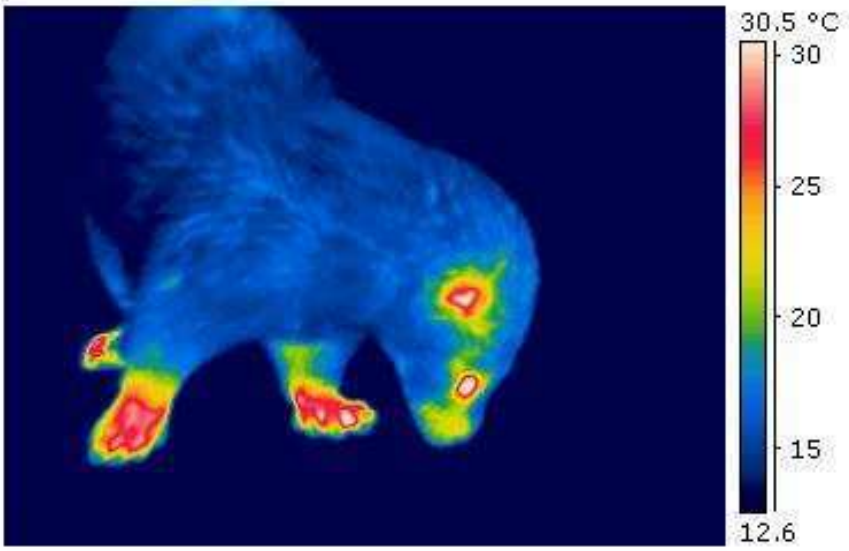


Fig. 112: Thermogram of Naima (Ta=12.5°C). The feet were twice as warm as the rest of the body (head excepted).
Ttrunk=14.5±1.1°C, Tfeet=28.7±1.5°C (n=5 randomly chosen measuring points).

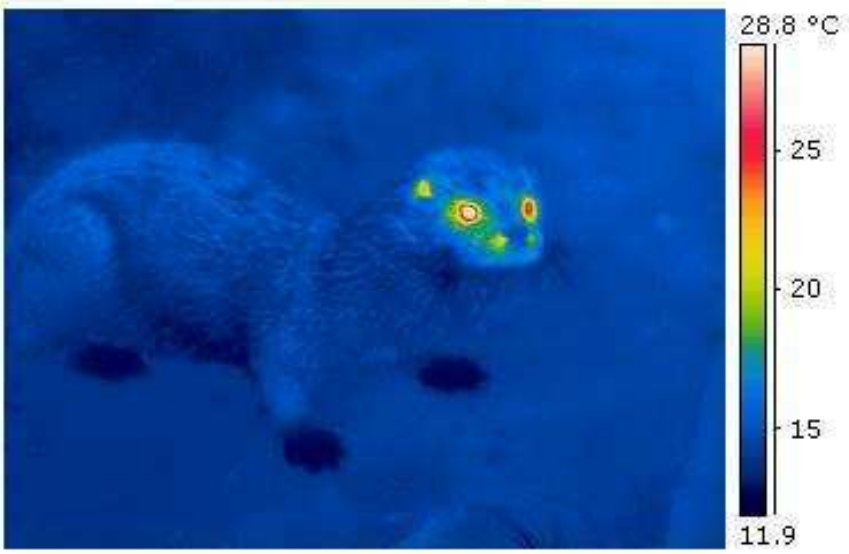


Fig. 113: Thermogram of Tomasz taken after a swimming bout (Ta=15.3°C, Tw=12.2°C). Both trunk and feet were beneath Ta. The feet were about 2°C colder than the rest of the body and had a surface temperature similar to Tw.
Ttrunk=14.4±0.3°C, Tfeet=12.5±0.1°C

The temperature at the surface of the feet was often heterogeneous; particularly the temperature of the interdigital webbings was mostly different from the temperature at the surface of the fingers. In a “cold feet” situation, both Tfingers>Tweb and Tfingers<Tweb (or Tfingers=Tweb) were observed, whereas in a “warm feet” situation, clearly Tweb>Tfingers (Fig. 114). In a cold feet situation, the greatest ΔT measured was Tweb about 3°C colder than Tfingers, whereas in a warm feet situation, the difference reached 10°C. However, Tweb could be measured on many warm feet but on only a few cold feet, because when Tfeet was above Ta, the otters tended to spread their feet more often, which made the recording of Tweb easier. The highest T measured at the surface of the webbings was 39.7°C.

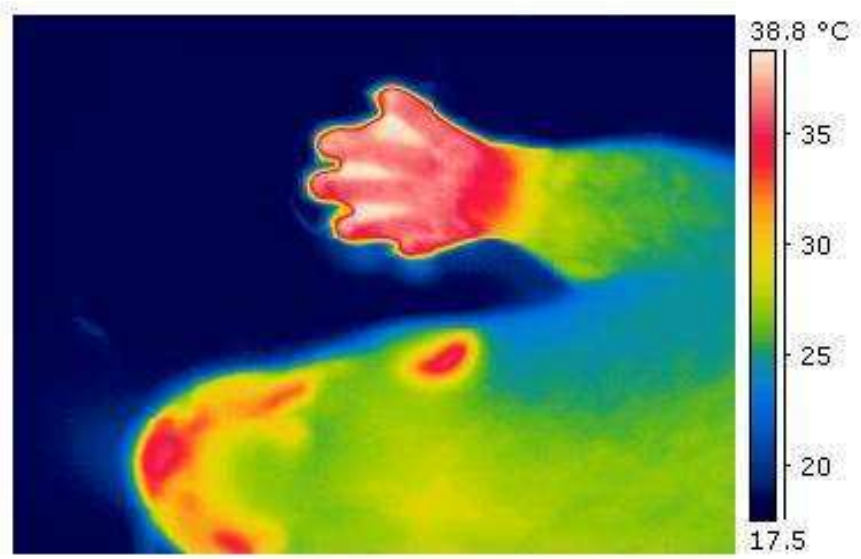


Fig. 114: Thermogram of Naima’s fore foot (Ta=20.3°C). The interdigital webbing was almost 3 degrees warmer than the fingers. Tfingers=36±0.6°C, Tweb=38.7±0.2°C

The T at the surface of the fore and hind feet was usually similar, except in a few cases. For example, when the otter scratched and dug heavily with the fore feet, these sometimes got warmer than the hind feet, and the hind feet sometimes got warmer when the otter stood on them and jumped heavily in order to catch something (in general food). A noticeable temperature difference between fore and hind feet was observed on only very few thermograms.
Most of the time, the legs showed the same surface temperature as the trunk. Heat loss from the legs increased when the otter stretched them, particularly when the fur was wet. Especially the inner part of the legs, which was often not visible on the thermograms, appeared to be warmer than the trunk, both under “warm feet” and “cold feet” conditions. For example, in Fig. 112, the inner part of the left leg was at 20.3±0.4°C, which was 5.8°C higher than the trunk but 8.5°C lower than the feet. An equivalent temperature difference between trunk and inner part of the legs was measured on 4 other thermograms of wet and dry otters taken at

different Ta. Smaller temperature differences were also recorded. The ankles had a surface temperature intermediate between that of the feet and of the leg-trunk, when the feet were warm (Fig. 115). On several thermograms taken when the otter had “cold feet” (at different Ta), the ankle appeared warmer than the feet and the leg-trunk (Fig. 116).

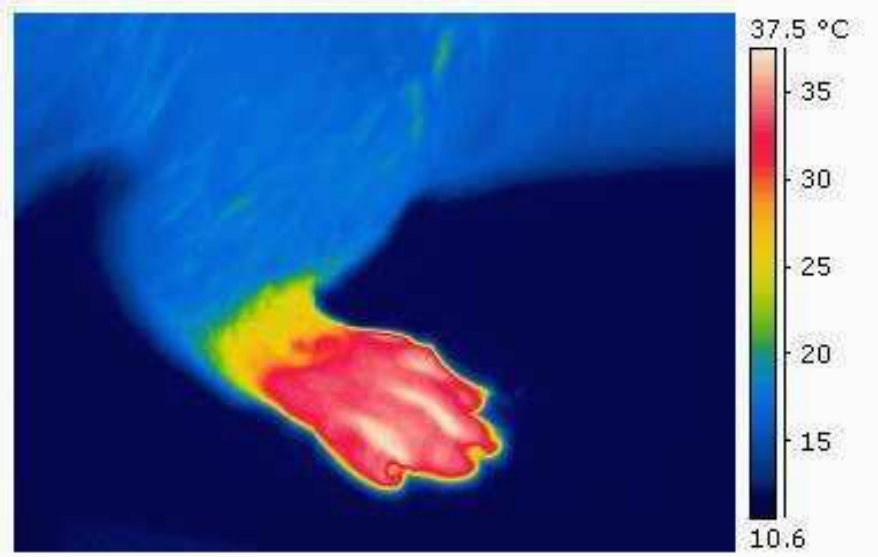


Fig. 115: Thermogram of Naima’s hind foot, taken 35 minutes after she had left the water (Ta=13.1°C). The ankle (yellow) was about 9°C colder than the foot and 8°C warmer than the leg and trunk (blue).
Tfeet=35±2.1°C, Tankle=25.8±0.5°C, Tleg-trunk=17.6±0.4°C

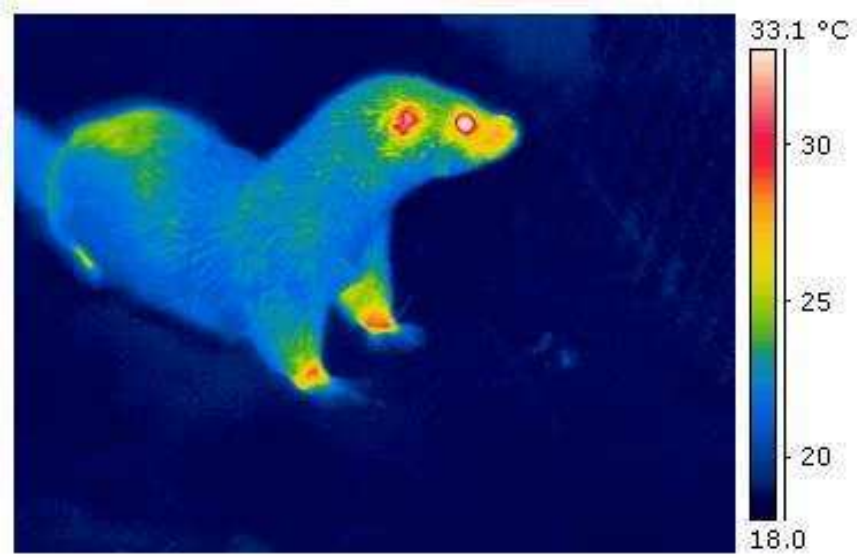


Fig. 116: Thermogram of Naima taken 2 minutes after she had left the water and quickly rolled in the grass (Ta=19.9°C, Tw=18.4°C). The ankles (yellow and red) were the hottest part of the body (head excepted).
Tfeet=21.1±0.8°C, Tankles=28.1±0.8°C, Tlegs=24.5±0.7°C, Tleg-trunk=22.3±0.5°C
Tlegs was measured at the inner part of the left leg and outer part of the right leg just above the ankle (green area), and Tleg-trunk was measured at the upper part of the right leg and trunk (blue area).

Temperature of the trunk

The trunk was not considered to be an important avenue for heat loss because its surface temperature was mostly about the water and/or air temperatures. When an otter left the water, Ttrunk was at Tw or slightly above, but usually below Ta (when Tw<Ta). Actually, when the difference between Tw and Ta was of several degrees, which was often the case, then Tw<Ttrunk<Ta during several minutes following the swimming bout. Then, Ttrunk increased up to Ta and eventually above, because the wet guard hairs began to clump together, which "opened" the fur (Fig. 117). This depended much on the way the otter moved; if the otter moved rather "unidirectionaly", then Ttrunk could remain below Ta for quite a long time, but torsions, movements in different directions, favoured openings of the hair coat and thus increases of Ttrunk. However, at Ta>10°C, mean Ttrunk usually did not get more than 5°C warmer than Ta. At Ta<10°C, the difference could reach 8°C (see 5.3.1.2&3 for examples of the fluctuations of Ttrunk at different Ta and in different situations). Note that at high Ta, mean Ttrunk more than 5°C warmer than Ta, could be due to heating of the fur by radiation from the sun, which could not be completely avoided outdoors, even when the sky was cloudy and the enclosure shadowy.

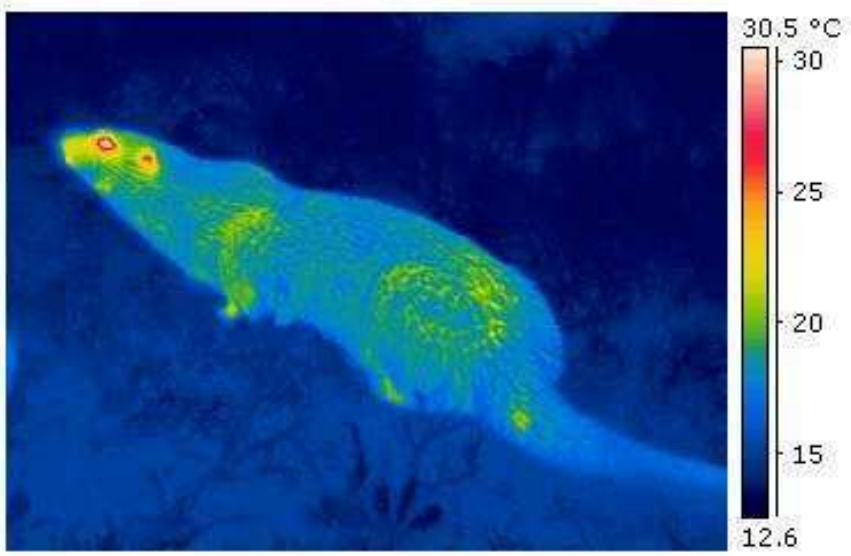


Fig. 117: Thermogram of Robert taken a few minutes after a swimming bout (Ta=15°C, Tw=13.1°C). The mean temperature of the trunk (blue area) was of 17.9±0.5°C, whereas on the areas where the wet hairs clumped together (green and yellow), the temperature reached 22.5°C (mean 20.7±0.7°C).

We could get only a limited number of recordings of a dry otter, because keeping an active otter dry for more than 30 minutes could be done only by locking it in a room without pool. At Ta>10°C, Ttrunk tended to stay quite constant during a period of activity and was equal to Ta or up to 5°C above Ta. Unfortunately, only few thermograms of a dry otter active on land could be made at Ta<10°C, and most of them were taken when the otter just came out of the

sleeping box. During one December session, the temperature of Naima's trunk was between 10 and 12°C immediately after she had left the sleeping box, and was about 7-9°C 10 minutes later (Ta=2.8°C). The temperature of Teufel's trunk was about 9-10°C at Ta=6.4°C.
On several occasions, it was possible to measure the temperature inside of the hair coat, for example when the otter had scratched itself and thus induced deeper openings of the coat, or when close-ups of the clumps of wet hairs could be made. Thermocouples would be necessary to measure the exact temperature of the otter skin, but IRT allowed us to conclude that the air layer within the otter fur had a temperature above 25°C, even after a swimming bout (Fig. 118). Indeed, temperatures between 25 and 30°C at areas just scratched or gaps between the hairs could be measured on many thermograms, even on a few of those taken in winter. On one thermogram taken a few seconds after a 9 minutes long swimming bout (Tw=13.9°C), temperatures up to 28°C could be measured on the back, between tufts of wet hairs.

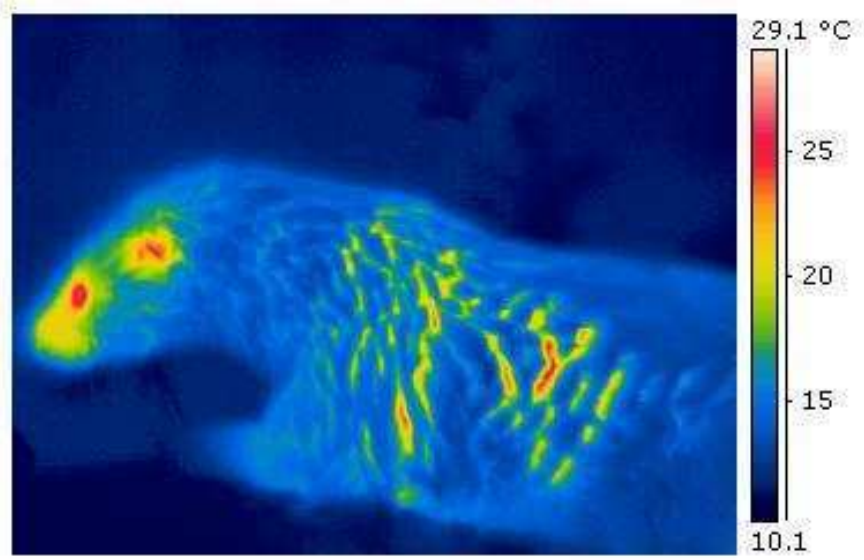


Fig. 118: Thermogram of Naima taken 7 minutes after exit from water (Ta=13.6°C, Tw=12.9°C). The temperature at the surface of the fur ranged from 12.5 to 14.5°C (blue area), whereas on gaps between tufts of wet hairs, the temperature reached 25.2°C (red areas).

Temperature of the head with emphasis on the peripalpebral region, vibrissal pads and ears

The ears, the peripalpebral region and the vibrissal pads appeared warm (above Ta) on every thermogram, even at low Ta and after a swimming bout (Figs. 119 & 120). The ears tended to be the warmest regions; their temperature reached 37°C (highest value measured), and they were clearly visible even on the thermograms taken when the otter swam at the water surface. The temperature of the mystical vibrissal pads reached 34°C. They tended to be slightly warmer than the peripalpebral region, including the base of the supraciliary and upper genal vibrissae, except for the rim of the eyes, which was as warm as the mystical pads. The temperature at the base of the lower genal vibrissae could be measured on only a

few thermograms, but was also above Ta (Tmax measured=34°C). During the coldest session (Ta=2.8°C), temperatures between 15 and 18°C were measured at the peripalpebral region and mystical vibrissal pads and up to 21°C at the ears, after a swimming bout (Tw=5.2°C). When the otter was dry, the mystical pads were between 20 and 25°C in all the winter sessions, and Tears up to 31°C could be measured.

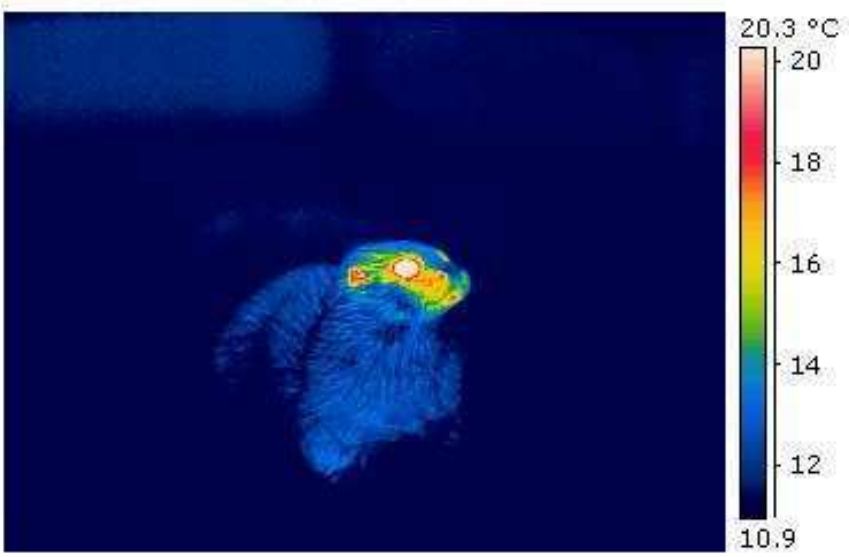


Fig. 119: Thermogram of Robert coming out of the water (Ta=13.3°C, Tw=11.3°C). The mean temperature at the surface of the trunk was of 12.1±0.2°C, whereas the mean temperature of the peripalpebral region and mystical vibrissal pads was of 17±0.7°C. The temperature of the ears ranged from 15.5°C to 19°C.

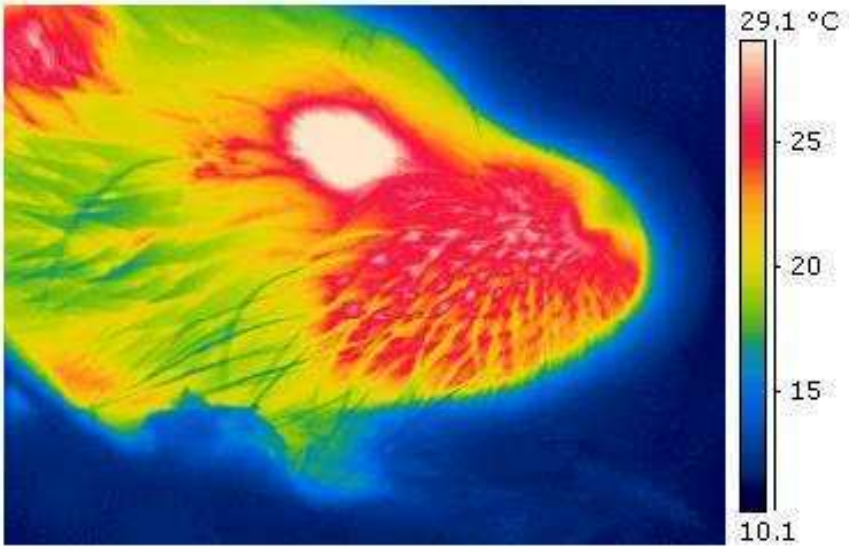


Fig. 120: Thermogram of Naima taken 23 minutes after she had left the water (Ta=13.6°C, Tw=12.9°C). The mean temperature of the mystical vibrissal pads was of 25.2±0.7°C (reached 28.2°C at the base of each vibrissae), whereas the mean temperature of the surrounding regions of the head was of 18.5±0.6°C. The mean temperature measured at the upper genal vibrissal pads was of 24.3±0.4°C.

The entire head, particularly the face and forehead appeared warmer than the trunk on thermograms of wet and dry otters taken at different Ta (Fig. 121). However, the difference between Tforehead and Ttrunk was moderate (generally less than 2°C in dry otters and less than 4°C in wet otters). Moreover, the forehead had a temperature similar to that of the trunk on many thermograms recorded in different conditions (otter dry or wet, different Ta). As for the trunk, heat loss from the forehead was influenced by the way the otter moved the head, particularly when the fur was wet.

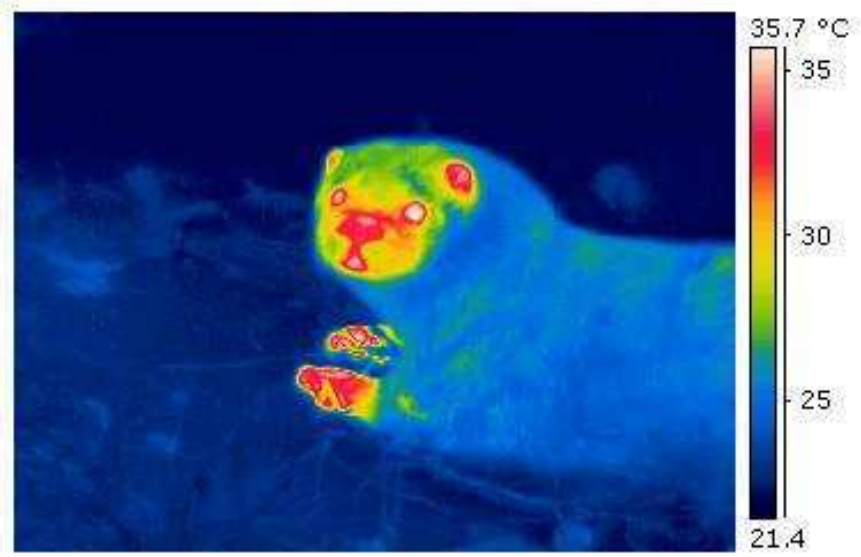


Fig. 121: Thermogram of Naima taken 24 minutes after she had left the water (Ta=21.2°C, Tw=19.9°C). The forehead was about 2°C warmer than the trunk.
Tforehead =27.3±0.2°C, Ttrunk =25.4±0.3°C

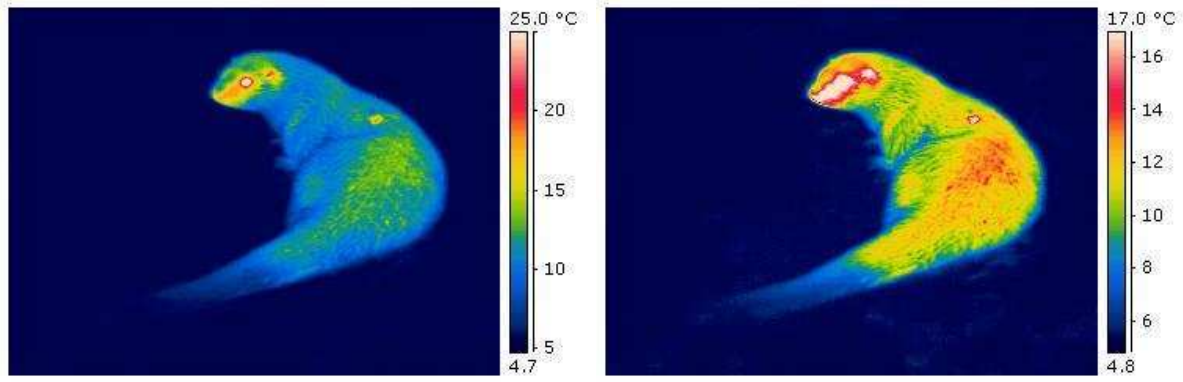
No particularity regarding the temperature of the hairless rhinarium was observed. Tnose was mostly above Ta except during the minutes following a swimming bout (in condition that Tw was several degrees colder than Ta). Tnose always increased very rapidly after a swimming bout, and for example, during a session at Ta=26.6°C, Tnose was about 30-31°C six minutes after the otter had left the water (Tw=19.2°C). Tnose<Ta was never recorded at Ta<10°C. The highest Tnose measured was 37.5°C. Tnose about 35-36°C was measured during the session at Ta=26.6°C, which was the highest Ta during a recording session (where Tnose could be measured). Thus, a brain cooling using the nose apparently did not occur, at least not at Ta below 27°C.

Temperature of the tail

The tail of wet otters was considerably colder than the trunk at low Ta (<10°C). When otters were dry, a continuous isothermal area was seen from the trunk to the tail, at least when Ta>10°C. This was also the case in wet otters during the spring-summer sessions, except on

three occasions in May. On one thermogram taken 10 minutes after Naima had left the water (Ta=13.6°C, Tw=12.9°C), the tail was about 2°C below Ta, whereas the back was 1°C above Ta. On another thermogram of Robert, taken shortly after a swimming bout (Ta=14.7°C, Tw=11.2°C), Ttail was similar to Ta, whereas Ttrunk was about 3°C above Ta. Ttail lower than Ttrunk was never recorded at Ta>15°C. On two thermograms taken at respectively Ta=19.9°C and Ta=29.4°C, small openings of the wet fur of the tail were at 28-31°C, about 4°C higher than the surface of the fur, which indicated that a warm insulating air layer was present under the surface of the fur, even at the tail.

The tail appeared colder than the trunk on every thermograms representing a wet otter taken during the winter sessions (Ta between 2.8 and 8.1°C), except in a few particular cases (very high activity or only short swimming bout after a time in the sleeping box). Differences up to 7°C between Ttrunk and Ttail were recorded. Actually, not the complete tail appeared colder but only the distal part (about 1/3 to 2/3 of the tail length), whereas the proximal part was at the same temperature as the back (Fig. 122). Within the "cold part", the temperature decreased toward the tip. Apparently, the temperature of the tail decreased down to Tw during a swimming bout, and when the otter came out of the water, Ttail stayed constant at a temperature similar to that of the water. Ttail was usually below Ta after a swimming bout (again with a few exceptions), whereas the temperature at the surface of the trunk was always above Ta at Ta<10°C. An increase of Ttail after a swimming bout was not recorded until the otter went back into the sleeping box. During one session in December (Ta=6.4°C, Tw=5.2°C), Olli left the water and jumped heavily on his hind feet to catch some food. This caused an increase of Tfeet up to 2-3°C above Ta, whereas Ttail remained similar to Tw.



A B

Fig. 122: Thermogram of Naima taken 2 minutes after she had left the water (Ta=7.3°C, Tw=5.7°C), represented using two different temperature scales. The temperature decreased toward the tip of the tail, which became "invisible". The temperature difference between the distal part of the tail and the back, including the proximal part of the tail, is better seen on B, where the yellow area is about 5°C warmer than the blue area.
Tback-proximal tail=11.6±0.8°C, Tdistal tail=6.4±1.2°C

5.3.1.2 Recording sessions with Naima

Thermographic pictures of Naima were taken during 26 sessions in spring (May), summer (June), and winter (December, January). Each session lasted 1 to 2, exceptionally 3 hours. On several occasions in spring, she spent 30 to 40 minutes in the indoor part of the enclosure, usually not accessible for otters (service room), which was the only way to get her active on land for such a long time. Examples of temperature fluctuations at the surface of the trunk and the feet are given in the graphs of Fig. 123 and illustrate observations that have already been reported. The temperature of the tail is also represented on two graphs (winter sessions). Ttrunk was relatively constant, while Tfeet showed rapid and important fluctuations. In winter Tfeet and Ttrunk were closer to each other, except when the otter rested in the sleeping box. In the first four graphs (spring sessions), Naima was indoors before (Fig. 123A, B) or after (C, D) a swimming bout. During those periods indoors, she was always active, walking around, exploring everything of the unknown room, being sometimes really excited. In the graph 123D, the high Tfeet only 7 minutes after the swimming bout, can be explained by a high level of activity and excitement. Indeed, Naima went into her sleeping box, and then scratched very heavily the wall of the wood funnel relying her sleeping box to the outside, as if she had tried to dig a hole in the wood plate. Then she was allowed to go inside.

Whereas for Ttrunk, the standard deviations tended to be almost negligible, they were more important for Tfeet, particularly when Tfeet increased or decreased. This was because the temperature at the surface of the different parts of the foot did not vary at the same rate (the chosen measuring points were equally distributed all over the foot surface). When Tfeet increased, the metatarsal/metacarpal region was the first part of the foot where the surface temperature increased. Then the interdigital webbing got warmer. While the webbing became the hottest area of the foot, the temperature of the fingers increased up to a temperature similar to that of the metacarpal/metatarsal region (Fig. 124). Then the temperature at the surface of the foot became more homogeneous. The webbing remained the hottest region of the foot but the temperature difference between webbing and fingers decreased (see Fig. 114 taken after 5:10 hours on land; the webbing was only 3°C warmer than the fingers). On some thermograms taken at an advanced “warm feet” stage, the fingers appeared slightly warmer than the metacarpal/metatarsal region (Fig. 115).

Fig. 123: Evolution of Tfeet and Ttrunk (Ttail is also included in two graphs) in different situations and at different Ta and Tw (spring: A-D, summer: E-G, winter: H-K). Naima sometimes came on land for a very short time during a swimming bout (1-3 minutes). Those short "breaks" on land were not indicated, but the results of some of the recordings, which could be made then, were represented. The error bars indicate the standard deviations.

Spring (Fig. 123: A-D)

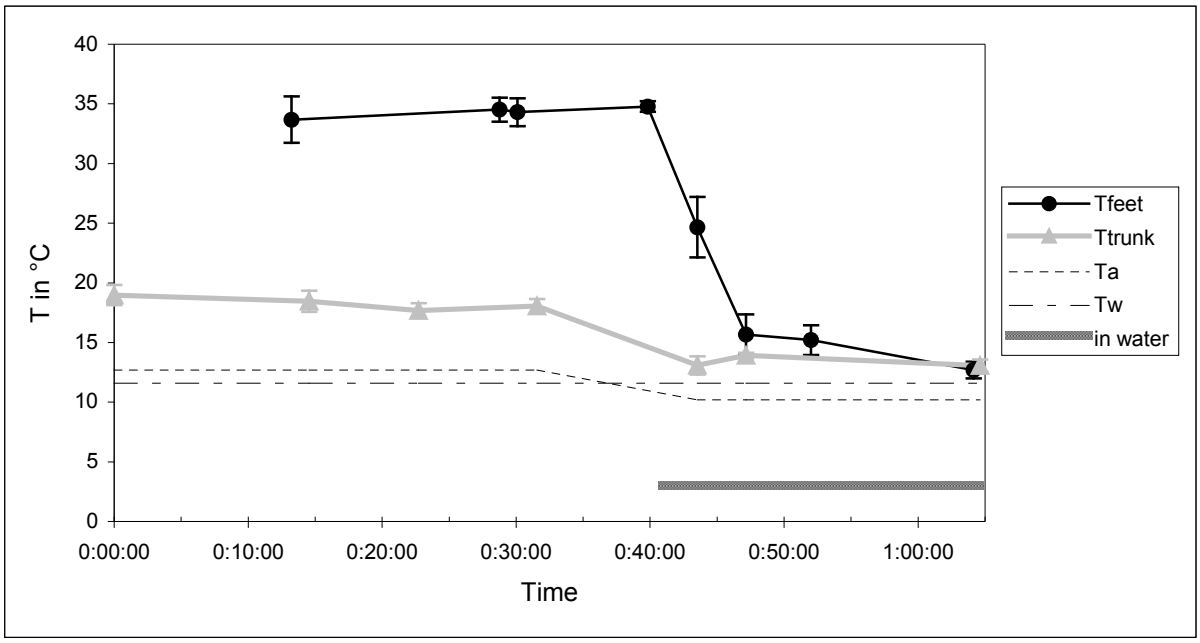


Fig. 123 A. At 0:00:00, Naima just came out of the sleeping box and was dry. She spent 40 minutes locked in the indoor part of the enclosure. Then when the door was opened, she went into the water, where she stayed until the end of the session. The change in Ta was because the temperature outdoors slightly differed from the temperature indoors.

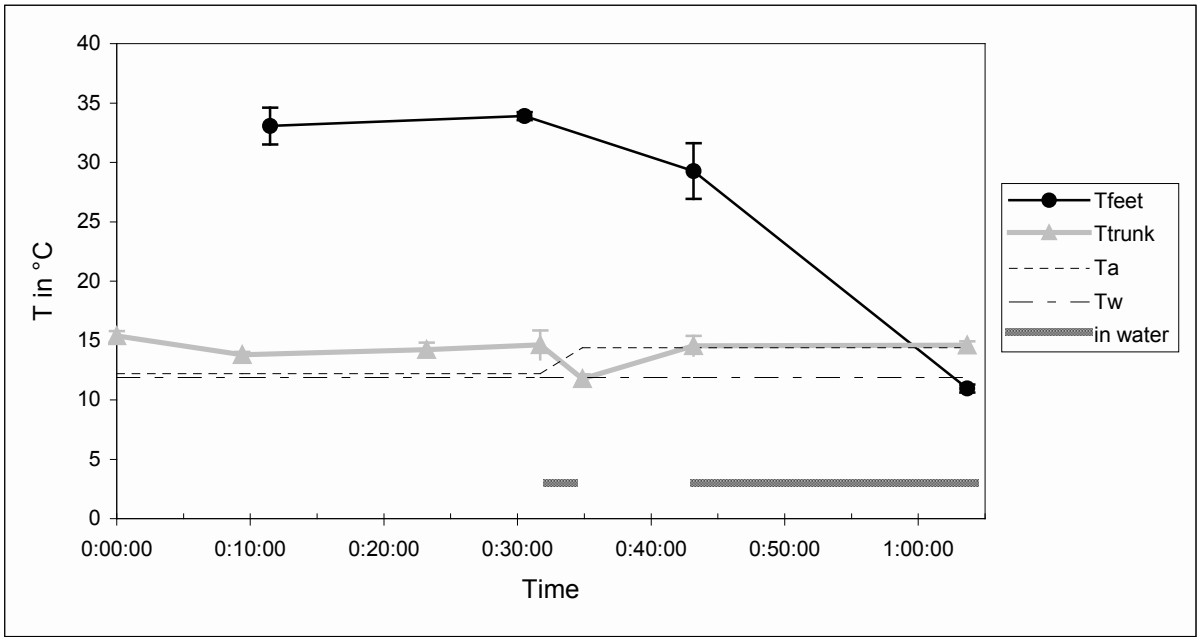


Fig. 123 B. At 0:00:00, Naima came out of the sleeping box and was dry. She spent 33 minutes locked in the indoor room, and then went into the water as soon as the door was opened.

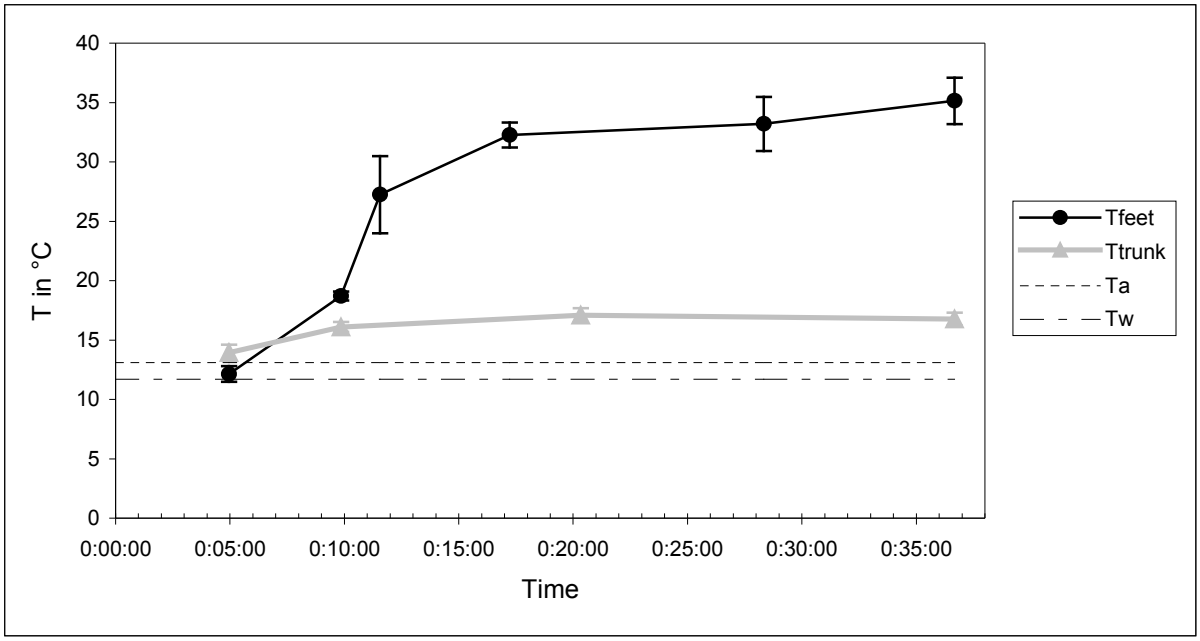


Fig. 123 C. At 0:00:00, Naima just came out of the water, where she spent 15 minutes in total during the last 30 minutes, always switching between a few minutes on land and a few minutes in water. At 0:05:00, Naima went indoors, where she stayed for 36 minutes. She was dry at the end.

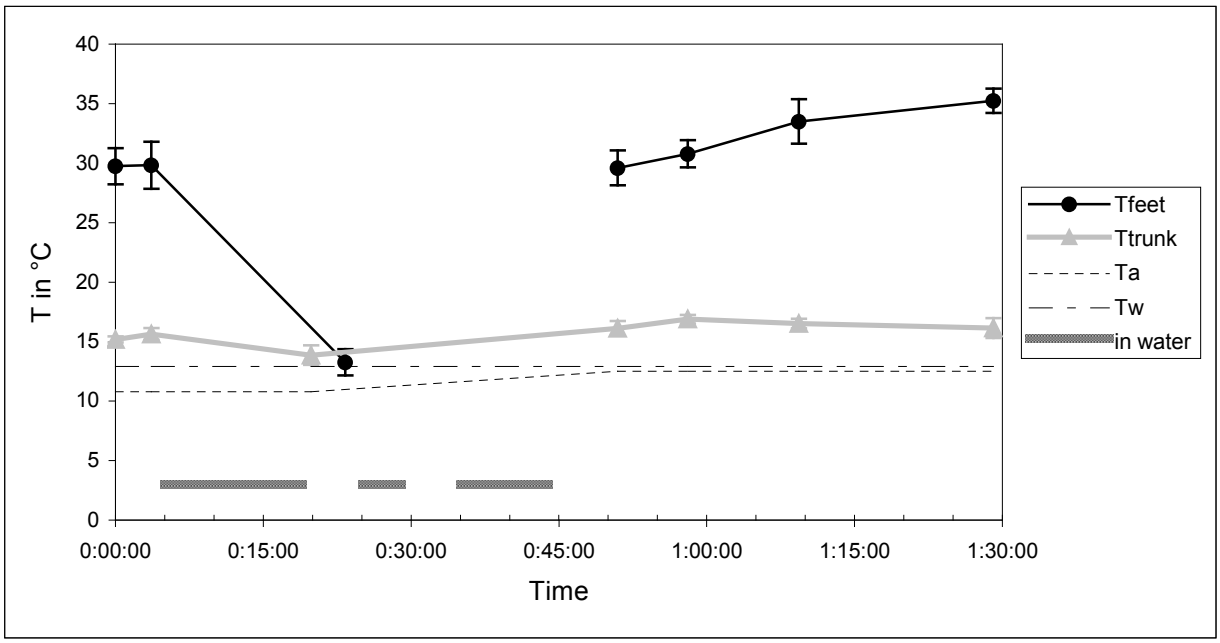


Fig. 123 D. At 0:00:00, Naima came out of the sleeping box and was dry. She then spent about 40 minutes mostly in the water. Two minutes after she came out of water, she went into her sleeping box, where she scratched very heavily against a wood plate situated at the entrance during 4 minutes. Then she went into the indoor room where she stayed 40 minutes. She was dry at the end. The interruption in the Tfeet line is because no recordings could be made during those swimming bouts or shortly after.

Summer (Fig. 123: E-G)

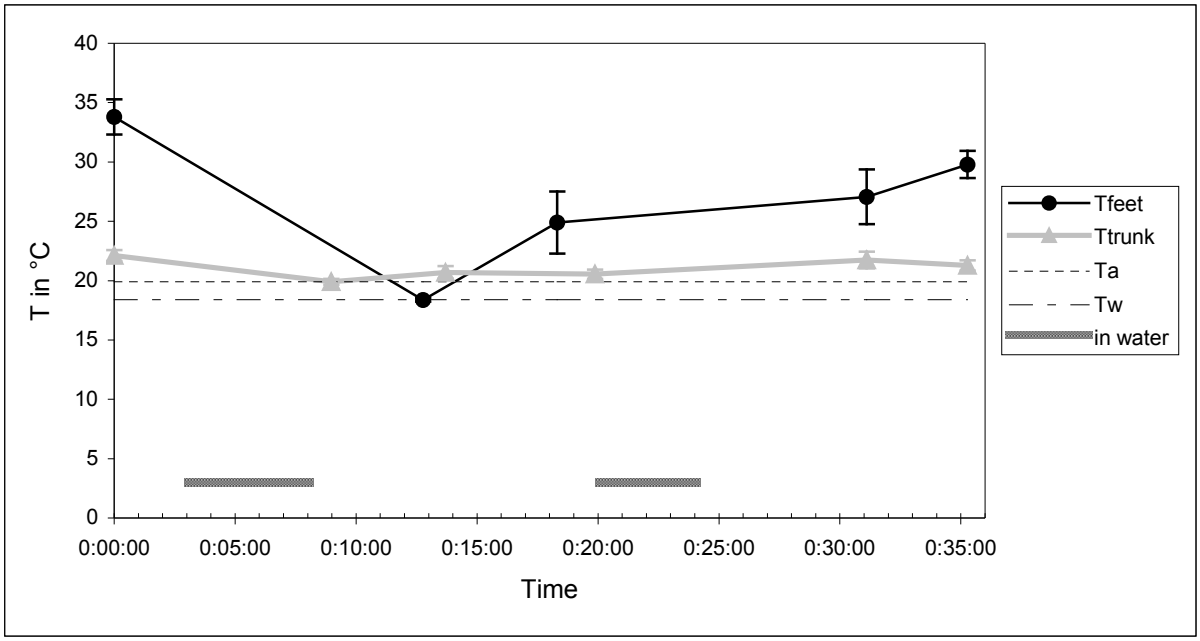


Fig. 123 E. Naima was active on land and slightly wet at 0:00:00

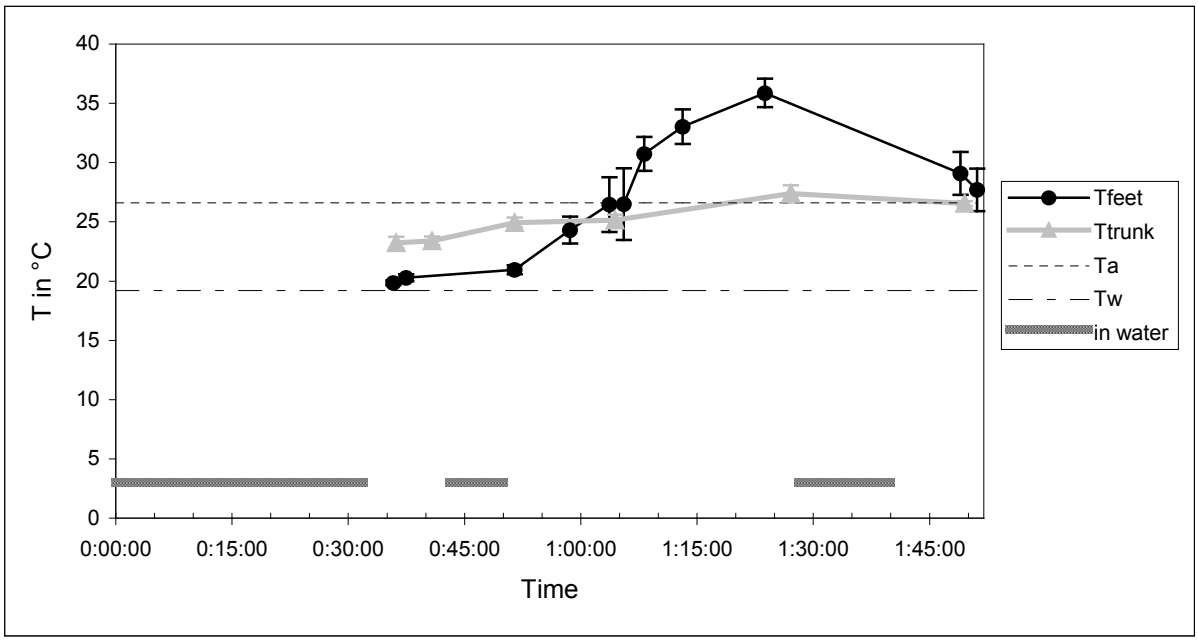


Fig. 123 F. At 0:00:00, Naima had just entered the water. Before the swimming bout, she was 9 minutes on land. She was dry at the end of the second period of activity (00:50 – 01:28).

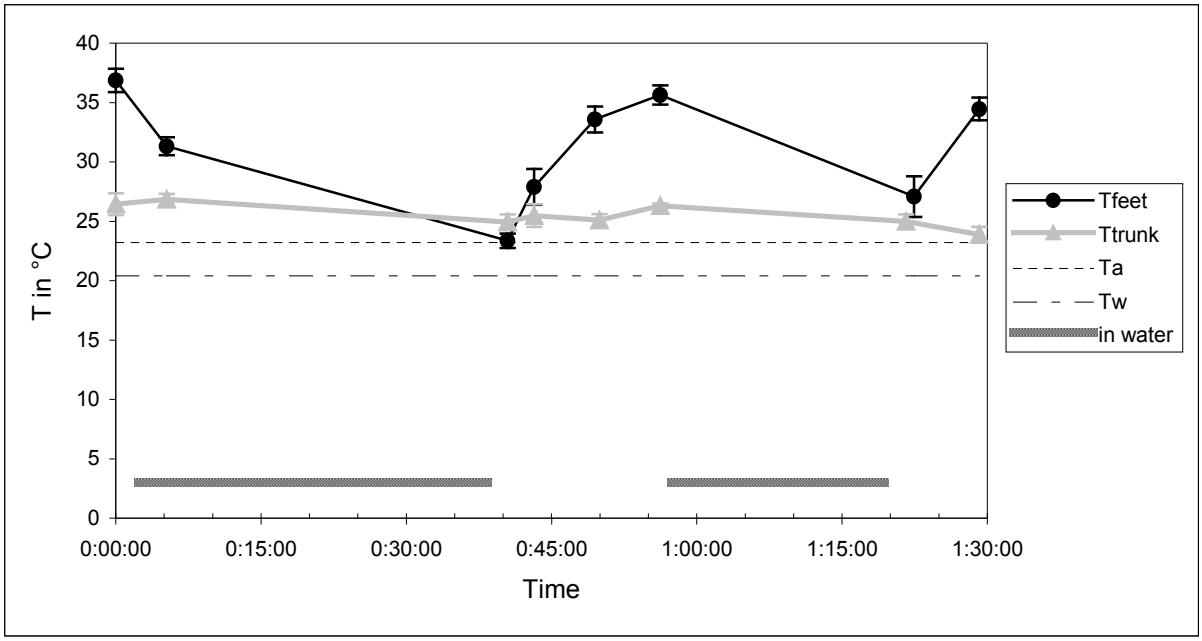


Fig. 123 G. Naima just came out of the sleeping box and was dry at 0:00:00. She groomed her fur after the swimming bout, and was dry at the end of the period of activity (00:38 – 00:57). She was almost dry at the end of the second period of activity.

Winter (Fig. 123: H-K)

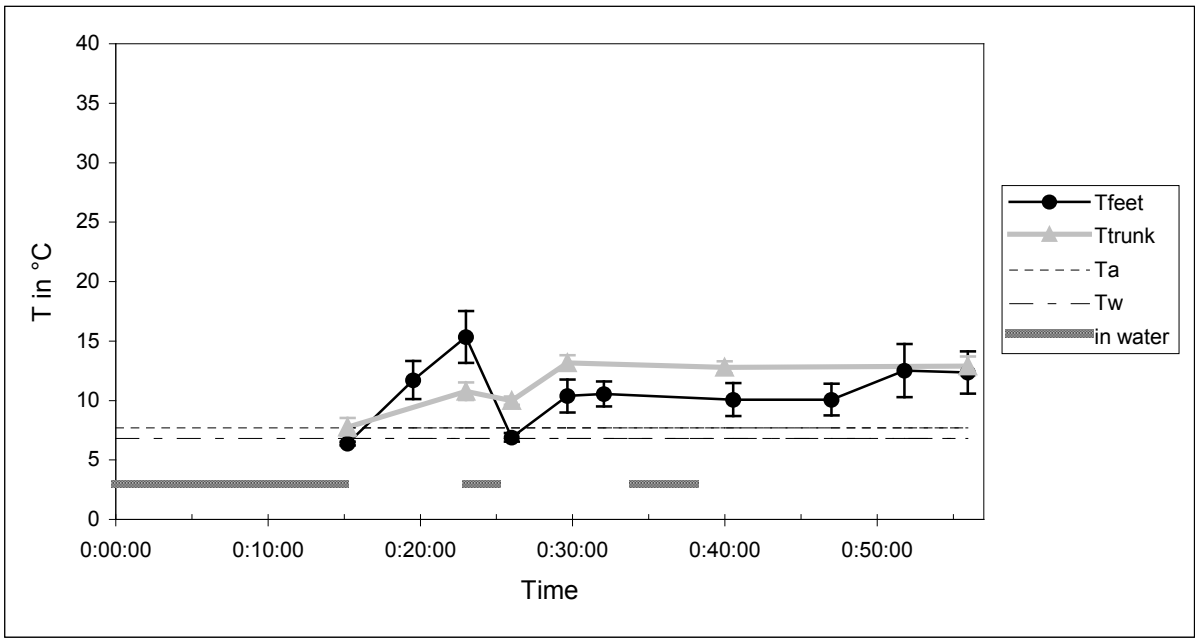


Fig. 123 H. Naima was active on land, and just had entered the water at 0:00:00

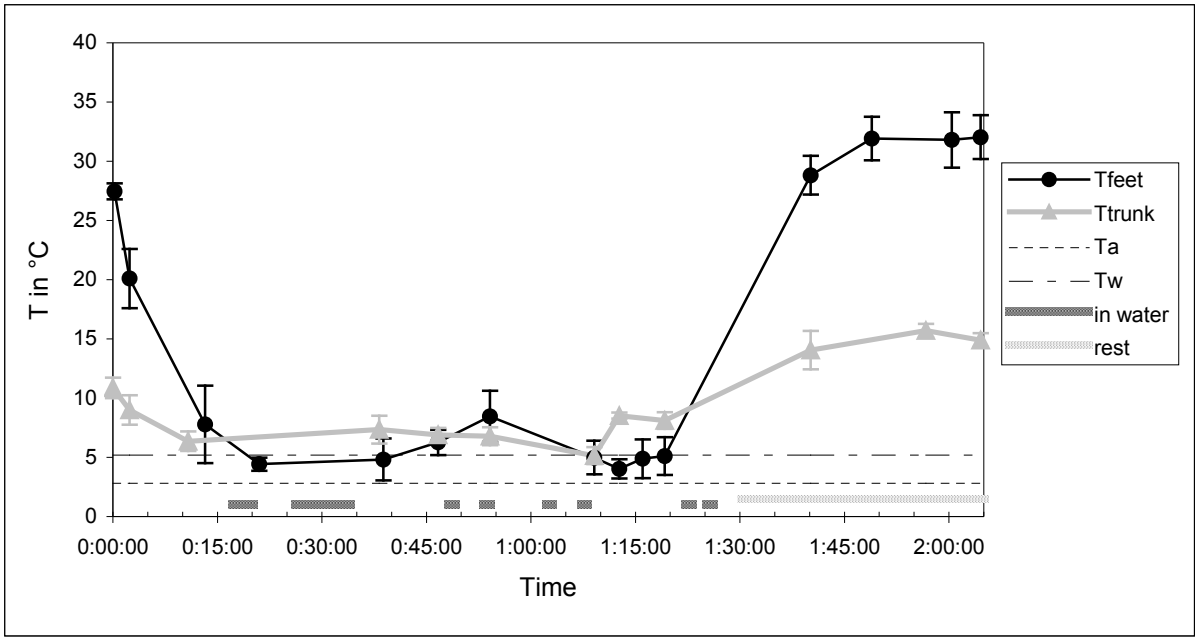


Fig. 123 I. At 0:00:00, Naima came out of the sleeping box and was dry. She often went into the water for very short periods (max 8 minutes, several times less than 1 minute). Then she went back to the sleeping box, groomed her fur and slept. The temperature of the empty box was about 5°C, but might increase when the otter was in the box.

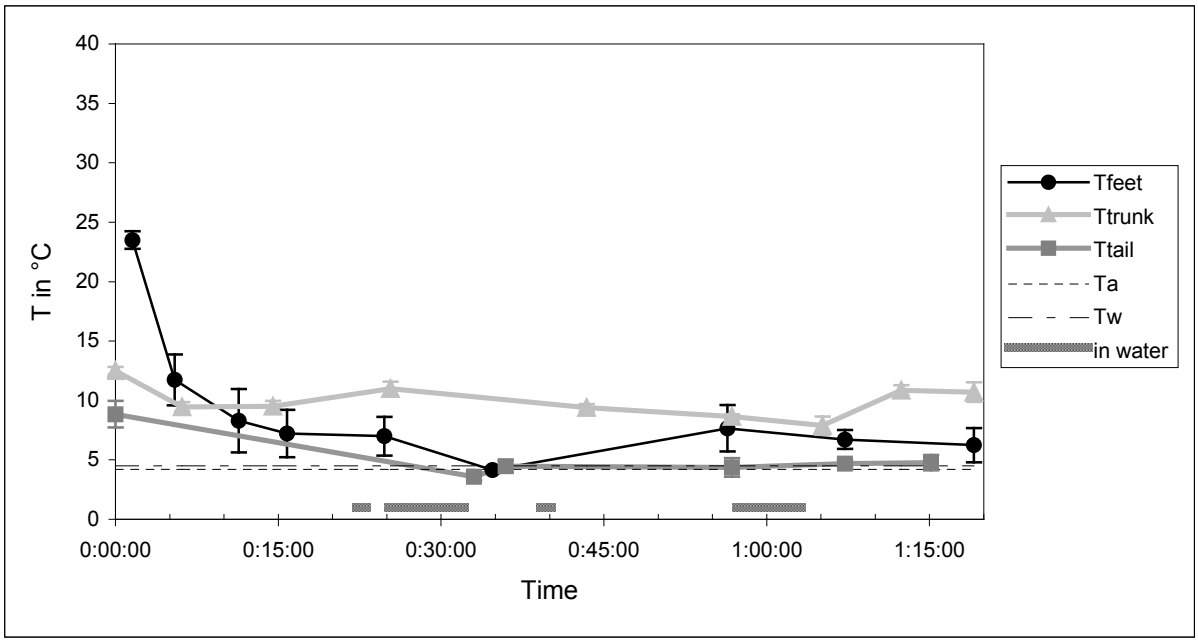


Fig. 123 J. Naima came out of the sleeping box and was dry at 0:00:00. The decrease of Ttail, which could be well documented during this session, is also represented on the graph. This decrease occurred only in the distal 1/3 of the tail

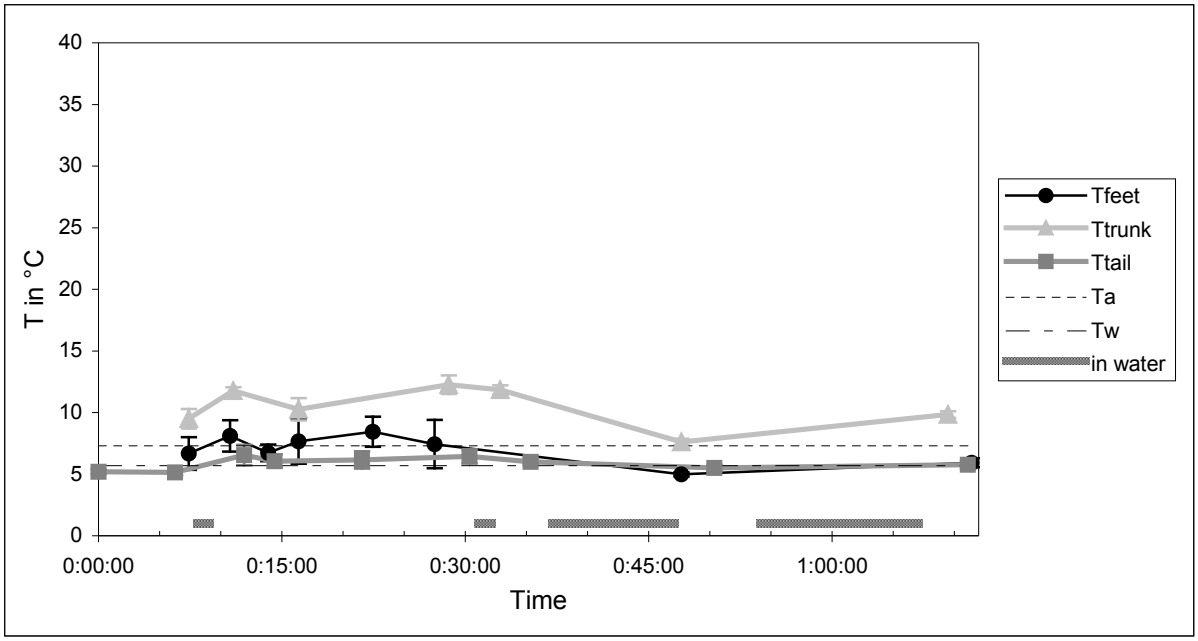


Fig. 123 K. At 0:00:00, Naima came out of the water. A Ttail lower than the mean temperature of the rest of the body was measured here in the distal 2/3 of the tail.

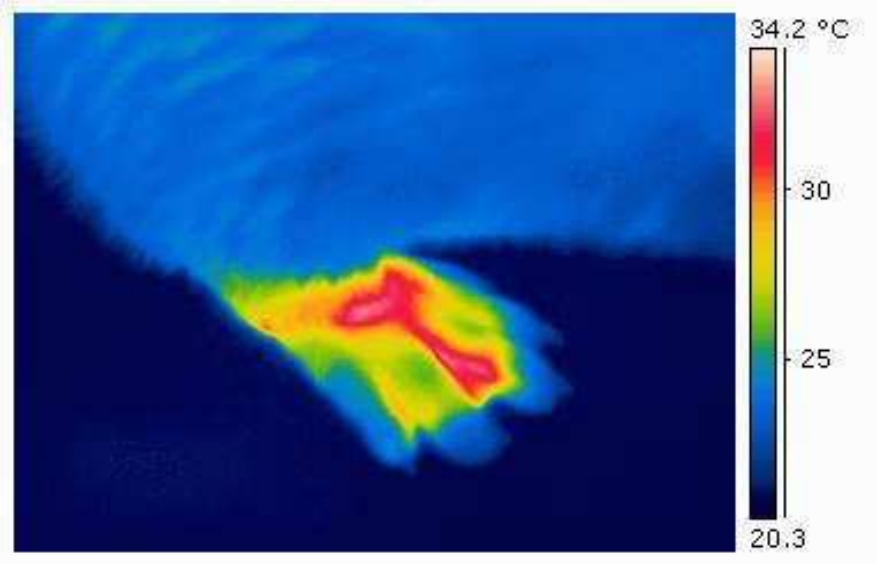


Fig. 124: Thermogram of Naima's hind foot, taken 3 minutes after she had left the water (Ta=21.2°C, Tw=19.9°C).

Measuring session without access to the water for 5.5 hours

During one measuring session, Naima was kept in an indoor enclosure (Ta=20.2°C) without pool for 5.5 hours. Only water for drinking was available. Naima was not fed prior to or during the session. She was wet, when she was captured, and the transport to the indoor enclosure lasted about 15 minutes. She did not groom her fur during the first two hours of the session, then she was left alone for one hour to allow quiet grooms and to rest. One hour later, the fur was dry and groomed.

During the first two hours, Naima was always active, walked around, scratched at the door, rolled on the floor, but did not show any sign of stress, just excitement and curiosity. Tfeet measured at the beginning of the stay in the indoor enclosure was 34.3±1.1°C, and actually remained about 34-35°C during the first two hours. The same was observed for Ttrunk, which remained constant around 23-24°C during the first part of the session.

After the break, Tfeet had risen to about 37°C and Ttrunk to 25-26°C. The temperature of both feet and trunk remained constant during the rest of the session. Naima was quiet during all the second part of the session. She sometimes scratched at the door, but spent most of the time lying on the floor. She always lay in a sprawling position, on her belly but also sometimes on her back (Fig. 125). She very often stretched her body to increase surface contact with the floor, the feet away from the trunk, sole against the cool floor and fingers spread. Thus, the interdigital webbings were good to see on almost every thermogram, and temperatures up to 39.7°C could be measured there (see also Fig. 114, taken after 5:10 hours in the indoor enclosure). Naima sometimes spread her fingers to the maximum, so that the complete webbings could be seen, but only for 2 or 3 seconds. Occasionally, she turned her feet, so that the plantar side was upwards and the dorsal side against the floor (Fig. 125C). The temperature of the hairless sole could be measured on several occasions and appeared to be similar to that of the webbings. Naima did not show any sign of stress, and did not seem to suffer from the absence of “bathing facilities”. She was never asleep during the recordings, except maybe once, but not for more than 5 min.

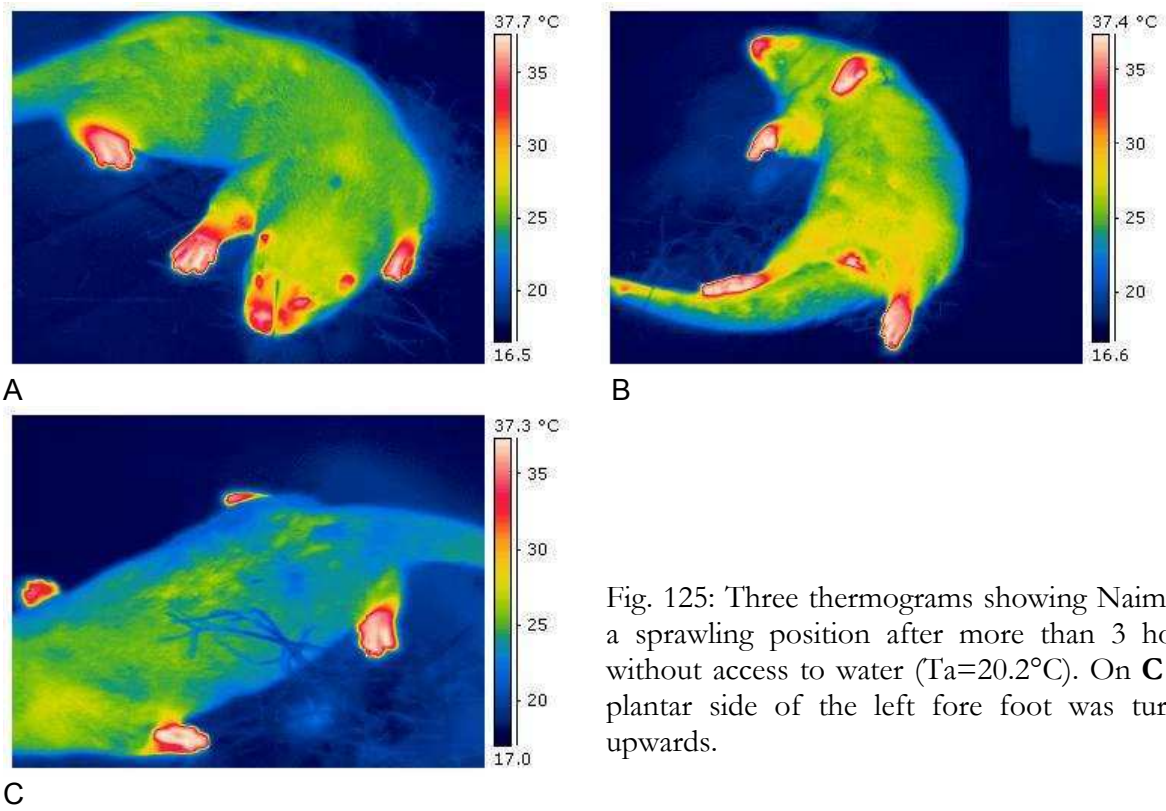


Fig. 125: Three thermograms showing Naima in a sprawling position after more than 3 hours without access to water (Ta=20.2°C). On **C** the plantar side of the left fore foot was turned upwards.

5.3.1.3 Recording sessions with Teufel

10th December (Ta=6.4°C Tw=5.0°C)

Teufel entered the water with Ttrunk=9-10°C and Tfeet=19-20°C. After 2 minutes in the water, Ttrunk was similar to Tw and the feet were still at 17-18°C. After 4 minutes of intense activity on land (running around, jumping, playing with the keeper, attacking pants and shoes), Ttrunk was around Ta (6-8°C) and Tfeet=23-26°C. One and a half minute later, Ttrunk had not changed, whereas some parts of the feet had reached 28.7°C.

5th January (Ta=6.4°C Tw=5.9°C)

Tfeet was at 11-13°C after a quick swimming bout (few seconds). After 8 minutes of intense activity on land, Tfeet=17-19°C. T of the interdigital webbings even reached 27°C (was at 14°C just after the swimming bout).

On several occasions, Teufel spent a few seconds in the pool and left the water with Tfeet between 13 and 19°C, despite of Tw=5.9°C. Then Tfeet increased rapidly during activity on land, whereas Ttrunk remained quite constant. First after a 15 minutes long swimming bout, the feet became noticeably colder with Tfeet=Tw. Then, 3 minutes after having left the water, Teufel went into the sleeping box where he groomed himself. He came out 9 minutes later with an almost dry fur, Ttrunk=Ta and Tfeet=12-14°C (fingers) and 16-18°C (webs). The mean Tfeet was of 15.1±2.5°C, which was 9.3°C warmer than the mean Tfeet of 5.9±0.2°C measured before Teufel went into the sleeping box.

The thermograms of Teufel showed how high Tfeet could be, and how fast it could increase, despite of a low Ta (6-7°C) and Tw (5-6°C), and despite of the otter being wet. This must be related to the very intense activity of Teufel, who was the most “excited” of all the animals studied. Fast increases of Tfeet were also observed in the other animals studied but at higher Ta. For example, while he jumped around like a kangaroo to catch the food bowl holt by the keeper, the T of Olli’s feet increased by 2.7°C in 2.5 min at Ta=12.8°C and Tw=11.8°C. On a hotter day (Ta=21.7°C and Tw=19.2°C), the T of Kuno’s fore feet increased by 4.1°C in 1 min 20 s, also during a feeding session. In both cases, the animals were in the pool a few seconds or minutes before and were wet (see also results for Naima).

The observations made with Teufel also showed that the temperature of the water contained in the hairs had no consequent influence on the temperature measured at the surface of the feet (the hairs of the feet are only a few mm long). Indeed, Tfeet higher than 15°C were measured despite of the otter just became wet in water being as cold as 5-6°C. Thus, we can conclude that when an otter left the water with a Tfeet similar or close to Tw, it was not because of the water soaked in the fur, but really reflected the temperature of the skin of the feet. Tfeet several °C higher than Tw, despite of the fact that the otter had just left the water,

were measured on a few thermograms of some of the other animals studied, taken immediately after short swimming bouts (mostly only a few seconds).

5.3.1.4 Abnormal thermograms

During the study, we had the occasion to document a clinical case. Each of the thermograms of Lukas showed abnormal areas of heat loss on the posterior part of the back, which could be seen even when the otter was swimming at the water surface (Fig. 126). Those areas were not large, but the temperature difference between them and the body trunk was important: the mean temperature of the abnormal areas was of 26.3±1°C (max 29°C), whereas mean Ttrunk=16.9±3°C, which represents a difference of ΔT=9.5°C (mean values measured on 4 thermograms, n=5 measuring points/thermogram). Lukas died a few days after the last measuring session. The exact cause of death was not determined, but Lukas suffered from liver infection, osteoarthritis and paralysis of the hind feet. The thermograms of Robert, taken four days before his death, did not show any abnormality. Lukas and Robert were both 9 years old, which is not a high age for captive otters.

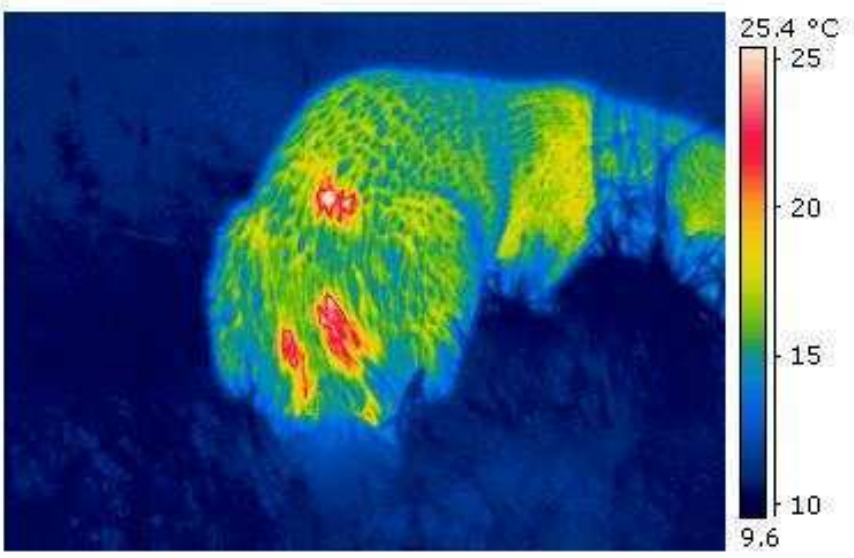


Fig. 126: Thermogram of Lukas taken five days before his death. The hot places (red and white areas) were not due to rubbing or scratching, but were observed on every picture, even during swimming bouts. Here, the mean temperature of those abnormal areas was of 25.3±1.1°C, whereas Ttrunk=15.6±0.9°C.

5.3.2 Thermographic recordings in *Pteronura brasiliensis*

Thermographic pictures of the two Giant otters were recorded during 3 sessions. Since it was not possible to distinguish between the individuals, the results for both otters were combined. During the first two sessions, the otters were in an indoor enclosure with pool (Ta=22.4°C, Tw=19.1°C). The otters went into the pool every couple of minutes or even seconds, and never stayed more than 5 minutes on land (usually less than 3 minutes). Despite of this,

Ttrunk around 24-25°C was recorded on the majority of the thermograms. Actually, when the otters just came out of water, Ttrunk was around 20-21°C, and then increased to temperatures up to 24-25°C in less than a minute (Fig. 127, Fig. 128).

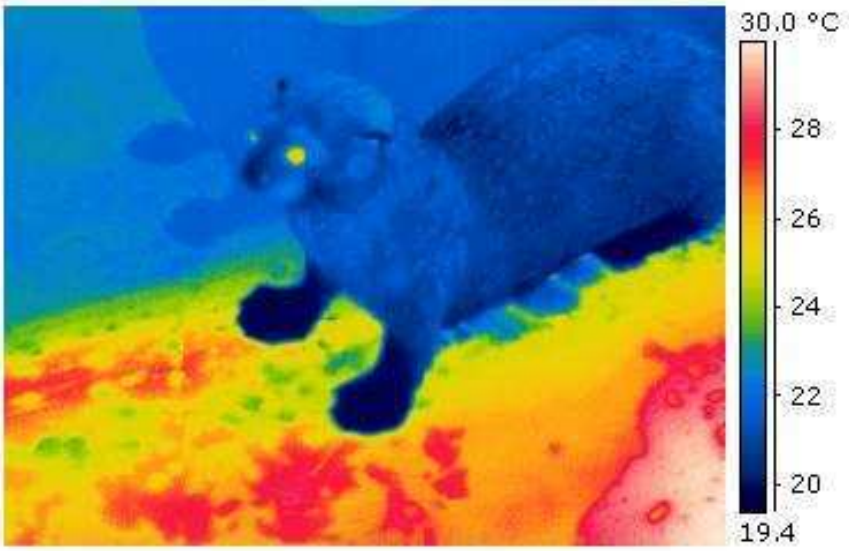


Fig. 127: Thermogram of a Giant otter, who just came out of the water (Ta=22.4°C, Tw=19.1°C). Ttrunk=20.8±0.2°C, Tfeet=19.7±0.2°C.

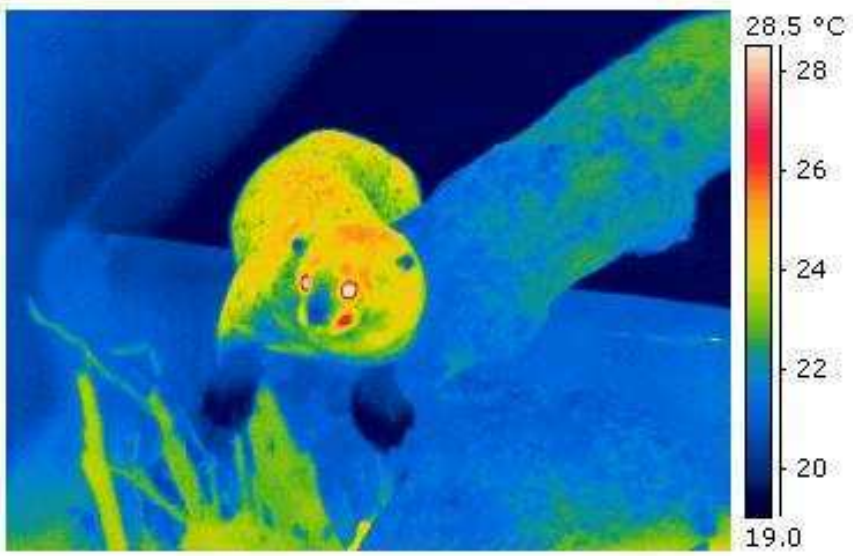


Fig. 128: Thermogram taken 30s after the Giant otter came out of water (Ta=22.4°C, Tw=19.1°C). Ttrunk=24.5±0.3°C, Tfeet=19.7±0.2°C, Tears=20.7±0.3°C
Tmystical pads=26.4±0.2°C, Tperipalpebral=24.2±0.9°C

Tfeet was always slightly beneath Ttrunk at the end of a swimming bout (Tfeet=19.5±0.2°C, Ttrunk=20.5±0.5°C, values measured on 4 thermograms taken within the 30 seconds following exit from water, n=5 measuring points/thermogram). Then, while Ttrunk increased very rapidly, Tfeet remained quite constant during the following minutes. The same was observed with the temperature of the tail (Fig. 129). The mean temperature measured at the surface of

the feet and the tail during the 5 minutes following the swimming bout was Tfeet=20±0.6°C and Ttail=20±0.5°C (mean values calculated using 5 randomly chosen thermograms). Once, the otters fed on land during 5 minutes, which permitted a continuous recording of the surface temperature during this period (Fig. 130). Ttrunk increased above Ta within the 20 s following exit from water, whereas Tfeet and Ttail increased much slower, and reached Ta toward the end of the 5 minutes period.

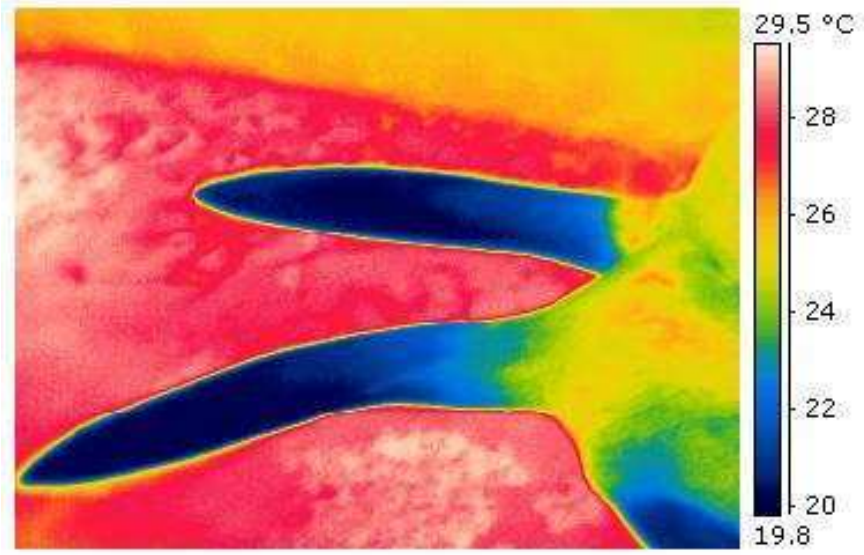


Fig. 129: Thermogram representing the tail of two Giant otters, taken less than 5 minutes after a swimming bout.
Upper otter: Ttail=20.3±0.4°C
Lower otter: Ttail=20.3±0.3°C, Tback=24.9±0.6°C
Ttail was measured on the distal 2/3 of the tail (dark blue area). The transitory part (light blue area) had a mean T of 22.6±0.2°C.

Actually, the feet and the tail were always the coldest parts of the body. An increase of Tfeet and Ttail above Ttrunk was observed only during the third session, where the otters were kept in a cage without water for 1 hour (Fig. 131). The otters sometimes scratched at the door relying the cage to the enclosure with the pool, but they remained relatively calm during most of the session. The otters just came out of water at the beginning of the session. As usually, Ttrunk increased very rapidly and was above Ta (24.5°C) at the end of the first minute following exit from water. Afterwards, Ttrunk increased slowly during the first 30 minutes and then remained quite constant during the last 30 minutes. Tfeet and Ttail increased quite slowly and continuously during the first 30 minutes, until they reached Ta. After 40 minutes, Tfeet and Ttail increased more rapidly and after 50 minutes, Tfeet and Ttail were between 1 and 3°C above Ttrunk.

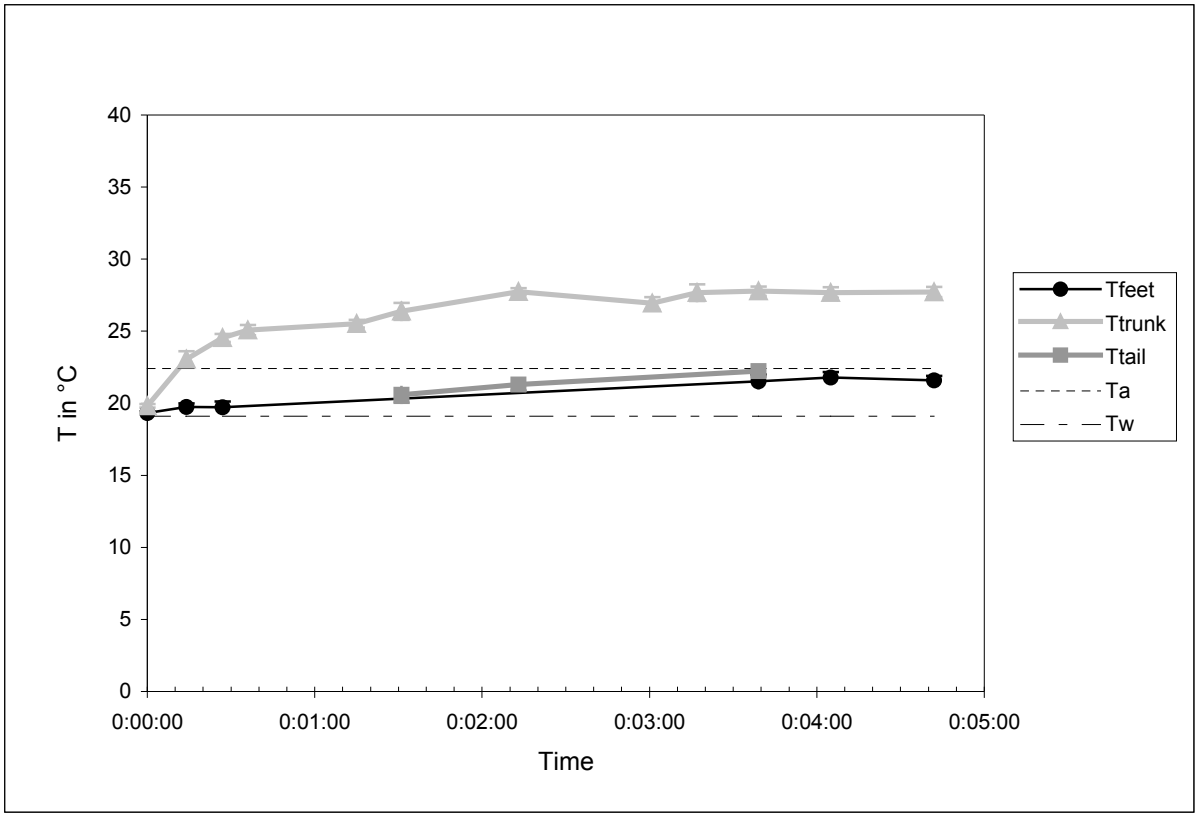


Fig. 130: Graph representing the development of Ttrunk, Tfeet and Ttail of two Giant otters, during the 5 minutes following a swimming bout

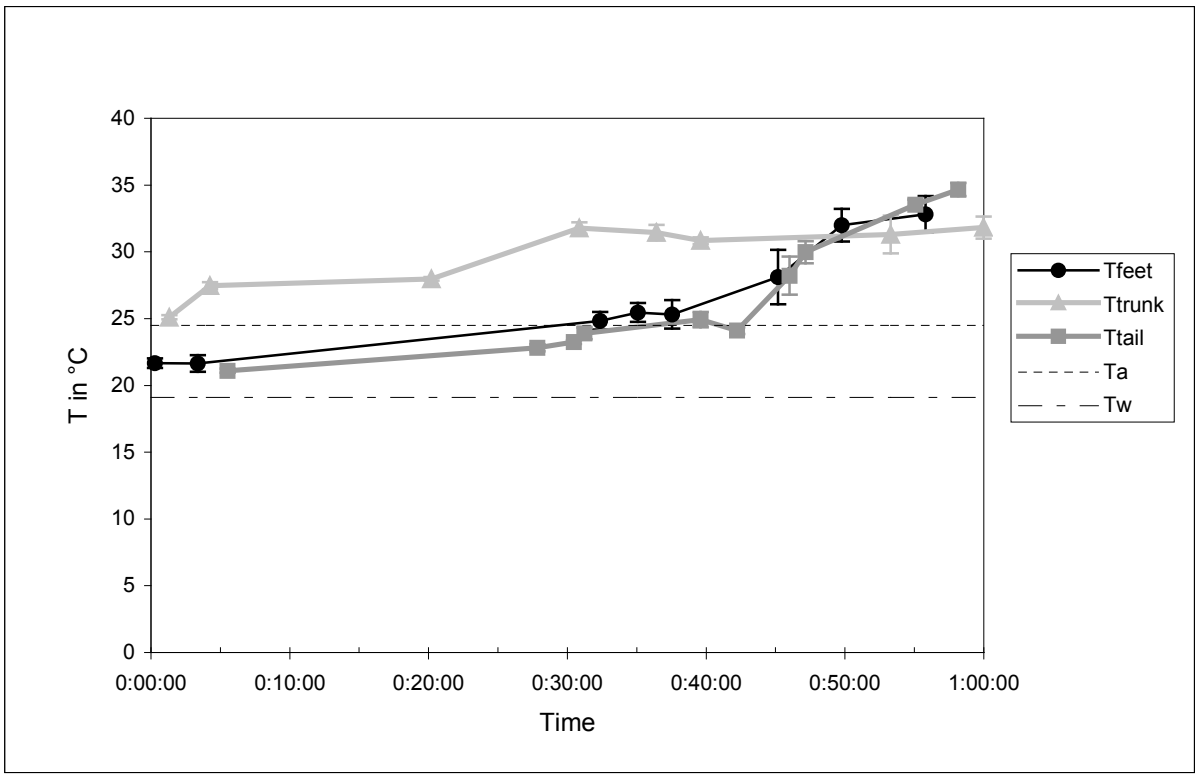


Fig. 131. Graph representing the development of Ttrunk, Tfeet and Ttail of two Giant otters, during the hour following a swimming bout. The otters had no access to water and were dry at the end of the session.

The temperature at the surface of the feet was quite homogeneous on most of the thermograms. Indeed, the interdigital webbings were mostly at the same temperature as the fingers, except at the end of the third session. Indeed, on one thermogram taken after 55 minutes out of water, Tweb was around 34°C, whereas Tfingers was between 30 and 31°C. During the minutes following exit from water, the temperature at the surface of the metacarpal/metatarsal region was similar to Tfingers or slightly higher (less than 2°C). On one thermogram taken at the end of the third session, Tfingers was between 26 and 28°C, whereas Tmetacarpal/metatarsal was between 31 and 32°C.

The temperature at the surface of the legs could be measured accurately on only very few thermograms. Tlegs was similar to Tfeet when the otter just came out of water. In the following minutes, Tlegs was slightly higher (about 1°C). On one thermogram taken during the third session 35 minutes after the beginning, Tfeet was between 24 and 26°C, Tlegs around 28°C and Ttrunk between 29 and 31°C. Another thermogram taken at the end of the session (55 minutes), showed Tfeet=32.8±1.4°C (max 34.4°C measured on the webbings), Tlegs between 33 and 34°C, and a small area of the inner part of the legs reaching 36°C.

The temperature measured at the forehead was similar to Ttrunk during the complete third session. Tears was slightly above Tw when the otters came out of water and then increased up to Ta after a few minutes. Temperatures above 25°C were measured on the mystical vibrissal pads within the minute following exit from water (see also Fig. 128). The peripalpebral region was also above Ta but somewhat colder than the mystical vibrissal pads, except for the supraciliary and upper genal pads. Tnose was always the coldest part of the body and was at Tw or slightly above after exit from water. During the third session, Tnose increased slowly and reached Ta after 25 minutes. Temperatures measured at different parts of the head at the beginning and at the end of the third session are given in Tab. 27. The temperature of the whole face and ears increased during the session, but at different rates; the ears had the most important increase and the nose the smallest.

Tab. 27: Mean temperature at different parts of the head measured just after a swimming bout and 50 minutes later (Ta=24.5°C, Tw=19.1°C). Tface was calculated using measuring points equally distributed all over the face, including the peripalpebral region and vibrissal pads, but excluding the nose.

Mean T in °C	After exit from water	50 minutes later
Tnose	21	26.5
Tmystical vibrissal pads	27.1	34.3
Tperipalpebral region	26.4	31.8
Tface	25.6	33.3
Tears	21.6	32.5

5.3.3 Thermographic recordings in *Amblonyx cinereus*

Thermal pictures of the group of 6 Small-clawed otters were taken on 3 sessions, which lasted about 2 hours. The ambient air temperature ranged from 21 to 24°C. The water was at 20.8°C during the first session and at 12.8 and 13.4°C during the second and third session. These otters were particularly boisterous. Like the Giant otters, they went into the water every couple of minutes. During the second session, they stayed almost one hour on land, while they slept during almost 30 minutes. During the third session, they stayed on land once for about 15 minutes.

Feet and tail were most of the time the coldest parts of the body. When the otters came out of water, Ttrunk was around Tw (max 1°C above) and increased to max. 4°C above Tw during the minutes following exit from water. Tfeet and Ttail remained constant at a temperature similar to Tw during the minutes following exit from water (at least 5 minutes). For example, on a thermogram taken about 5 minutes after exit from water (Ta=21°C, Tw=20.8°C), the feet were at the same temperature as the water, whereas the trunk was about 3°C warmer (Ttrunk=23.3±0.6°C, Tfeet=20.7±0.2°C). On another thermogram taken during the same session, also about 5 minutes after exit from water, the tail was at Tw, whereas the trunk was about 4°C warmer (Ttrunk=25±0.2°C, Ttail=20.9±0.1°C) (see also Figs. 132 & 133). Note that here, the complete tail had a temperature different from that of the back.

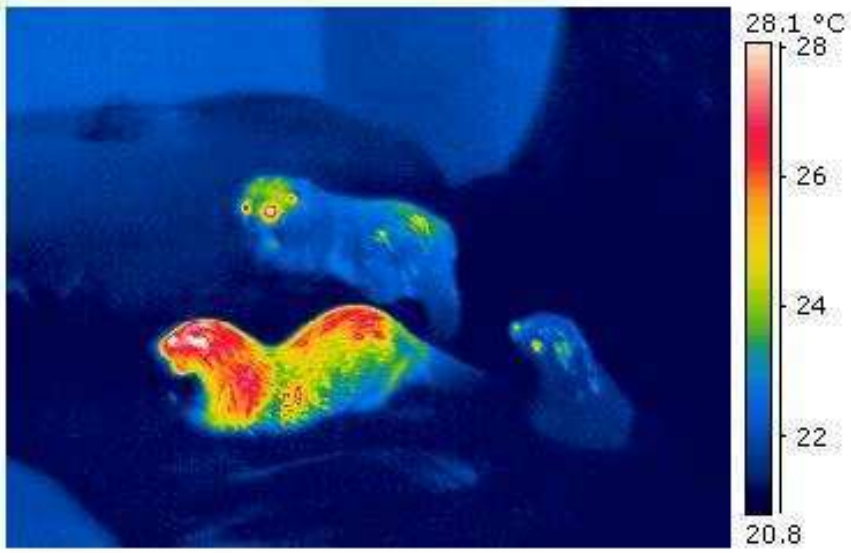


Fig. 132: Thermogram representing three Small-clawed otters half in the water (right) or shortly after exit from water (Ta=21°C, Tw=20.8°C). The otter with the highest Ttrunk (yellow and red) was the first to come out of water. The feet, which cannot be distinguished from the background, had a temperature less than 1°C above Tw. The tails are still in the water.
Upper otter (blue): Ttrunk=22.3±0.2°C, Tforehead=24±0.2°C
Tperipalpebral region=25.1±0.5°C, Tears=26.2°C (n=1), Tnose=21.9°C (n=1)
Lower otter (yellow and red): Ttrunk=24.8±0.4°C
Otter half in water (bottom right corner): Ttrunk=21.4±0.1°C

During the second session, the 6 otters slept lying together during about 30 minutes (Ta=22.1°C, Tw=12.8°C). Tfeet could be measured on a few thermograms, and values going from 14°C to 22°C were recorded. Tfeet as low as 14°C was recorded also at the end of the period of rest. Actually, the temperatures measured at the surface of the body might depend on the position of the otter within the "heap", because the animals warmed each other. For example, in Fig. 134, the otter on the top of the heap had relatively cold feet. Increase of Tfeet up to 22°C was also observed during the last session, when the otters spent about 30 minutes on land (Ta=24°C, Tw=13.4°C). A noticeable increase of Ttail had not been observed, and Ttail similar to Tw was recorded even on thermograms taken after 45 minutes of activity and rest on land.

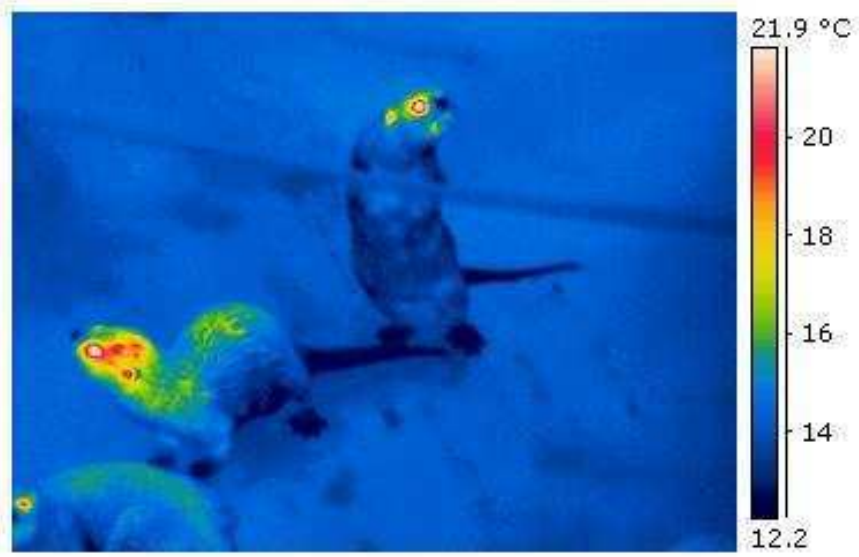


Fig. 133: Thermogram of two Small-clawed otters taken less than 5 minutes after exit from water (Ta=22.1°C, Tw=12.8°C).
Otter on the right: Ttrunk=13.3±0.3°C, Tfeet=12.4±0.1°C, Ttail=12.5±0.1°C
Tperipalpebral region=16.8±0.4°C, Tears=17.7 °C (n=1), Tnose=12.5°C (n=1)
Otter on the left: Ttrunk=14.1±0.2°C, Tfeet=12.6±0.1°C, Ttail=12.5±0.1°C
Tperipalpebral region=17.8±0.5°C, Tears=20.3°C (n=1), Tnose=13.2°C

The peripalpebral region was one of the warmest area of the body. Tperipalpebral region lower than 16°C was never recorded, even when the otters came out of water at 12.8°C. When Tw=20.8°C, Tperipalpebral of an otter who just came out of water was about 5°C above Tw (see Fig. 132). During the first session with Ta=21°C and Tw=20.8°C, Tperipalpebral was always above Ta, whereas during the other sessions, when the water was much colder than the ambient air (Ta=22.1°C, Tw=12.8°C and Ta=24°C, Tw=13.4°C), Tperipalpebral was never above Ta and reached Ta only during longer periods of activity and/or rest on land (at least 15 minutes).

Unlike in the other species studied, the mystical vibrissal pads could not really be distinguished from the surrounding area of the head (see Fig. 135). The mystical vibrissal pads were “visible” only when the otters stayed on land for at least 10 minutes, often longer, but were not thermally clearly defined against the rest of the head (see Fig. 134). During the sessions were Tw was between 12 and 14°C, the mystical vibrissal pads never showed temperatures above 23°C, even after 45 minutes on land.

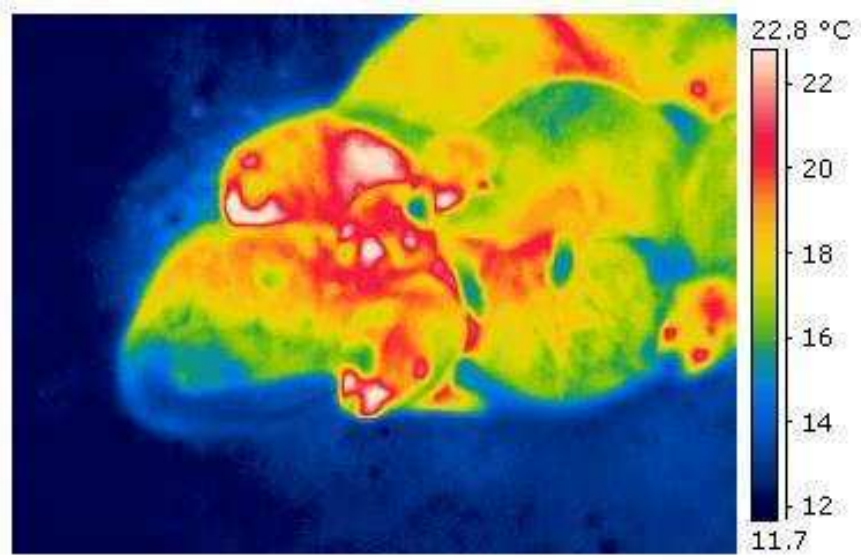


Fig. 134: Thermogram of a group of Small-clawed otters sleeping together, taken about 30 minutes after exit from water. The otters had been resting for about 15 minutes.
Ta=22.1°C, Tw=12.8°C
Otter on the top of the heap (with blue feet): Ttrunk=17.1±0.5°C, Tfeet=15.2±0.2°C
Tperipalpebral region=20.5±0.4°C, Tears=19.6°C (n=1), Tnose=14.8±0.4°C
Otter in the foreground: Ttrunk=18.3±0.2°C, Tfeet=17.4±0.5°C, Ttail=13.2±0.3°C
Tperipalpebral region=21.7±0.7°C, Tmystical pads=22.1±0.5°C
Tears=21.2°C (n=1), Tnose=16.8°C (n=1)

The ears were warmer than the surrounding area of the head on most of the thermograms. During the first session (Ta=21°C, Tw=20.8°C), T as high as 30.4°C was measured on the ears of an otter sitting in water. During the two other sessions (Ta=22.1°C, Tw=12.8°C and Ta=24°C, Tw=13.4°C), Tears was several degrees warmer than Tw during the minutes following a swimming bout but usually not higher than 21°C. It was of interest to see that even after 40 minutes of activity and rest on land, Tears was not higher than 23°C (Ta=22.1°C, Tw=12.8°C) (see also Fig. 134). Temperatures higher than 23°C were also not recorded during the 30 minutes following a swimming bout at Ta=24°C, Tw=13.4°C.
During the first session (Ta=21°C, Tw=20.8°C), Tnose was always at Ta or slightly above, but never above 23°C. During the second and third session (Ta=22.1°C, Tw=12.8°C and Ta=24°C, Tw=13.4°C), Tnose was always several degrees colder than Ta, but this was because the water was much colder than ambient air. Tnose increased slowly after a swimming

bout, and even after 40 minutes of activity on land, Tnose was not higher than 17°C (Ta=22.1°C, Tw=12.8°C) (see also Figs. 133 & 134). A higher Tnose was also not recorded during the 30 minutes following a swimming bout at Ta=24°C, Tw=13.4°C.

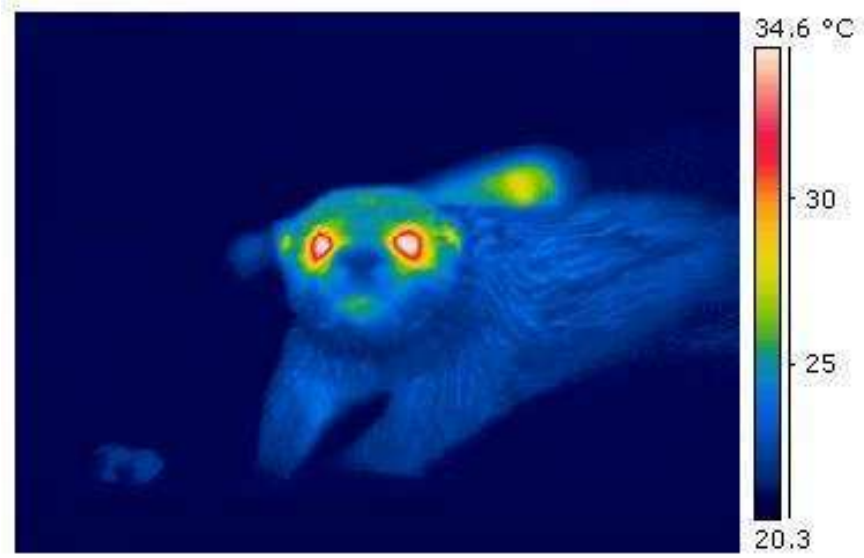


Fig. 135: Thermogram of a Small-clawed otter taken shortly after exit from water (less than 5 minutes). Ta=21°C, Tw=20.8°C
Ttrunk=22.5±0.4°C, Tforehead=24.8±0.1°C
Tmystical vibrissal pads=24.2±0.5°C, Tnose=22.1±0.2°C
Tperipalpebral region (yellow)=27.7±0.6°C

5.4 Discussion

5.4.1 Surface temperature and thermal windows in otters

The feet represented the major thermoregulatory area in otters. Particularly in the Eurasian otter (*Lutra lutra*), the feet were the only region where important temperature fluctuations could be observed and where considerable heat dissipation could occur. Indeed, the highest temperatures were measured there, and the feet were up to 20°C above Ta. In water, where maintenance of body temperature is a greater challenge than in air, Tfeet decreased to Tw, in order to reduce heat loss. Decrease of Tfeet out of the water was observed only in winter at Ta below 10°C (Tfeet will be further discussed in 5.4.4). A second heat loss avenue was the inner part of the legs, and to a lesser extent the lower legs.

Heat loss also occurred through the head, particularly in the face region. However, the forehead was normally at the same temperature as the trunk or only a few degrees above (max 3°C), and the face, particularly the peripalpebral region and the mystical vibrissal pads, cannot be considered as thermal windows, because they were always above Ta, but not because of thermoregulatory needs (see 5.4.5).

The body trunk is also not considered to be a thermoregulatory region, because the temperature at its surface tended to follow Ta, which is generally observed in well-furred mammals

(HAMMEL 1955). Even when the otter was wet, Ttrunk, which was equal to Tw just after the swimming bout, tended to remain around Ta. However, Ttrunk up to 5°C above Ta was also observed, even when the otter was dry and at Ta above 10°C (see 5.3.1.2). Minimal differences between Ttrunk and Ta were recorded in summer, when Ta>20°C and Tw>15°C. In winter, Ttrunk was up to 8°C above Ta when the otter was wet. The behaviour of the otter influenced Ttrunk because torsions, rubbing and scratching induced openings of the fur and thus increased heat loss, particularly when the otter was wet. When the otter was lying on the floor, the fur and, thus, the insulating air layer was compressed, which also increased heat loss. Grooming restored the insulating capacity of the fur, but heat loss might increase during the grooming process. Further studies, including measurements of the trunk surface temperature of otters kept dry for longer periods at different Ta, would be necessary to better quantify heat loss from the trunk.

Ttail higher than Trunk was never recorded in the Eurasian otter during this study. Actually, Ttail was mostly similar to Ttrunk when the otter was dry. We could see on a few thermograms, that an insulating air layer having a temperature of at least 28°C was present under the surface of the fur, even at the tail. However, those thermograms were taken at Ta and Tw above 15°C. At colder temperatures, particularly below 10°C and/or after longer swimming bouts, this insulating air layer apparently disappeared because Ttail decreased to a temperature below Ttrunk and equal to Ta and/or Tw (Ttail will be further discussed in 5.4.4).

A different pattern was observed in the Giant otter (*Pteronura brasiliensis*). Here, more heat was lost through the whole body because of the rapid increase of Ttrunk after a swimming bout, but also because of the large surface of the animal (Giant otters can be more than 2 meters long with the tail, weighting only 30 kg). At Ta=22.4°C and Tw=19.1°C, Ttrunk was at least 5°C above Ta, less than 2 minutes after exit from water. During the session where the Giant otters where kept in a cage without water for an hour, Ttrunk increased slowly up to about 31-32°C (7°C above Ta) during the first 30 minutes and then stayed constant. The feet and tail were usually at Tw or slightly above during the minutes following a swimming bout. Tfeet and Ttail reached Ta after more than 30 minutes on land, and were above Ttrunk only after more than 45 minutes. Actually in this session, heat loss from the feet and tail begun only when heat loss from the trunk became stagnant. In this tropical species, which normally does not occur at temperatures below those in our experimental conditions, a considerable amount of heat could be lost through the whole body, even at high Ta. A decrease of Tfeet and Ttail down to Tw occured during swimming bouts in order to reduce heat loss. Feet and tail were used to dissipate excessive heat quite late, compared to the Eurasian otter. Indeed, feet and tail were still below 35°C after 1 hour on land at Ta=24.5°C, but we expect a further increase of Tfeet and Ttail at higher Ta and/or longer time on land. The Giant otter was the only species in which Ttail above Ta was recorded.

The Small-clawed otter (*Amblonyx cinereus*) exhibited a kind of intermediary pattern, compared to the two previous species. Tfeet and Ttail decreased down to Tw during a swimming bout. Afterwards, Tfeet increased quite slowly compared to the Eurasian otter, and Tfeet above Ta were not recorded during this study. However, this must be related to the fact that the otters never stayed on land more than 5 minutes during the first session, and to the relatively low Tw during the second and third session. Tfeet above Ttrunk could be recorded in some of the individuals when they slept on land. Ttail always remained at a temperature similar to that of the water, even after 45 minutes of activity and rest on land. Ttrunk increased more slowly than in the Giant otter but more rapidly than in the Eurasian otter. Indeed, during the first session (Ta and Tw around 21°C) Ttrunk increased to max 4°C above Ta and Tw during the 5 minutes following exit from water. At similar air and water temperatures (see Fig. 123), the trunk of the Eurasian otter was quite constant at a T almost equal to Tw during the complete session. During the second and the third session, Ttrunk of the Small-clawed otter never reached Ta, even after 45 minutes of activity and rest on land, but this has also to be related to the relatively cold water. Further measurements, particularly recordings of single individuals and without access to water, would be necessary to get a more accurate pattern of body surface temperature variations in the Small-clawed otter.

5.4.2 Thermal windows in endotherms

Like otters, many animals use their appendages as “thermal windows” to regulate heat loss. Indeed, feet and legs are important for heat dissipation in birds (STEEN & STEEN 1965a, BRYANT 1983, MARTINEAU & LAROCHELLE 1988, SPEAKMAN et al. 1997). Ostrich, emu and cassowary, additionally, use their beak, neck and also casque for the last one, to regulate heat loss (PHILLIPS & SANBORN 1994). Despite of not being the hottest region of the body, the wings can contribute to more than 50% of overall heat loss because of their relatively large surface (WARD et al. 1999); the same was observed for the wings of bats (LANCASTER et al. 1997). In Polar bears (*Ursus maritimus*), heat is dissipated from the bare footpads and claws and the sparsely furred area of the snout (ØRISTLAND et al. 1974). In euthermic woodchucks (*Marmota monax*), vasomotion in the feet and to a lesser extent in the pinna is used to regulate heat loss (PHILLIPS & HEATH 2001). The pinna is a particularly important thermoregulatory surface in rabbits (HILL & VEGHTE 1976, MOHLER & HEATH 1988) and elephants (PHILLIPS & HEATH 1992). The lower legs and paws are the most important thermoregulatory surface in foxes (KLIR & HEATH 1992). Heat loss also occurs through the face, nose and front of the pinna in the Arctic fox (*Alopex lagopus*), and through the face, nose, whole pinna and dorsal head in the Red fox (*Vulpes vulpes*) and the Kite fox (*Vulpes macrotis*). In the Arctic fox, 22% of the body surface is important for heat exchange, 33% in the Red fox and 38% in the Kite fox.

The tail is the major thermoregulatory body region in the Muskrat (*Myocastor coypus*) (JOHANSEN 1962b, KRATTENMACHER & RÜBSAMEN 1987) and in the Beaver (*Castor fiber*) (STEEN & STEEN 1965b). Cetaceans use their dorsal fin, pectoral flippers and fluke as thermal windows, but can also lose heat through the whole hairless body (HAMPTON et al. 1971, MCGINNIS et al. 1972, CUYLER et al. 1992, WILLIAMS et al. 1999, MEAGHER et al. 2002, PABST et al. 2002). The flippers are also the most responsive surfaces in varying heat dissipation in seals (HART & IRVING 1959, GALLIVAN & RONALD 1979, RYG et al. 1993), but the body trunk can also function as an important heat dissipator (KRUMBIEGEL 1933, ØRISTLAND 1968, MOLYNEUX & BRYDEN 1975, KVADSHEIM & FOLKOW 1997). Particularly surprising is the occurence of more or less circular shaped thermal windows, which enlarge and fuse, on the trunk of hauled-out seals, well documented in the work of MAUCK et al. (2003).

5.4.3 Insulating capacity and importance of fur integrity

Infrared thermography allows to “see heat”, and thus gives us a good “representation” of the insulating capacity of the otter hair coat. Indeed, the existence of “two layers” around the body of the otter could be observed very clearly on many thermograms of the Eurasian otter, particularly when the otter was wet, and scratched or rubbed its fur, or when torsions created deeper gaps between tufts of wet hairs. On the thermograms where the warm “inner layer” was visible between the tufts of wet hair, it looked as if the “external layer” would be fissuring. The inner layer is formed by the air trapped within the extremely dense wool (secondary) hair part of the fur, and warmed by the heat produced by the body, which is thus mainly conserved instead of being lost to the surrounding air. This heated insulating air layer is maintained even in water. The external layer is made of the upper part (shield) of the guard (primary) hairs, which are longer than the wool (secondary) hairs. In water, the large and flat shields lie parallel to the body, and form a kind of wet film, which protects the underlying layer from becoming cold and wet. This layer of PH shields confers to the otters their slippery aspect when they leave the water.

We could measure on many thermograms a thermal gradient of more than 10°C between the surface of the fur and the inner layer. A temperature of 28°C was measured within the inner hair coat layer of an otter who just came out of water at 13.9°C. Temperatures above 25°C were also measured in winter. Those measurements gave us the temperatures at different points situated within the inner layer, which might be somewhat beneath the temperature of the skin. KRUUK & BALHARRY (1990) measured the temperature in the air layer immediately adjacent to the skin, when cold water at 6°C was run along the outside of fur sections that were stretched over a copper vessel maintained at 35°C. The mean temperature inside the fur near the skin, measured using thermocouples, was 29.1±1.1°C. In Sea otters, a sub-

cutaneous temperature of 36.5°C was recorded in water of 15°C (COSTA & KOOYMAN 1982).

Several mammals living in cold climates (arctic or mountains) have a fur the thermal conductivity of which, in air, is lower than that of otter fur, but in water, the otter hair coat shows the best insulating capacity, at least for *L. lutra*, *L. canadensis* and *E. lutris,* which takes first place (SCHOLANDER et al. 1950, MORRISON et al. 1974). However, this insulating system is fragile. Indeed, we could see on the thermograms, that each disturbance in the external protecting shield induced an increase of several degrees of the surface temperature of the disturbed area, which means a considerable increase of heat loss. Many studies showed that fouling of the pelt, oiling and washing processes induced a decrease in body temperature and an increase in oxygen consumption, and were even lethal in some cases (KOOYMAN et al. 1977, COSTA & KOOYMAN 1982, STOSKOPF et al. 1997). In our study, abnormal areas of heat loss could be seen on the thermograms of the Eurasian otter Lukas, who died a few days after the recordings. The exact cause of death was not determined, but Lukas suffered from liver infection, osteoarthritis and paralysis of the hind feet. We do not pretend that the defect of the fur caused the death, but the abnormal heat loss could have contributed to weaken the animal. In case of important disturbing of the fur, measures like increasing the food quantity or keeping the animal inside by cold temperatures should be considered. Infrared thermography could be used to control the insulating capacity of the fur, for example after surgery or oil contamination. This could be particularly useful when otters are released to the wild. In Monterey Bay (California), IRT was used to control recovery of the Sea otter fur insulating capacity after different washing processes (JOHNSON, pers. communication).

5.4.4 Heat loss from feet and tail

In otters, vasomotion in feet and tail is the principal heat loss regulation mechanism. In the particularly well-furred Eurasian otter, the sparsely furred feet are the only part of the body where considerable heat loss can occur. This was discussed by former authors (TARASOFF 1974, BAITCHMAN & KOLLIAS 2000) but, as far as we know, has never been measured. When the otter is in water and/or at low Ta, a vasoconstriction in the feet skin prevents excessive heat loss. Vasodilatation occurs in the feet when excess heat has to be dissipated. This effective thermoregulatory surface is relatively small, but Tfeet can be considerably above Ta, and this happens very rapidly when the otter is in a heat load situation. Otters also have large paws, compared to other animals of similar size, and the interdigital webbings increase the surface area available for heat loss. In our study, Tfeet was usually above 30°C (often above 35°C) during activity on land or rest, quite independently from Ta, except in winter, where Tfeet decreased toward Ta, when the otter came out of the sleeping box. However, in winter the floor of the enclosure was often wet and muddy, and thus the feet proba-

bly became wet, which would increase evaporative heat loss. Those measurements should be repeated when the otter walks on a dry floor. In our study, greatest heat loss occurred at ambient temperatures between 10 and 15°C, when Tfeet was about 20°C above Ta. During the trial without access to water, the ambient air was at 20°C and Tfeet was about 17°C above Ta after 2 hours of stay on land. Tfeet well above Ta was also recorded in the Woodchuck, but Tfeet was not above 30°C until Ta was above 15°C and the highest Tfeet-Ta, which was of approximately 15°C, was recorded only at Ta between 15 and 20°C (PHILLIPS & HEATH 2001). In foxes, the feet were never more than 10°C warmer than Ta, at Ta above 0°C (KLIR & HEATH 1992).

Unlike the feet, the tail of the Eurasian otter is well insulated. With Ta above 15°C, the temperature near the epidermis was several degrees above the temperature at the surface of the fur, even at the tip of the tail and even after a swimming bout. However, this insulating layer must be very thin, and is apparently insufficient at lower temperatures, particularly in water below 10°C. Thus, a vasoconstriction occurred in order to prevent heat loss and the skin cooled down to Tw and/or Ta. This decrease in Ttail occured only in the distal part of the tail (max 2/3), which must be related to the shape of the tail. Hair length and density at the base of the tail is similar to that of the back (see chap. 1 and 3). Data are not available for the whole tail but the hairs, at least the PHs, have a quite constant length all along the tail. Anyway, the decrease of the diameter toward the tip of the tail reduces the possibility of maintaining an insulating air layer.

In the Giant otter and Small-clawed otter, the tail is covered with very short fur and is thus poorly insulated. In these species, feet and tail cooled down to Tw during a swimming bout. In the Giant otter, heat loss from feet and tail occurred only more than 30 minutes after exit from water (at Ta=24.5°C, Tw=19.1°C), which corresponds to the moment where heat loss from the rest of the body became stagnant. Like in the Eurasian otter, not the whole tail cooled down to Tw but only the distal 2/3. However, whereas in *Lutra lutra* the base of the tail was at a temperature similar to that of the back, here the proximal part of the tail showed a temperature intermediary between the back and the distal part of the tail.

In the Small-clawed otter, the tail has a quite constant diameter and is only slightly thicker at the base (more “rat-like”), and thus the whole tail cooled down to Tw during a swimming bout. Heat loss through feet and tail could not be documented during this study, but Tfeet increased during staying on land after a swimming bout and reached Ta in some of the individuals. Ttail stayed quite constant during the 45 minutes following a swimming bout but we expect a use of the tail to dissipate excess heat at higher temperatures and/or longer stay on land.

SOKOLOV (1962) considered the tail to be a site for dissipating excess heat in the majority of semi-aquatic forms. This was demonstrated in the European beaver (STEEN & STEEN

1965b), the Muskrat (JOHANSEN 1962b) and the Coypu (KRATTENMACHER & RÜBSAMEN 1987). For example, a beaver (*Castor fiber*) placed in air at 16°C had a temperature at the tip of the tail slightly above Ta and maintained a normal rectal temperature of 37±0.2°C. In air at 25°C, Ttail reached 35°C within 30 min and the rectal temperature was about 2°C above normal. However, this increase of body temperature did not occur when the beaver was allowed to dip its tail in cool water at 6°C (STEEN & STEEN 1965b). In the Muskrat (*Ondatra zibethicus*) placed in air at 25°C, the rectal temperature remained normal but increased to 41-42°C when the tail blood flow was occluded (JOHANSEN 1962b). In the Coypu *(Myocastor coypus*), Ttail, which was around Ta at Ta between 15 and 20°C, increased to about 33°C at Ta=25°C. The body temperature did not change but began to increase at Ta above 30°C (KRATTENMACHER & RÜBSAMEN 1987). In submerged beavers and muskrats, the surface temperature of tail and feet is similar to that of the water (JOHANSEN 1962b, FISH 1979, MACARTHUR 1984, MACARTHUR & DYCK 1990).

Curiously, less attention has been paid to heat loss from the feet. However, FISH (1979) measured that in *O. zibethicus*, the temperature of the hind feet was equal to Ta at Ta=25°C, but increased to 35°C at Ta=30°C. Note that in the Eurasian otter, an increase of Tfeet above Ta occurred at much lower temperatures, which may indicate a better heat tolerance in the muskrat. The plantar surfaces of minks (*Mustela vison*) resting in air at 23.8°C were around 37°C (WILLIAMS 1986). When submerged in water at 24.6°C, they were about 2-3°C above Tw. A heavily sedated mink resting in water, did not exhibit peripheral vasoconstriction, and the skin temperature of the feet remained close to body temperature. Thus, the Tb of this individual decreased at a rate twice as high as in minks demonstrating peripheral vasoconstriction.

In the Platypus (*Ornithorhynchus anatinus),* the tail is also used for heat loss, even at low Ta (GRANT & DAWSON 1978). Indeed, at Ta=5°C, Ttail was around 24°C; in water, Ttail was equal to Tw. The feet were at Tw or within the 2°C above Tw at Tw=15°C, but the difference increased with decreasing water temperature, and at Tw=5°C the feet were between 3 and 6°C above Tw. In the Australian Water Rat (*Hydromys chrysogaster*), Tfeet and Ttail were equal to Tw at Tw above 20°C (FANNING & DAWSON 1980). At lower water temperatures, the difference increased up to 4°C. In air between 0 and 35°C, the temperature at the surface of the feet was above Ta and increased with increasing Ta. Ttail was quite similar to Ta at Ta below 20°C, but increased above Ta at higher ambient temperatures.

The ability to lose or to retain heat depends on the anatomy of the peripheral circulatory system. Indeed, in a countercurrent heat exchange system, the cold venous blood returns from the extremities to the body core through veins that are associated to arteries, and thus part of the heat from the arterial blood is transmitted to the venous blood instead of being lost to the ambient air (or water). When heat has to be dissipated, the venous blood returns through

superficial veins where heat is lost to the environment. The efficiency of this system depends on how elaborated it is. The most complex, and thus most efficient countercurrent heat exchangers are found in the appendages of pinnipeds, cetaceans and sirenians, where a venous plexus surrounds the arteries (rete mirabile) (SCHOLANDER & SCHEVILL 1955, BARNETT et al. 1958, TARASOFF & FISHER 1970, ROMMEL & CAPLAN 2003). Additionnally, arteriovenous anastomoses (AVAs) of the cutaneous microvasculature allow arterial blood to enter directly the venous part of the circuit without passing through capillary beds. During exposure to heat, AVAs are open and divert part of the increased blood flow from the capillaries into superficial veins. They are closed during exposure to cold (JESSEN 2001). AVAs are present in many tissues and organs, but are more numerous in the skin, and abundant in acral regions like ears, nose, feet and flippers. AVAs are equally distributed in the skin of some seals, so that in those species the whole body surface is important for heat dissipation (MOLYNEUX & BRYDEN 1975).

A quite elaborated countercurrent arrangement of veins and arteries and AVAs were found in the hind feet and tail of the beaver (CUTRIGHT & MCKEAN 1979). Some anatomical specialisations in the vascular system of the extremities, which allow countercurrent heat exchange, were also reported for the platypus (GRANT & DAWSON 1978). Unfortunately, only very little is known about the peripheral circulatory system of otters. Actually, information is available only for *Lontra canadensis* and *Enhydra lutris,* but we expect the circulatory systems of *Lutra lutra* and *Lontra canadensis* to be quite similar because of the anatomical and ecological similarities between both species. The skin is relatively thin in the feet, particularly in the interdigital webbings (TARASOFF 1972, TARASOFF 1974, BAITCHMAN & KOLLIAS 2000). In both species, the interdigital webbings are highly vascularized. TARASOFF (1972, 1974) considers that the vascularization of the hind feet of *Lontra canadensis* is comparable to that of a terrestrial carnivore. He observed that the hind feet of *Enhydra* are more vascularized than in *L. canadensis* but less than in pinnipeds. He did not find retia mirabilia comparable to those in pinnipeds and added that, “the occurrence of arteriovenous anastomoses has not been studied in those species”. However, our study showed that blood flow in the feet of *Lutra lutra* changed very rapidly in response to changing thermal conditions, not only in the interdigital webbing, and that heat loss decreased to minimal values during a swimming bout, even in water below 5°C. FANNING & DAWSON (1980) observed that in submerged Australian Water rats, the feet were at temperatures close to or equal to Tw, despite of the absence of particular countercurrent heat exchangers, and concluded that the low skin temperatures must be achieved principally thanks simple vasoconstriction. However, this species is much less adapted to long swimming bouts in near freezing water and to low air temperatures than the Eurasian otter. We could not find any information on the circulatory

system of Giant otter and Small-clawed otter. So far, further examination of the circulatory system of otters, particularly in the appendages is needed.

5.4.5 Surface temperature of the sensory organs

In the Eurasian otter, surface temperatures of ears, peripalpebral region and vibrissal pads were never lower than 15°C, even after a swimming bout and in winter. In this connection, it has to be noted that those temperatures measured at the surface of the fur, indicate substantially higher temperatures of the skin. This selective heating occurs in order to insure a good functioning of the sensory organs, because cooling would impair sensitivity (GREEN et al. 1979, GESCHEIDER et al. 1997). Thanks to their vibrissae, otters can detect prey under water even under strongly reduced light condition (GREEN 1977). DEHNHARDT et al. (1998) showed, using IRT, that in seals, which also have to deal with the large cooling power of water, the appropriate operating temperature of the vibrissal pads is maintained, even in water at 1.2°C. The same was demonstrated for the follicle crypts on the rostrum of dolphins (HAMPTON 1971, MAUCK et al. 2000).

In the Giant otter, the mystical vibrissal pads were also warmer than the surrounding regions of the head but in this species, the whole face and the body trunk were several degrees above Tw less than a minute after exit from water. The ears apparently cool down to water temperature during a swimming bout and get slowly warmer after exit from water. However, regarding the fact that Giant otters occur in water usually above 20°C - in Peru for example, they occur in waters that are around 28°C all year round (SCHENCK 1997) - an impairing cooling of the ears may not happen.

A selective heating also occurs in the Small-clawed otter but is apparently less efficient than in the Eurasian otter and does not concern all the sensory organs. Indeed, after a swimming bout in water at Tw between 12 and 14°C, the temperatures of the sensory organs were generally above 15°C but tended to stay below 20°C for quite a long time and increased very slowly compared to the Eurasian otter. We expect the Small-clawed otter to have difficulties to maintain its sensory organs at an appropriate temperature in cold water, probably below 10°C. The peripalpebral region was the warmest region of the body after exit from water (ears excepted). The temperature of this area also increased slowly after exit from water at Tw=12-14°C. After a swimming bout at those temperatures, the eye itself remained between 20 and 25°C during the session. “Cold eyes” (about 20°C) were also observed in the Eurasian otter just after swimming bouts in winter, but Teye increased above 25°C within minutes. A selective heating of the mystical vibrissal pads apparently does not occur in this species, because those areas were not warmer than the surrounding regions after a swimming bout. The mystical pads became warmer during stay on land but stayed below 23°C in the sessions were Tw was between 12 and 14°C, even after 45 minutes on land, and were not ther-

thermally clearly defined against the rest of the head. This may be because Small-clawed otters do not rely on the sensitivity of their vibrissae as much as Eurasian otters and Giant otters. Indeed, Eurasian otters and Giant otters are principally fish eaters and catch their preys with their mouth, whereas the Small-clawed otters, the less aquatic otters, eat principally invertebrates that they catch with their fore paws. RADINSKY (1968) demonstrated for the brain of *Lutra* and *Pteronura* an expansion of the coronal gyrus, indicating a somatic sensory specialisation of the head region, presumably in the form of extremely sensitive vibrissae, whereas the primary somatic sensory projection area of the forelimb is enlarged in *Amblonyx*.

Since Small-clawed otters use their fore paws to catch their food, the tactile sensitivity of their fingers should be maintained. A selective heating of the fingers apparently does not occur because Tfeet followed Tw. However, since Small-clawed otters occur in warm regions, their manus probably rarely cools down to a temperature that could impair the tactile sensitivity of the fingers. Moreover, the fingers may not require a temperature as high as the vibrissal pads to insure an appropriate sensitivity. However, there is another otter species, which uses its fore paws to get food, and which lives in a much colder environment (water temperatures can be near 0°C). Indeed, Sea otters search the sea floor for molluscs, crabs and sea urchins and often use a stone like a hammer to detach the shells fixed to the rocks. Then they open their preys at the surface with a great dexterity. According to RADINSKY (1968), Sea otters also have an enlarged primary somatic sensory projection area of the forelimb. Sea otters may not register impairment of sensory function of the fingers at the same temperature as man, but a decrease of the temperature of the fore paws down to 0°C could possibly impede the manual skills of the Sea otter. This aspect still remains to be examined.

5.4.6 Temperature of the nose

In many species, a decrease of the nose surface temperature below Ta occurs at high ambient temperatures in order to prevent a dangerously increased brain temperature. For example, KLIR & HEATH (1992) measured that the nose temperature of the Red fox and Kite fox increased with increasing Ta, but at Ta higher than 22°C, Tnose decreased. In the Arctic fox, the increase of Tnose reversed when Ta got higher than 20°C. In the euthermic woodchuck, Tnose fall below Ta with exposure to Ta above 30°C (PHILLIPS & HEAT 2001). When a countercurrent brain cooling occurs, the cooled venous blood that returns from the nose, cools the arterious blood which supplies the brain when it drains through the cavernous sinus (TAYLOR & LYMAN 1972). In the Eurasian otter, Tnose was mostly above Ta, except after a swimming bout in colder water. A drop of Tnose was not observed in this study, even not after more than 5 hours without access to water and also not at 26.6°C, which was the high-

est Ta during a recording session. Thus, a brain cooling using the nose apparently does not occur, at least not at Ta below 27°C. This has also not been observed in the Giant and Small-clawed otter. Like in the Eurasian otter, Tnose was around Tw after exit from water and increased during stay on land. In the Giant otter, Tnose was above Ta after 25 minutes on land at Ta=24.5°C (about 5°C above Tw). In the Small-clawed otter, Tnose was below Ta even after 45 minutes on land, but this has to be related to the relatively cold water temperature (about 10°C colder than Ta). In both species, the temperature of the nose increased very slowly compared to the Eurasian otter.

5.4.7 Adaptive value of body surface temperature variations and general remarks on otter thermoregulation

Few studies compared the surface temperature regulation between species belonging to the same taxonomic group, but occurring in different climatic regions. An IRT study of surface temperature in three species of foxes showed that the species living in the coldest regions had the smallest thermoregulatory effective surface area, and the species living in the hottest and most arid climate the largest (KLIR & HEATH 1992). This tendency could also be recognized in our study. The Giant otter can loose the greatest amount of heat, not only because it has the largest and less haired appendages (also largest relatively to the body), but also because it can dissipate a considerable amount of heat through the whole body, due to the short hair coat, which allows a greater heat transfer than in the other species. Indeed, the Giant otter has hairs that are half as long as those of the Eurasian otter, and is one of the two otter species with the shortest and thinnest hairs (see chap. 2). Moreover, it has a higher surface to volume ratio than the other species. The Giant otter is a diurnal tropical species living in the Amazon Basin, and thus the ability to loose a considerable amount of heat when on land is advantageous. However, this species is also subjected to aquatic cooling, and thus vasoconstriction in feet and tail reduces heat loss in water. Moreover, the fur is short but dense, and may provide a sufficient insulation in water above 20°C.

The Small-clawed otter is closer to the Giant otter than to the Eurasian otter, when looking at the climatic region where it occurs and the characteristics of its hairs (see chap. 2), but it generally can encounter a wider range of environmental conditions, and, unlike the Giant otters, it also occurs along sea coasts and high in the mountain (KRUUK 2006). Heat loss from the whole body is intermediary between that of the Giant otter and of the Eurasian otter. BORGWARDT & CULIK (1999) found that the resting metabolic rate in water was comparatively higher in *A. cinereus* than in *L. lutra,* and noted that their findings point to “poorer insulative capacity of the fur and underlying skin or to less well-developed heat saving mechanisms in the vascularization of the appendages”. However, as far as we know, information on the vascularization of the appendages of *A. cinereus* is not available, but our IRT study

showed that Small-clawed otters are perfectly capable of reducing heat loss from the appendages to minimal values when in water.

In the Eurasian otter, the surface available for heat loss is relatively small and this could be a limiting factor in air. However, Eurasian otters occasionally make quite long trips on land and can be met more than 10 km away from the next water body (ROSOUX & GREEN 2004). Remember that Eurasian otters are as well insulated in summer as in winter (see chap. 3). They also encounter high temperatures and sometimes drought within their home range (BROYER et al. 1988, RUIZ-OLMO et al. 1999). Their nocturnal activity reduces their exposure to hot temperatures, but Eurasian otters are not strictly nocturnal and in certain areas, particularly in coastal habitats, they are even active mostly during daytime (KRUUK 2006). We could speculate that they probably avoid longer trips on land at high ambient temperatures, which could lead to overheating. Death caused by hyperthermia has been observed in an Eurasian otter after anesthesia (REUTHER & BRANDES 1984). The body temperature of this animal had reached 43°C. In a captive otter, a body temperature of 41°C was recorded on several occasions, once during a visit to the veterinary, and also in other stressful situations (pers. observation). Both hyperthermia and hypothermia have been observed in studies on River otter (*Lontra canadensis*) anesthesia (SPELMAN et al. 1997).

REUTHER & BRANDES (1984) expected the abdomen to have a thermoregulatory function because of the thinner hair coat. However, the PHs from the abdomen are thinner than those from the back but this difference is not significant, and the hair density on the belly is similar to that of the rest of the trunk (see chap. 1 and 3). We could measure the surface temperature of the belly only on a few thermograms, and it was a few degrees higher than at the back on some pictures, but also similar to that of the back on some others. The temperature at the abdominal body surface may be influenced by the fact that when the otter lies on it, which is mostly the case, the insulating air layer is compressed. Further studies would be necessary to see if heat loss from the belly is higher than from the rest of the trunk, but it is unlikely that a considerable amount of heat can be lost through this region.

A heavy panting during a heat stress situation was reported for the Eurasian otter and the Sea otter (BARABASCH-NIKIFOROW 1947, REUTHER & BRANDES 1984). Panting has never been observed during our study, even not at high ambient temperature, during intense activity or after 5 hours on land. The ambient temperature, length of activity on land and/or intensity of activity (or stress) threshold for the use of panting were apparently not reached during our study. During the trial without access to water, the Eurasian otter Naima did no show any sign of stress and did not seem to suffer from heat load, but postural adjustments allowed to increase heat loss. Naima always lay in a sprawling position, mostly on her belly, sometimes on her back, with her arms stretched, feet against the floor. Her fingers were always spread, and on several occasions, she spread them to the maximum for a few sec-

onds, and thus stretched the webbings, which increased the surface available for heat loss. She would probably spread her fingers completely for longer periods at higher ambient temperatures. Naima also sometimes turned her feet, so that they lay sole up against the floor, which increased heat lost from the upper side of the feet to the floor by conduction. In all the trials where otters were prevented from access to water (also for the Giant otters), they went straight to the water, as soon as they were released in their usual enclosure.

The Sea otter has at its disposal a greater body surface for heat loss, particularly due to the large hind flippers, which are sparsely furred and heavily vascularized (IVERSEN & KROG 1973, MORRISON et al. 1974, COSTA & KOOYMAN 1982, ESTES 1989). In Sea otters, the surface area of the four extremities amounts to 17% of total body surface (IVERSEN & KROG 1973). This area is also considerably adjustable because closing of the hind flippers results in a 25% reduction of the surface (COSTA & KOOYMAN 1982). However, Sea otters have a better-insulated hair coat and a higher metabolism than Eurasian otters, and thus they have an only narrow thermal tolerance in aerial environment (IVERSEN & KROG 1973, KOOYMAN et al. 1977). MORRISON et al. (1974) reported a heat death that occurred at only Ta=22.5°C (in air). This temperature is just above the upper limit of the thermoneutral zone (TNZ), estimated to range from at least –19°C to 21°C. It has to be noted that this individual maintained a state of excitement during the two hours of exposure to 22.5°C, which may have contributed to the fatal temperature increase. The body temperature of the dead animal had reached 44°C. IRVING & KROG (1955) measured that in a Sea otter kept in air at 7°C, the temperature at the extremities was around 22°C. During rest, Sea otters always hold their feet out of water, which allows reducing heat loss and may also allow gaining heat from solar radiation (TARASOFF 1974, LOUGHLIN 1977, COSTA & KOOYMAN 1982). A study of body surface temperature using IRT is under performance in Santa Cruz, California (JESSUP, pers. communication).

As far as we know, the thermoneutral zone (TNZ) of the Eurasian otter has not been clearly determined. In water below 15°C, the metabolism increases with decreasing water temperatures (KRUUK et al. 1994b). PFEIFFER & CULIK (1998) calculated that for an Eurasian otter swimming at a speed of less than 1.3 m/s (mean swimming speed of *Lutra lutra*: 0.89 m/s) in water of 12-15°C, the energy cost of thermoregulation is higher than the energy cost of exercising. However, Eurasian otters swim for long periods in ice-cold water, and KRUUK (1995, 1997) observed that water temperatures had no effect on body temperature and on the length of swimming bouts, even in water at 2°C. In air, otters do not seem to be impeded by subfreezing temperatures and even sleep lying on the ice (see Fig. 136). We could not find any information on the upper limit of the TNZ. Our study otter Naima did not suffer from heat during more than 5 hours in air at 20°C, but adopted postures that increased heat loss and the mean Tfeet stayed constant around 37°C, with the webbings reaching a temperature

near 40°C. Thus, an important further increase of dry heat loss through the feet is not conceivable.

The mean body temperature (Tb) of an inactive Eurasian otter is about 38-38.5°C (REUTHER & BRANDES 1984, KRUUK 1997, KRÜGER & KUHN 2003). Nonetheless, the body temperature undergoes important fluctuations depending on the activity. Indeed, KRUUK (1997) found in wild and captive otters, that the Tb tended to increase by about 1.2°C at the beginning of a period of activity, and then fell during swimming down to Tb at rest, the mean swimming bout length being about 30 minutes and the water temperature below 16°C. In a captive female with a mean inactivity Tb of about 38.5°C, Tb rose by 1.5-2°C during periods of activity (KRÜGER & KUHN 2003). During these periods of activity, which lasted from 45 minutes to almost 4 hours, the otter always switched between water and land, being rarely more than 5 minutes on land and almost never more than 5 minutes in water.

Fig. 136: Male captive Eurasian otter sleeping on the ice

We saw that in several semi-aquatic species hyperthermia occurs in air above 30°C (see 5.4.4). KRATTENMACHER & RÜBSAMEN (1987) measured that in the Coypu, the TNZ of which ranges between 20 and 30°C, the body temperature increased to more than 3°C above normal at Ta=35°C, and referred to WARKENTIN (1970), who observed that in the wild, coypus usually stay in water at Ta above 34°C. In the Australian Water rat, hyperthermia occured also in air at 35°C (FANNING & DAWSON 1980). In *Castor fiber*, Tb was 2°C above normal after 30 min in air at 35°C (STEEN & STEEN 1965b). MACARTHUR (1989) found that in *Castor canadensis*, the TNZ may go from 0°C to 28°C, and also reported that the animals were able to maintain a constant Tb in air at –20°C. He quoted several authors

who observed an aversion of beavers to subzero temperatures. The lower critical temperature in water is near 20°C (MACARTHUR & DYCK 1990).

Beavers are less well insulated than otters (see chap. 3), but they are generally larger, which is thermoregulatory advantageous at low temperatures. Beavers also have advantages and disadvantages over Eurasian otters regarding heat loss. First, they dispose of a larger body surface available for heat loss than the Eurasian otter, in view of the fact that tail and feet account for about 18% of total surface (MACARTHUR & DYCK 1990). Moreover, unlike *L. lutra*, they do not have an elevated BMR that compounds the risk of hyperthermia. On the other hand, they possess a unique morphological feature that protects larynx and trachea from water intrusion during underwater foraging, but also precludes open mouth breathing and panting during heat stress (MACARTHUR 1989).

Eventually, considering our observations, available data on otter thermoregulation and data from other semi-aquatic mammals, we expect the Eurasian otter to suffer from hyperthermia during longer periods of activity in air at Ta above 30°C. The lower limit of the TNZ in air is probably clearly below 0°C. Further studies would be necessary to determine the exact TNZ of the Eurasian otter and to evaluate the air temperature at which it may encounter difficulties to regulate its body temperature.

5.5 Conclusion

The Eurasian otter relies mainly on its feet to dissipate excess heat, whereas the Small-clawed otter, and, particularly, the Giant otter can loose more heat through the whole body. In all three species, the temperature of the feet decreases to a temperature similar to that of the water when the animal is submerged, so that in water heat loss from the feet is reduced to minimal values. The tail also exhibits a very labile surface temperature. Indeed, the Giant otter, and probably also the Small-clawed otter, use their tail to dissipate excess heat. In those species, the tail follows the same thermoregulatory pattern as the feet when the animal is in the water. In the Eurasian otter, the insulating air layer, trapped below the surface of the fur, exists even at the tail. However, in colder water (10°C in this study) or during longer swimming bouts, this insulating air layer is not maintained, and then the skin of the tail cools down to water temperature. Despite of the heat loss that this induces, a selective heating of the sensory organs insures their good functioning, particularly in the Eurasian otter.

During our study, we did neither encounter subfreezing temperatures nor temperatures above 30°C. Moreover, the otters almost never stayed on land longer than a few minutes, except for resting, and thus recordings of a dry otter could be done almost only when the access to the water was prevented, which was not always possible. So, further studies would be necessary to complete our information on surface temperature regulation in the Lutrinae, including exposure of the animals to a wider range of air and water temperatures as in our

study, and further recordings of otters kept dry. In particular the data on tropical species need to be completed. As far as it is possible, the animals studied should be kept individually during the recording sessions. However, great caution should be accorded to the temperatures at which the animals are exposed. Whereas Eurasian otters are not impeded by subfreezing temperatures, longer exposures to high temperatures could lead to overheating. Even in the tropical species, longer exposures to high temperatures without access to water should be considered carefully. Small-clawed otters can be exposed to temperatures colder than those encountered in our study, but subfreezing temperatures should be avoided. In captivity, Small-clawed otters are usually not exposed to temperatures below 5°C, and this threshold is probably higher in many zoos. To expose Giant otters to temperatures colder than those that they encounter in their natural habitat is not really conceivable. An IRT study of body surface temperature associated with recordings of body core temperature, using intraperitoneally inserted transmitters, would be an interesting follow up study. Moreover, the control of the body core temperature could allow to prevent dangerous hypo- or hyperthermia during exposure to different ambient and/or water temperatures.

When planning an IRT study of otters, one has to keep in mind that those animals are as excited as they are exciting. Their very boisterous nature associated with their rather small size makes thermal recordings difficult, particularly the focussing of the thermocamera. Even tame animals do not stay still in front of the camera and may be bothered by the experimenter after a while. Thus, otters are very interesting but difficult study objects for IRT.

CONCLUSIONS

This study demonstrated that the primary hair (PH) characteristics are quite constant within an otter species. Even the PHs from different body regions are quite similar to each other, except for the hairs from the head, which moderately differ, mostly by being shorter and thinner. The greatest differences are observed between the PHs of Eurasian otters coming from a temperate region and those coming from a tropical region.

The PHs of the different otter species share many common characteristics, but they also show some significant divergences. The hair structure seems to be influenced by phylogenetic relationships and by adaptive pressures. Generally, the species living in colder climates have longer primary and secondary hairs, but the microscopic features of the hairs also play an adaptive role.

This work confirmed the exceptional high hair density of the otter fur. The Eurasian otter (*Lutra lutra*) has between about 60,000 and 80,000 hairs/cm², and the Sea otter (*Enhydra lutris*) between about 120,000 and 140,000 hairs/cm². Most of the hairs are secondary (wool) hairs (hair coat with only about 1% of PHs). The hair density is quite constant over the body (appendages excluded), except for the Sea otter that has fewer hairs on the head (about 60,000 hairs/cm² at the cheek). In the Eurasian otter, hair density is not influenced by the sex of the animal, and there is no relevant seasonal variability.

An additional adaptive feature which improves the thermal insulative quality of the hair coat of otters, is found related to the cuticle of the secondary hairs. Here, the shape and arrangement of the scales allow a flexible interlocking of adjacent hairs, which facilitates the trapping of the insulating air layer. This characteristic is apparently not or only slightly influenced by factors like climate or relation to the aquatic environment.

Our infrared thermographic study showed that the air layer trapped in the fur of the Eurasian otter could be maintained at a temperature above 20°C, even in water colder than 10°C. The Eurasian otter relies mainly on its feet to dissipate excess heat, whereas species living in warmer climates have greater possibilities to lose heat. From the later group, for example, the Small-clawed otter, and, particularly, the Giant otter can dissipate more heat throughout the whole body. In all three species, heat loss from the feet is reduced to minimal values when the animal is submerged, by a decrease of the temperature at the surface of the feet to a temperature similar to that of the water. The Giant otter, and probably the Small-clawed otter, also use their tail to dissipate excess heat.

Generally, the difference between the insulative properties of the hair coat of the different species may be principally due to differences in hair length and hair density. However, the data on tropical otters need to be completed. There is still no information on the hair density in otter species living in warm climates, due to a lack of material to study. An interesting fol-

low up study would be to measure the thermal conductivity of the fur of each otter species in air and in water, because this information indicates the insulative value of the hair coat, which is actually influenced by a combination of all the hair coat parameters. Unfortunately, here again, the accessibility of appropriate material may be problematic for many otter species.

Although some answers are still missing, we could emphasize the high level of adaptation of the hair coat of the different otter species. Even the microscopic structure of the hairs apparently plays an adaptive role. We could also observe that otters possess effective mechanisms to regulate heat loss through the less-furred parts of their body.

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In the days when the earth was new and there were no men but only animals, the sun was far away in the sky. It was so far away that there was no summer. It was so far away that the trees and the grasses did not grow as they should.

He-Who-Made-the-Animals saw how it was that there was not enough sun to heat the earth, and so he fashioned a snare. The Sun did not see the snare in his path, walked into the snare, and the snare held him fast.

Then the sun was close to the earth. In fact, the snare held the sun so close to the earth that there was no night. Day after day the sun shone and the earth dried and the grasses withered. There was not enough food or water for the animals and they desperately called a council. "Sun," the animals said, "You give too much heat to the earth."

"Set me free from this snare" the Sun said, "and I will go away."

"But if you go away, then there will not be enough heat." "Set me free," the Sun said, "and I will come to the edge of the earth in the morning and in the evening; then at noon-time I will stand straight above the earth and warm it then."

The animals sat around the council fire and they said, "Who is going to set the sun free?"

"I shall not do it," Wildcat said. "Whoever sets the sun free must go so close to the sun that he will be burned to death." Lynx said, "Whoever sets the sun free must chew the leather thong that holds him; the sun will burn him to death before he can do it."

"I shall not do it," said the deer, the wolf and the raccoon. "I shall do it," Otter said. "How can you do it?" said the animals. "You are too small, your teeth are for fish, and your fur has already burned away." None of the other animals liked the otter because he played too much.They did not think he was brave.

"Let him try," Bear said. "He will burn to death, but we will not miss him. He is of no use to us. He looks silly now that his fur is gone." The animals laughed.

Ignoring the taunts, the otter set off to the place in the sky above the earth where the sun was held by the snare. Otter took many days to get to the sun. The sun burned him. The sun was so bright, Otter had to close his eyes. When he reached the sun, Otter began to chew on the leather thong that held the sun. His skin was burning and blistering, his eyes were hot stones. But Otter did not stop chewing.

Suddenly he chewed through the leather. The animals saw the sun rise into the sky. The animals felt the cool winds begin to blow on the earth. Otter had freed the sun from the snare.

Time passed. Otter lay in the center of the council ring. There was no fur at all left on his body. His skin was burned and scorched and his flesh was falling off his bones. His teeth were only blackened stumps.

He-Who-Made-the-Animals also stood in the center of the council ring. "Otter," he said, "the animals will not forget what you have done for them. I will see that they do not forget," and he gave Otter new strong teeth, tireless muscles, keen eyesight, and a powerful tail to help him in his hunting and in his play. He did not have to give him bravery. But he gave him new fine fur that was like down on his skin, and a second coat of fur to guard the first so that he would not get cold in water or in winter. Then he gave him joy so that he would always be happy in his otter's life, and Otter has so remained until this day.

An Otter Legend derived from the Cree Indians
Contributed by John Mulvihill
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