

A contribution to the understanding of the self-rubbing behaviour in psammophiid snakes (Squamata: Psammophiidae)

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Abstract. The family Psammophiidae is unique in its behaviour among snakes in applying a special secretion of their nasal glands on their own abdomen and flanks. This so-called self-rubbing behaviour has not been experimentally analysed so far. We provide evidence that self-rubbing behaviour is temperature-dependent and helps to protect the snakes against desiccation due to the lipids contained in the nasal secretion. A SEM study of the scale ultrastructure of several psammophiids shows that the complex ultrastructure serves to optimally keep the secretion on the scale surface. We furthermore document the occurrence of self-rubbing behaviour for two species of *Rhamphiophis*, viz. *R. rostratus* and *R. rubropunctatus*, bringing the number of psammophiid genera from which this behaviour is known to seven (of the eight currently recognised genera), from two more species, viz. *Psammophis elegans*, and *Psammophylax acutus*.

Key words. Squamata, Colubroidea, Psammophiidae, scanning electron microscopy, gas chromatography, behaviour.

Introduction

Snakes of the family Psammophiidae have a vast distribution in Africa, the western and central Palearctic region as well as the Oriental region, mostly inhabiting arid regions (BROADLEY 1962, BRANCH 2005, HARTMANN et al. 2011). Until 2008, their systematic status and taxonomic rank used to be uncertain for a long time. The eight genera recognized then, viz. *Dipsina*, *Dromophis*, *Hemirhagerhis*, *Malpolon*, *Mimophis*, *Psammophis*, *Psammophylax* and *Rhamphiophis*, were either considered a tribe of various subfamilies (e.g., Colubrinae, BRANDSTÄTTER, 1995) or a subfamily in their own right (BOURGEOIS 1968, KELLY et al. 2008, VIDAL et al. 2008).

The molecular phylogenetic work of KELLY et al. (2008, 2009) grouped the above mentioned genera in one family, Psammophiidae, and clarified some relationships between and within genera. PYRON et al. (2010) granted them only a lower hierarchical rank, i.e., a subfamily Psammophiinae within the family Lamprophiidae. Here, we adopt KELLY's (2008, 2009) view because of the many apomorphic character states of this group of snakes, both in morphological and in physiological ecological respect. *Dromophis*, because it appeared to be nested within *Psammophis*, was synonymised with the latter (see also HUGHES 2004) while one species of *Rhamphiophis*, viz. *R. acutus*, turned out to cluster with *Psammophylax* and was consequently transferred to that genus. Likewise, KELLY et al. (l.c.)

found a rather deep split between the *Malpolon monspessulanus* group ("true" *Malpolon*) and "*Malpolon*" *moilensis*, thus supporting the recognition of a separate generic status for the latter. The generic name *Scutophis*, coined by BRANDSTÄTTER (1995) in his PhD thesis for the latter species, turned out to be antedated by *Rhagerhis* PETERS, 1874, which is consequently the correct nomen for this monotypic genus (BÖHME & DE PURY 2011). Taxonomic problems still remain on the species level mainly of *Psammophis*, particularly in the so-called *P. sibilans* complex, where the taxonomic concept is not yet stable.

Morphologically characteristic of all Psammophiidae is the extremely small, filiform or even "vestigial" (SHINE et al. 2006) hemipenis of the males (DOWLING & SAVAGE 1960) (Fig. 1). This makes the identification of sexes more difficult than in other snakes, where males are characterised by swollen hemipenial pockets on their ventral tail base (MATTISON 2007). On the other hand, one can assume that courtship and mating behaviour might be also more complex and more ritualised in psammophiid snakes, to make a successful copulation with these minute organs possible. This has been demonstrated so far at least for *Malpolon monspessulanus* (DE HAAN 1982, 1999).

The most unusual characteristic of the Psammophiidae, however, is their behavioural system of "polishing" or "self-rubbing", by means of which the secretion of their nasal glands is smeared along their flanks and bellies by zigzagging head movements (DE HAAN 1999, COTTONE & BAUER

2009; Figs. 11 & 12, this paper). This nasal secretion is composed of lipids, proteins, fatty acids and K⁺ and Na⁺ ions (DUNSON et al. 1978). Until now, self-rubbing behaviour has been observed in species of six genera: *Hemirhagerrhis* and *Mimophis* (DE HAAN 2003), *Malpolon* (DE GRIJS 1898, DE HAAN 1999), *Psammophis* (DUNSON et al. 1978, DE HAAN 1982, BRANCH 1988, BRANDSTÄTTER 1995, 1996), *Psammophylax* (STEEHOUDER 1987, COTTONE & BAUER 2009), and *Rhagerhis* (DE HAAN 1982). Corresponding behavioural data for *Dipsina* and *Rhamphiophis* are still missing.

The function of self-rubbing left space for various speculations. Considering that the secretion applied is rich in lipids (DUNSON et al. 1978), several authors considered self-rubbing as protecting the skin against water loss (BRANCH 1988, LAHAV & DMI'EL 1996, WELDON et al. 2008). Accordingly, *Malpolon monspessulanus* (DUNSON et al. 1978) and *Psammophis schokari* (LAHAV & DMI'EL 1996) were shown to have extremely low rates of evaporative water loss compared to other snakes living in the same habitat. This hypothesis received further support from some anecdotal reports that self-rubbing frequency was lower when the snakes were exposed to higher humidity (STEEHOUDER 1992, BRANDSTÄTTER 1996) and became higher when snakes were exposed to higher temperatures and drier conditions (DAREVSKY 1956, RIEPPEL 1973, DE HAAN 1982, STEEHOUDER 1987, 1992).

Lipids are well known to have a protective function for the reptilian skin. Situated in the mesos layer of the stratum corneum (IRISH et al. 1988, WELDON et al. 2008), they provide an efficient barrier to water permeation (BURKEN et al. 1985, ROBERTS & LILLYWHITE 1980, 1983, BAEYENS & ROUNTREE 1983) and reduce water loss through the skin (KATTAN & LILLYWHITE 1989, EYNAN & DMI'EL 1993, DMI'EL 1998). The lipids reach the scale surface through minute pores in the ultrastructure of the skin (CHIASSON et al. 1989).

Another hypothesis concerning the function of self-rubbing in psammophiids was put forward by DE HAAN (1999, 2003) for *Malpolon* and by COTTONE & BAUER (2009) for *Psammophylax*. According to these authors,

self-rubbing is used to mark substrates, territories and conspecifics. Marking of hunting routes or territories occurs automatically when the snakes crawl on the surface after self-rubbing. Marking of conspecifics, as observed in *Malpolon* under captive conditions, happens when one individual crawls over another, so that the particles of the dried secretion collect in its grooved dorsal scales (DE HAAN 1999, 2003), a trait unique to the genus *Malpolon*. According to these authors, this marking behaviour is particularly important during the mating season when the secretion is supposed to be a chemical marker for females and other conspecifics.

The concept of "territoriality" in snakes is a difficult one to evaluate due to the definition of territoriality itself. In mammals or in birds, a territory is defined as an area defended throughout the activity period of the animal. However, this definition cannot generally be applied to snakes (GILLINGHAM 1987, RIVAS & BURGHARDT 2005). At present, territoriality in snakes is only known during the mating period, like in *Vipera berus* (ANDRÉN, 1986) or *V. aspis* (SAINT-GIRONS, 1996). The term territoriality has thus to be applied with precaution, also in the case of psammophiids and their self-rubbing behaviour.

The objectives of this paper are: (1) to perform a SEM analysis of the ultrastructure of psammophiid scales and to compare it with non-psammophiids from similar and different habitats, (2) to analyse the composition of the nasal secretion in *Psammophis* species and *Rhagerhis*, and (3) to carry out a detailed analysis of self-rubbing in psammophiids, meaning to determine under which conditions it is performed, and to discuss its possible functional significance.

Material and methods

Scanning electron microscopy

The ultrastructures of the dorsal scales of 17 psammophiid snakes (six *Psammophis mossambicus*, four *P. schokari*, one *Malpolon insignitus fuscus*, two *Psammophylax a. acutus*,

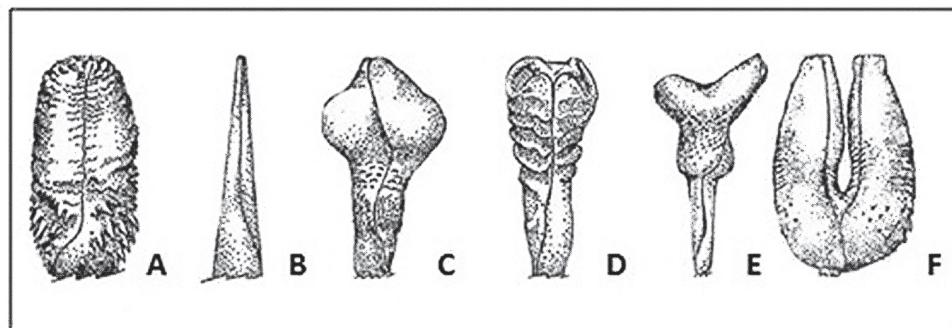


Figure 1. Hemipenial morphology showing the unornamented and filiform hemipenis of *Psammophis sibilans* (B) compared to the ornamented and structured one of: (A) *Spalerosophis diadema*, (C) *Spilotes pullatus*, (D) *Charina bottae*, (E) *Pantherophis obsoletus*, and (F) *Crotalus viridis* (after DOWLING & SAVAGE, 1960).

four *Rhagerhis moilensis*), and ten non-psammophiid reference taxa (with partly convergent morphologies such as *Hemorrhois hippocrepis*, *H. algirus*, *Masticophis mentovarius*, *M. flagellum*, *Philodryas psammophideus*, *Liophis typhlus*, *Demansia psammophis*, *Nerodia rhombifera*, *Natrix natrix*, *Thamnophis sirtalis*) were analysed. Oberhautchen samples from preserved specimens or shed skins of live specimens were analysed (BURSTEIN et al. 1974, IRISH et al. 1988). Two or three vertebral scales and one lateral scale (first row of scales in contact with the ventral scales, slightly larger than the dorsal scales) were removed from about 7 cm before the cloaca for this purpose. The centre of each scale was analysed.

The samples were coated with 20–20 nm of gold (4A) in a Hummer VII Scanning Electron Microscope Sputtering System (Anatech LTD) during 5 minutes of exposure. All samples were viewed at 0° tilt, 25 kV and 70–90 µA with an S-2460N Scanning Electron Microscope (Hitachi Scientific Instrument) (see JOSEPH et al. 2007). Specimen examination was performed at low (600–1250 ×) and medium (2000 and 5000 ×) magnification for the description of the general scale pattern. Published studies of scales had previously shown that pores become visible usually at magnifications of 4000–5000 × and have a dimension inferior to 1 µm (STILLE 1987, CHIASSON et al. 1989, JOSEPH et al. 2007).

Gas chromatography

To analyse the composition of the nasal secretion of *Psammophis mossambicus* and *Rhagerhis moilensis*, the secretion of two snakes of each species was collected during one week to be analysed by gas chromatography-mass spectrometry (GC/MS). This method allows the separation of the various components of a given solution. Each solution has a given retention time, which is the time a component of the solution requires to pass through the system. The mass-to-charge ratio (m/z) is a physical quantity, showing the number of molecules or fragments in a given solution (expressed in atomic mass units or amu).

The middle ventral and lateral parts of the body of the snakes were scraped off at random times with a cover glass to collect dried secretion (DE HAAN & CLUCHIER, 2006). The slides were then placed in 3 ml of hexane to dissolve the nasal gland secretion. Each solution was stored in a freezer until analysis by GC/MS. Before analysis, the hexane was removed by rotary evaporation so that a thin layer of residue was deposited on the recipient. This residue was dissolved in 50 µl of methanol. 1 µl of these 50 µl was then used for GC/MS. Gas chromatography was carried out on a Perkin Elmer AutoSystem XL gas chromatograph with a Perkin Elmer Turbo Mass mass spectrometer. The column was a fused silica PE-1 column (30 m × 0.32 mm × 0.25 µm). The carrier gas was helium (2 ml, split 1:30). Injection temperature was 230°C. The oven temperature was kept at 80°C for 3 min isotherm and then programmed to ramp up to 320°C by steps of 10°C/min. Then the temperature of 320°C was maintained for 8 min.

Two additional control samples, once only hexane and one hexane plus glass slides, were also measured to assure that neither the solvent nor the glass were contaminated.

Behaviour

Seventeen psammophiid snakes (six *Psammophis mossambicus*, four *P. schokari*, one *Malpolon insignitus fuscus*, four *Rhagerhis moilensis*, and two *Psammophylax a. acutus*) were studied in respect of their behaviour. Except of the *M. i. fuscus*, which was a large female, it was unfortunately impossible to identify the sexes of the snakes with certainty, due to the lack of distinct sexually dimorphic characters. In contrast to *Malpolon*, the other psammophiids have no marked sexual dichromatism, and the tiny hemipenes of all psammophiids, i.e., the lack of swollen tail bases in males, would make sexing possible only post mortem of the specimens by dissecting the body cavity; we refrained from killing our study animals after the experiments because of ethical reasons.

“Continuous recording” with cameras was chosen as a method to record self-rubbing behaviour (MARTIN & BATESON 1993). This method has the advantage of leaving the snake undisturbed so that it can perform its behaviour without disturbance by an observer. Two observation terraria (with dimensions of 114 × 80 × 120 cm and 100 × 80 × 70 cm, respectively) were used for the experiments. Both contained a bedding of soil or sand about 2 cm deep. During the shedding cycle or when a snake was ill, no experiments were conducted with this snake. All terraria were cleaned and disinfected (with Sterilium) between the individual experiments.

Self-rubbing in this study was defined as follows: Touching the nostril to the belly or flank followed by zigzagging movements of the head along the entire body at least to the cloaca.

Marking behaviour: Preliminary observations were made to determine the activity periods of the snakes, whether they would perform RB (rubbing behaviour) in the presence of an observer, the location they would perform it in, and how much time they needed to perform RB when they were placed into a new terrarium. The first part of the experiment was to determine whether self-rubbing behaviour would be used as a means of marking a new, unknown area (here: a terrarium) as has been proposed for *Malpolon monspessulanus*. One *M. insignitus fuscus*, six *Psammophis mossambicus*, four *P. schokari*, two *R. moilensis* and two *Psammophylax a. acutus* were tested thus. It was expected that self-rubbing would be more frequent on the first day when the snake marked its “new territory”. The frequency of RB on the first day was compared with a paired sample t-test to the mean frequency of the four following days. The data were analysed using SPSS Statistics 18 Software (SPSS Inc., USA). The snakes were filmed for 8 h/day.

The second part of this experiment was meant to determine whether the scraped-off secretion of one snake would

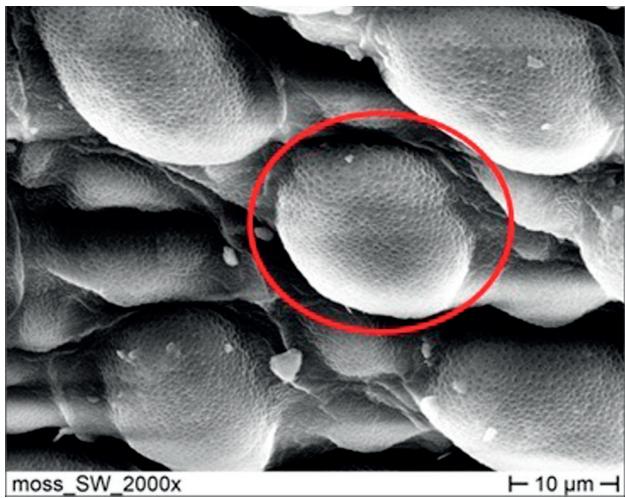


Figure 2. Porous cells of the stratum corneum in *P. mossambicus*. The red circle designs on of those porous cells (2000 ×).

elicit any reaction from a second, conspecific snake. For this experiment, a glass plate was placed in the middle of the terrarium to make the secretion on it visible (BRAND-

STÄTTER 1995). Four adult *P. mossambicus* and four adult *Rhagerhis moilensis* were tested. For each species, the gender of only two snakes (females) could be determined on the basis of their smaller sizes (*Rhagerhis*) and because one had previously laid eggs (*Psammophis*). For each species, one snake was chosen at random to be placed at first into the cleaned terrarium for four hours. Our preliminary experiments had shown that RB is mainly performed during the first 60–90 minutes after being placed into a new terrarium. After four hours the first snake was removed and the terrarium was checked, wearing gloves, whether any traces of secretion were visible on the glass plate. Then, the second snake was placed into the terrarium for the same period of time, without cleaning the terrarium between both snakes. The video recordings were then scanned for whether or not the second snake had followed the trails of the first one.

External factors: In order to determine whether self-rubbing behaviour might depend on external (extrinsic) factors, the temperature was varied in an experimental design. To induce self-rubbing behaviour, each snake was exposed to four different temperatures during a period of one week each. Temperature was provided by light bulbs of 40, 75, 100 and 80 W (reflector) mounted 30 cm above the ground. This created temperatures of 27.24, 31.72, 38.62

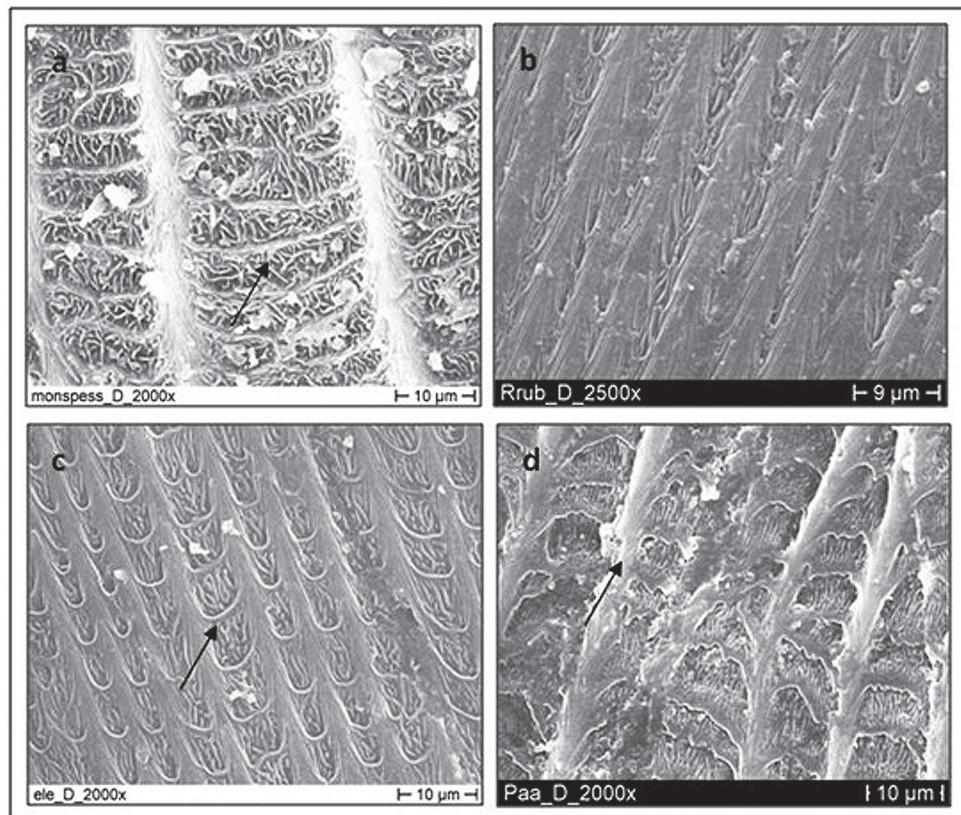


Figure 3. The four main types of micro-ornamentation in psammophiids: (a) scalar (*M. i. fuscus*, 2000 ×), (b) caniculate (*R. rubropunctatus*, 2500 ×), (c) cup-like flat (*P. elegans*, 2000 ×) and (d) cup-like ridged (*P. a. acutus*, 2000 ×). The arrows show (in a) the linear junction between the ridges, (in c) the curved junction between the ridges, and (in d) the raised ridges.

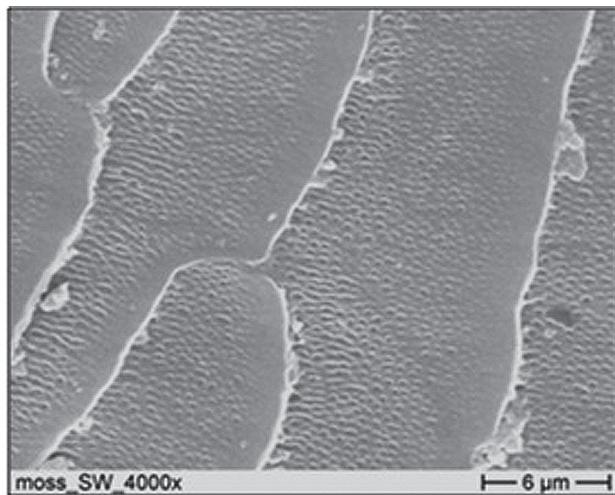


Figure 4. The basis of the oberhautchen: several stripes with parallel orientation have slightly denticulated anterior fringes. The surface of these stripes is peppered with small pits.

and 67.16°C, respectively, on the ground under the bulb. Each snake was exposed at random to these temperatures. All snakes had the option to escape the heated area. To obtain frequency data of self-rubbing behaviour (number of self-rubbing events performed per hour), the total number

of self-rubbing performances shown by each snake at the given temperature was divided by the observation time for this condition. The data obtained thus were analysed with the rank Spearman correlation coefficient test (SPSS Inc., USA 2009).

Results

Scanning electron microscopy

The ultrastructure of the scales differed with the scale region studied (apical vs. basal). The basal attachment zone of the scale comprises highly porous cells of the stratum corneum responsible for metabolite exchange (POCKRANDT 1936), which are clearly visible at 2000 × magnification (Fig. 2, for *Psammophis mossambicus*). The microstructure of the central parts of the scale consists of longitudinal parallel ridges ("alignments of indentation" after PAUWELS et al. 2000), extending from the basal to the apical parts of the scale, well visible at 600–800 × magnification. The spaces between the ridges of these vermicular structures vary slightly with the species at hand (Fig. 3). The basis of the oberhautchen itself consists of several parallel strips with slightly denticulate anterior margins. The surface of the oberhautchen is spattered with small pits in a grid-like pattern; this pattern corresponds posteriorly to the vermicular structure between the striations (Fig. 4). No pores could be

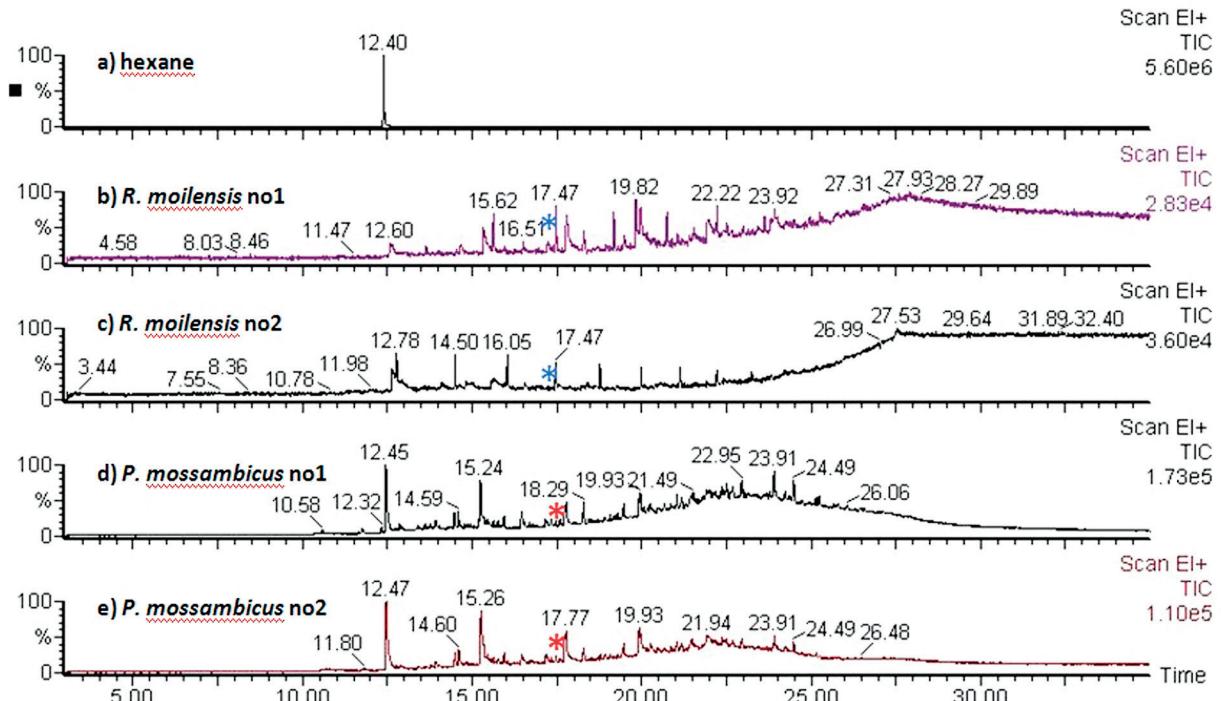


Figure 5. Gas chromatograms of: (a) the control samples hexane and hexane with glass slides; only one graph is represented here; (b) *S. moilensis* no1, (c) *S. moilensis* no2, (d) *P. mossambicus* no1, and (e) *P. mossambicus* no2. The retention times in minutes are indicated on the x-axis. The relative ion abundance (or intensity) is on the y-axis. The peaks indicated in red were analysed for their composition. TIC: total ion chromatogram.

identified on the surfaces of the median dorsal scales of the psammophiid snakes or any of the control taxa used, neither at $5000 \times$ nor at higher magnifications.

Gas chromatography

The chromatograms received from the secretion of the two *R. moilensis* were similar to each other, as was also the case with those of the two investigated *P. mossambicus* specimens. They differed, however, when compared between

species (Fig. 5). In *R. moilensis*, the only peak with the same retention time was the one at 17.47 min. A peak appearing at 17.77 min was detected in both *P. mossambicus*.

Scans of these peaks in *R. moilensis* revealed differences between the two specimens: *R. moilensis* no2 (Fig. 7) showed masses (*m/z*) of 300 amu and more which were absent in the scan of *R. moilensis* no1 (Fig. 6). A difference of this type was also detected in the scans of the *P. mossambicus* samples, but proved to be much smaller than in *R. moilensis*: In *P. mossambicanus* no1, an additional (*m/z*) of 296,8 amu appeared (Figs. 8 and 9).

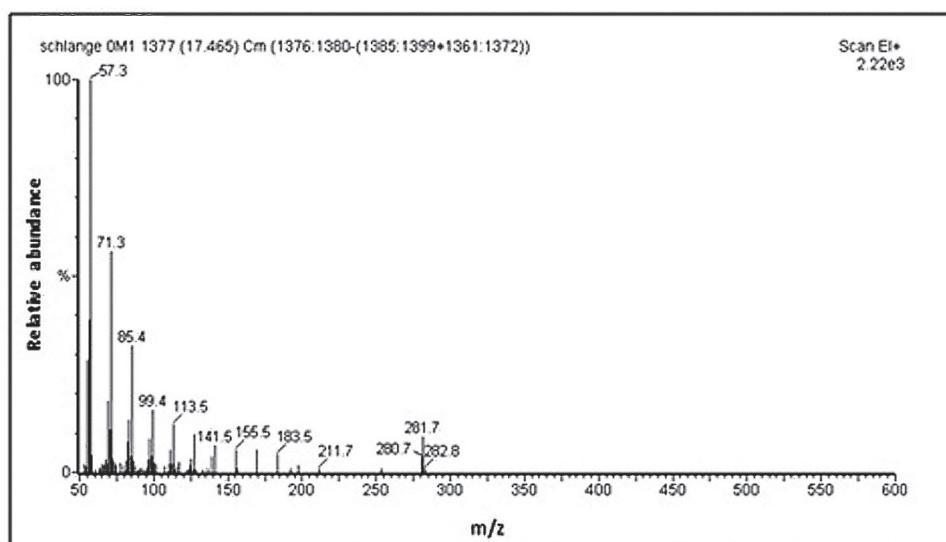


Figure 6. Mass spectrum (*m/z*) of the peak at a retention time of 17.47 min. in *S. moilensis* no1. Four instances of mass-to-charge > 200 amu were identified.

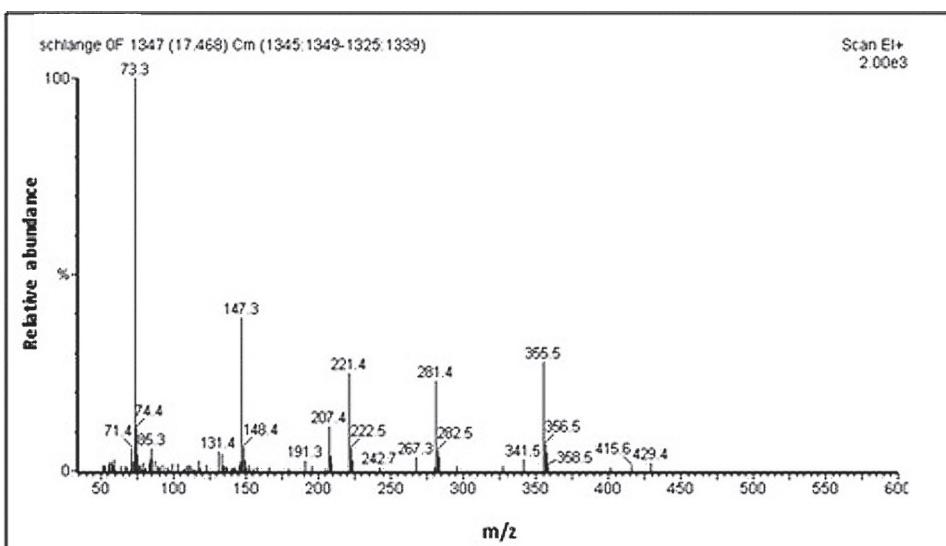


Figure 7. Mass spectrum of *S. moilensis* no2 at 17.47 min. Thirteen fragments with a mass > 200 amu were detected, although it is the scan at the same retention time as for *S. moilensis* no1.

The masses could not be assigned with certainty to one defined lipid since it had to be considered that these masses could also be those of fragment ions of a lipid with a higher molecular mass. Because of this reservation, no specific type of lipid could be identified in the two species studied.

Behaviour

Marking behaviour: In the first part of the experiment, rubbing frequency of a snake on its first day in an unfam-

iliar environment was compared to the mean rubbing frequency displayed within the following days when the snake had become used to its new environment. Rubbing frequency was not significantly higher on the first day than during the following days in the 15 psammophiids tested (overall paired sample t-test, $T_{14} = 0.158$; $N = 15$, $p = 0.877$).

The second part of the experiment was conducted to see whether the secretion acted as an olfactory trail marker for conspecifics. None of the individuals of both species studied had left visible traces of secretion on the glass plate, although all snakes had performed at least one rub-

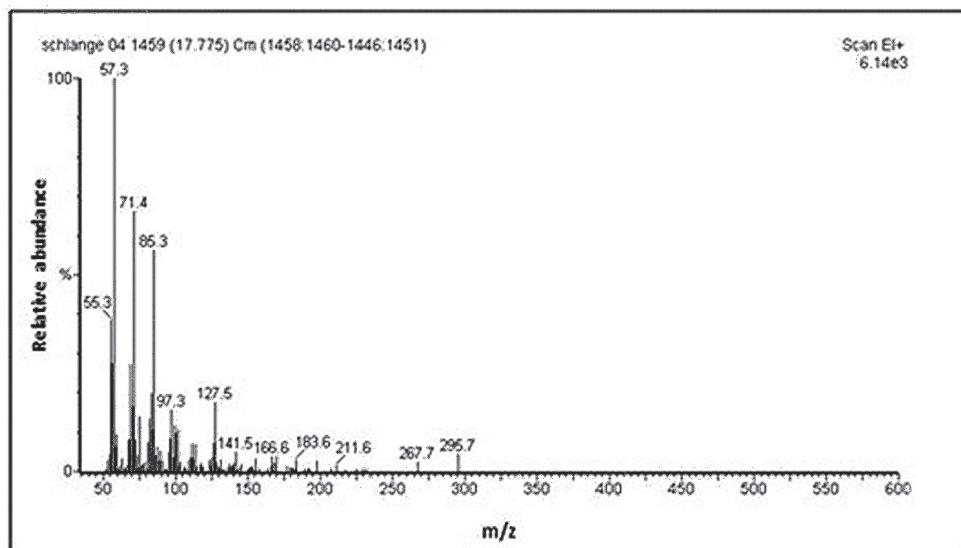


Figure 8. Mass spectrum of the peak at retention time 17.77 min. of *P. mossambicus* no1. Only three fragments with a mass-to-charge > 200 amu were detected. Masses-to-charge 267.7 and 295.7 were found in both snakes (see Fig. 9).

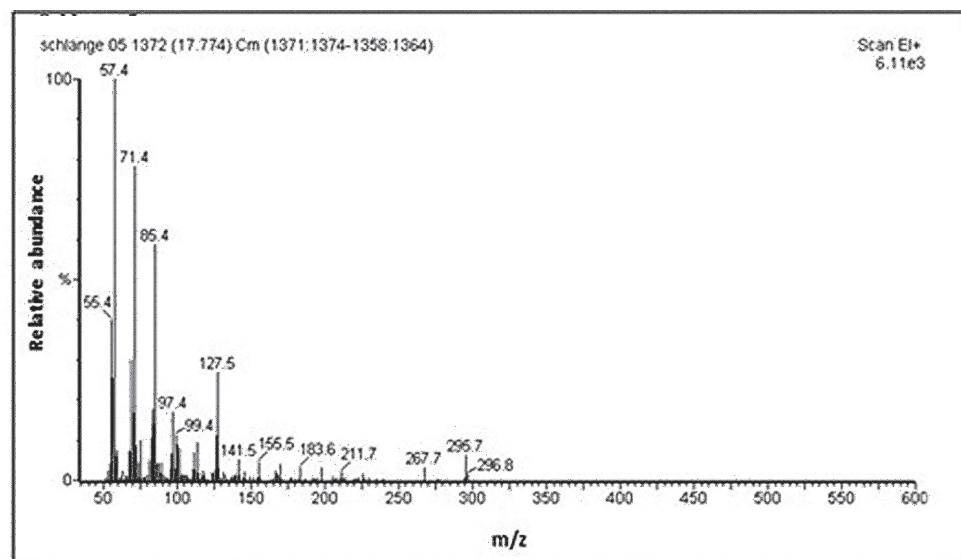


Figure 9. Mass spectrum of the secretion of *P. mossambicus* no2 at 17.77 min. The fragments with the masses-to-charge (m/z) 267.7 and 295.7 amu were also found in *P. mossambicus* no1.

bing sequence and crossed the glass plate several times. All snakes behaved as they did in their control runs, independent from whether they were used first or second. As stated above, we were not sure about the sexes of the individuals in the “couples”, but there was no sign in the behavioural responses that differences between males and females should have played a role here.

External factors: The dependency of rubbing behaviour on ambient temperatures was tested. Rubbing frequency increased in a linear manner with increasing temperature in the psammophiids tested (Fig. 10).

The intraspecific analyses showed a correlation between temperature and rubbing frequency in *Psammophis mossambicus* and *P. schokari*. No dependence of rubbing frequency on temperature was seen in *Malpolon insignitus fuscus*, *Rhagerhis moilensis* and *Psammophylax a. acutus*.

Rubbing behaviour of *Rhamphiophis rostratus* and *R. rubropunctatus*, *Psammophis elegans* and *Psammophylax a. acutus*: Rubbing behaviour could be observed for the first time in *Rhamphiophis rostratus*, *R. rubropunctatus*, *Psammophylax a. acutus*, and *Psammophis elegans*. *Rhamphiophis* and *Psammophylax* performed the behaviour with only one nostril in a movement similar to that described above for *Malpolon* and *Rhagerhis* (Fig. 11). The individual of *Rhamphiophis rostratus* observed sometimes rubbed its

body down to the tail tip twice, alternately using each nostril (Tab. 1). The durations of rubbing sequences in these species can also be seen in Tab. 1. In *Psammophylax a. acutus*, the head movements were less distinct as compared to the representatives of the other genera. Both individuals often rubbed their bodies incompletely, for example limiting an instance to 5 cm of the body and attending to other body parts only hours later. These short or minor rubbing sequences were not counted as normal rubbing behaviour. In *Psammophis elegans*, rubbing behaviour was performed just as in *P. mossambicus* and *P. schokari*, with both nostrils being used in an alternating manner to apply the nasal gland secretion (see Fig. 12); for durations see Table 1.

Discussion

Scanning electron microscopy

The SEM analysis of the scale microstructure revealed that pores are absent from the surface of dorsal scales in both psammophiid snakes and our control taxa. So far, the statement of CHIASSON et al. (1989) that all snakes have pores in their dorsal scales to allow the passage of lipids to the surface could not be substantiated in our study. When removing the oberhautchen from the scale, no imprints of pore-

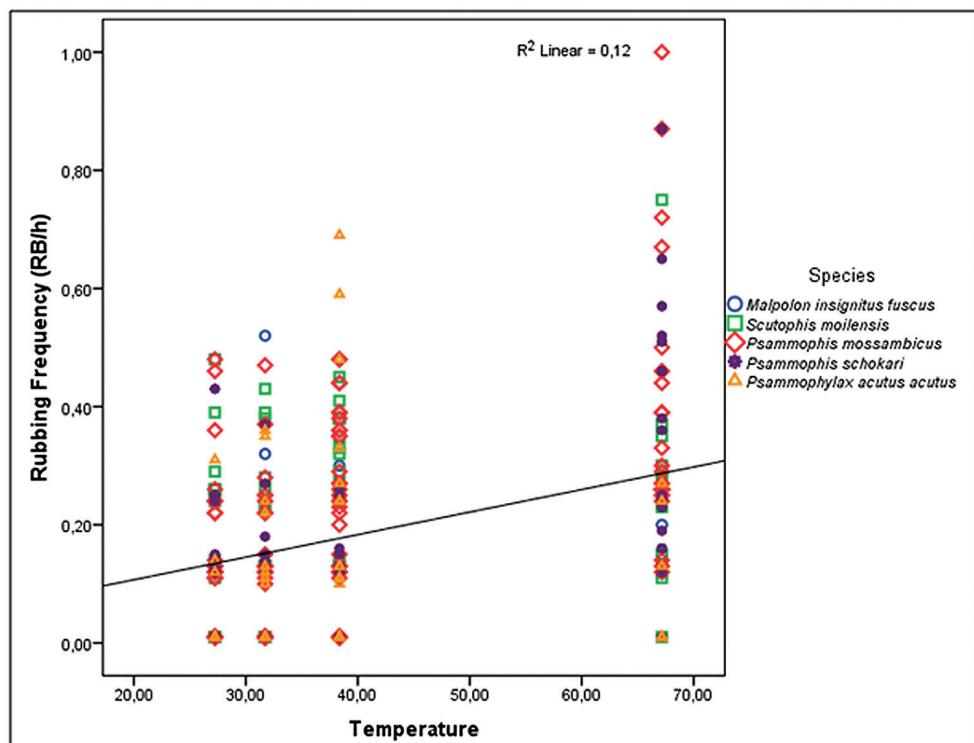


Figure 10. Influence of temperature on rubbing behaviour in the psammophiids studied: The overall analysis of the 17 snakes studied showed that the frequency of rubbing behaviour was dependent on temperature.

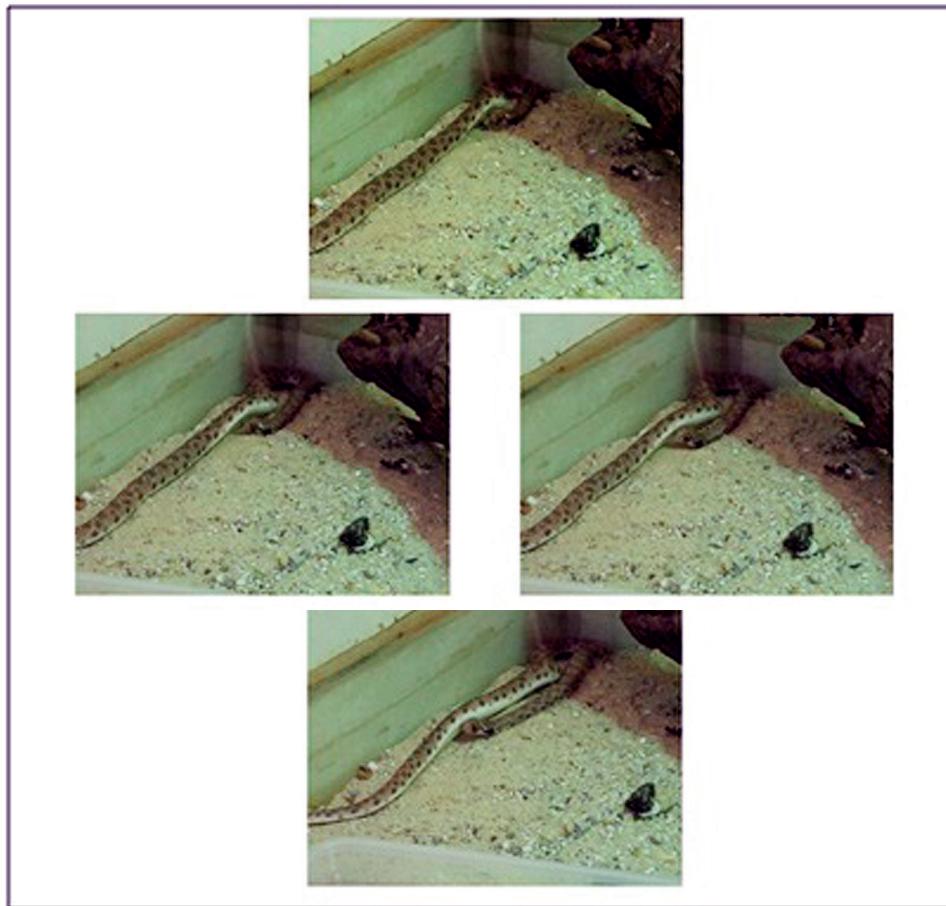


Figure 11. Sequence of a rubbing “act”, here in *Rhagerhis moilensis*. The movements are similar to those observed in *Rhamphiophis* and *Psammophylax*. The belly is slightly lifted from the ground to facilitate the application of the nasal gland secretion with zigzagging head movements.

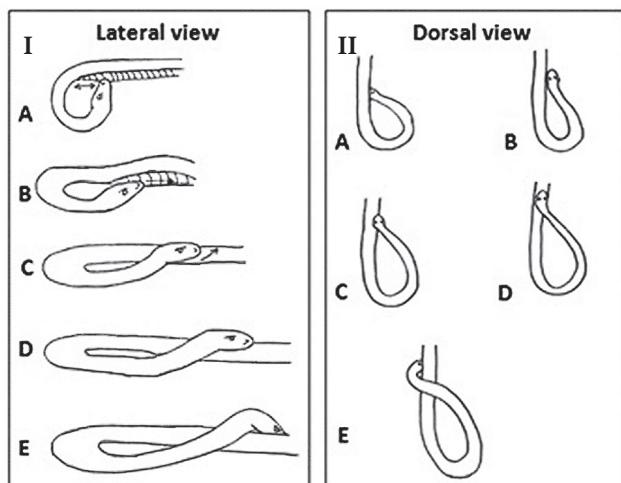


Figure 12. Rubbing sequence of *Psammophis elegans*. (I) Lateral view, (II) dorsal view. (A) First sequence, rubbing of the throat only; (B) The secretion is applied on the ventrals; (C) The nare is dragged along the flank; (D) The chin has contact with the back; (E) the second nare is used to apply the secretion on the ventrals and the other flank. Drawings based on video recordings.

like structures were present on the scale surface, which could have indicated a connection between the epidermal layers and the outer skin surface. Moreover, ultra-thin sections for transmission electron microscopy showed clearly that there is no connection (e.g., by means of canals) between pores and the lipid-secreting layers of the dermis (AMEMIYA et al. 1996, ALIBARDI 2005, EBERT 2007). Studies conducted on scaleless snakes showed that they were optimally protected against desiccation in spite of their abnormality (LICHT & BENNETT 1972, BENNETT & LICHT 1975, ROBERTS & LILLYWHITE, 1980). Indeed, the protective mechanism of the skin against water loss lies in the epidermal layer of the skin and not on its surface (see Introduction).

With regard to rubbing behaviour, the delicately sculptured micro-ornamentation might serve to retain the nasal glands’ secretion on the scale surface. So far, the working hypothesis that the scale ultrastructure of psammophiid snakes differs from those of other snake groups could be confirmed. However, our control taxa did not show any porous scale pattern either, so that the hypothesis that all snakes have pore-bearing scales should be refuted.

Self-rubbing behaviour in psammophiid snakes

Table 1. Analysis of rubbing behaviour: Duration, number of head movements, and methods used.

Species	No. of individuals observed	No. of observations	Mean duration (sec.)	Standard deviation (sec.)	Mean no. of head movements	Standard deviation	Method used
<i>Psammophylax acutus acutus</i>	2	19	65	± 88	25,11	± 6,85	each nostril once, one by one
<i>Rhamphiophis rubropunctatus</i>	1	9	154	± 39	64,11	± 11,07	only one nostril
<i>Rhamphiophis rostratus</i>	2	13	134	± 36	51,00	± 17,89	each nostril once, one by one

Gas chromatography

The GC/MS analysis of the nasal secretion of *Psammophis mossambicus* and *Rhagerhis moilensis* revealed uniformity within species and differences between species. The presence of lipids in the nasal secretion could be clearly determined in the two species studied. However it could not be clearly identified which lipids exactly they are. The peaks noted can only be assigned with certainty to substances with the help of reference substances (often the “pure” substance itself). This, in turn, can only be done when it is known which substance classes are comprised in the secretion; the spectra of these substances have then to be compared with the chromatograms of pure substances to identify precisely the mass producing the peaks and so refer them with certainty to a given substance class. Since no such comparative substances were at our disposal, no clear assignment of the masses-to-charge found was possible. In the present case, it can therefore only be hypothesised that lipids are present in the secretion of both species, because they perform RB just as *Malpolon monspessulanus* and lipids were found in its secretion. It cannot be said whether these are, e.g., fatty acids or aldehydes, though. It was rather curious that we obtained a peak at 12.40 min in the control samples (hexane alone and hexane with glass slides), since no peak is actually to be expected for this pure substance and glass does not dissolve in hexane. So far it is evident that an impurity must have been present in the solution. Therefore, in the present case, we can only state that lipids are present in the secretion of *Psammophis mossambicus* and *Rhagerhis moilensis*, as has already been documented for *Malpolon monspessulanus* (DUNSON et al. 1978), but we cannot say yet to which class of lipids they belong.

Ideally, each substance will have the same retention time in each sample when the parameters of GC/MS are the same. However, particularly for such a complex “mixture” as in the case of snake skin secretions, it is highly probable that various substances have the same retention time – as it is the case in both *Rhagerhis moilensis* specimens used in this study. Indeed, MASON et al. (1987) already demonstrated through the extraction of lipids from the skin of male and female *Thamnophis sirtalis parietalis* that there are differences at the level of the composition of the skin lipid, depending on the sex of the individual analysed. This allows the males to differentiate between males and females, e.g., during the mating/breeding period.

Behaviour

Marking behaviour: In the first part of this experiment, the assumption that rubbing frequency would be elevated on the first day as a result of the snake having to “mark” its new environment could not be confirmed. Our results showed no significant difference between the rubbing frequency on the first day and the following four days. Under this circumstance, the term “territoriality” for snakes has to be used with precaution (GILLINGHAM 1987, FORD & HOLLAND 1990, RIVAS & BURGHARDT 2005). Territorial defence has been reported from adders, but was observed only during the reproductive period, and the “space” defended was only the immediate area around a receptive female (CARPENTER 1977, ANDRÉN 1986). Here, it would be more reasonable to use the term proposed by DUVALL et al. (1992): “successive female defensive polygyny”, considering that males defend only their females and not any other possible resources. This term could then also be applied to *Malpolon* (DE HAAN 1999, DE HAAN & CLUCHIER 2006) and to the other psammophiids as far as fighting occurs during the mating season. Moreover it has to be considered that even juvenile psammophiids perform rubbing behaviour a few hours after hatching, which is certainly too short a period for establishing a “territory” (BRANDSTÄTTER 1996).

The second part of the experiment was based on DE HAAN’s (1999) observation that the snakes would rub themselves two or three times before crawling through a less familiar environment. A similar behaviour was expected here since the snakes were placed into an unfamiliar terrarium. However, no traces of secretion could subsequently be identified, neither on the blotting paper on the walls of the terrarium, nor on the glass plate laid out in the middle of the terrarium. It is entirely possible that the traces described by BRANDSTÄTTER (1995) were applied to the glass directly from the snake’s nostril and not scraped off the body when it crawled over it. This, in turn, might be due to the smooth surface of the glass on which the secretion is not abraded. Our videos showed that there was no recognisable change in the behaviour of the second snake, and it behaved as it did in the control run. There could be different for this: (1) the snakes behaved differently due to the transport between home and experimental terrarium. They wanted to escape the new terrarium independently from the previous presence of another snake; (2) the sexes of both snakes were identical – no interest in following the

scent trail; (3) following of either male or female trails takes place, but only during the mating season and this experiment was conducted outside of the mating season: (a) The first snake was a male and the second a female – no interest in following the trail; (b) The first snake was a female and the second a male – the male had no interest in following the female because it was outside of the mating period; (4) the second snake did react to the presence of the first, but in a manner not identified as a response by the observer.

External factors: The increase in rubbing behaviour frequency with temperature in the present study is in accordance with past observations (DAREVSKY 1956, RIEPPEL 1973, DE HAAN 1982, STEEHOUWER 1987) and supports the hypothesis of DUNSON et al. (1978) that rubbing behaviour has a primary function against desiccation. This is corroborated by the statement of GREEN et al. (1997) that high temperatures are more dangerous to thin- and slender-bodied animals as the surface : mass ratio has a major impact on the rapidity of cooling or heating, respectively. Protection through a lipid-rich secretion would enable psammophiids to stay active during the hottest hours of the day (STEMMLER 1972; KRAMER & SCHNURRENBERGER 1963 for *Malpolon*) in two ways: actively through rubbing the nasal secretion on the body, and passively by lipids extruding from the skin, as is the case in *Thamnophis sirtalis parietalis*, in which pheromones are extruded through the skin to the dorsal surface (GARSTKA & CREWS 1981).

However, IRISH et al. (1988) suggested that lipids would reach the surface through pores in the epidermis, and HAZEL et al. (1999) demonstrated that some pits found in the scales of *Boa constrictor* and *Morelia spilotes* were in fact continuous pores that permitted in an experiment the passage of viscous liquid.

Therefore, the morphology of these “depressions” or “pits” themselves should be clearly defined since HAZEL et al. (1999) and EBERT (2007) already noted morphological differences in the “pores” analysed. On the one hand we would have pores, larger in diameter, for the passage of substances (as described by HAZEL et al.) and on the other hand we would have pits (or micropits to avoid any confusion with the pits described by UNDERWOOD (1967)), parts of the micro-ornamentation of the scale as analysed by AMEMIYA et al. (1996), ALIBARDI (2005), and EBERT (2007).

This study cannot be concluded without having a short look on the rubbing behaviour in some frogs. The majority of them cannot control evaporative water loss so that they rely entirely on the proximity of water (BARBEAU & LILLYWHITE 2005). However, some arboreal frogs from arid regions are able to cover themselves with a skin secretion to survive dry periods. Self-rubbing in frogs is known from several species of various families, e.g. *Chiromantis*, *Hyla*, *Hyperolius*, *Litoria*, *Phylomedusa*, *Polypedates*, *Pyxicephalus*, *Scaphiopus*, *Spea* and others (BLAYLOCK et al. 1976, DREWES et al. 1977, McCCLANAHAN et al. 1978, CHRISTIAN et al. 1988, LILLYWHITE et al. 1997, BARBEAU & LILLYWHITE 2005).

Outlook

We want to state here that we are fully aware of the number of snakes observed certainly being too small (due to the difficulty to obtain these snakes) to state clearly the function of the rubbing behaviour of psammophioid snakes. However, the statistics used for the experiment give an indication how the results could be with a greater number of snakes. We have to emphasize again here the fact that the sexes of these snakes are extremely difficult to identify in living animals. To kill them merely for identifying their sexes was not justifiable for the objective of this study.

The overall analysis of the 17 psammophioids investigated in the present study support the hypothesis that their rubbing behaviour depends on temperature and serves primarily the prevention of evaporative water loss. The numerous examples of frogs protecting themselves against desiccation by rubbing a secretion on their bodies corroborate this hypothesis. This conclusion does, however, not exclude the possibility that further morphological specialisations such as the unique, grooved scales in *Malpolon*, may alter the primary function of the nasal secretion in this genus towards an additional function as an intraspecific communication means (DE HAAN 1999). On the other hand, comparative studies on psammophiids adapted to moister environments (*Psammophis phillipsi* (s. str.), *P.* (formerly *Dromophis*) *lineatus* or *P. condanarius* and *P. indochinensis*) could provide additional arguments for or against our conclusion.

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