

Variably occurring parietal pits in psammophiid snakes (Squamata: Serpentes): convergent expression of ancestral skin traits?

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Abstract. “Sense-organ-like” parietal pits (PPs) in snakes have so far been documented in several psammophiid species as well as the Asiatic *Atretium schistosum* (Natricinae). Hypotheses regarding the function of these structures have been suggested but support for any is weak due to sampling limitations of species exhibiting these, and previous inconclusive histological examinations. Here we present data from additional sampling (N=842 for seven species) on the variable expression of PPs throughout psammophiids and discuss possible explanations for their occurrence pattern in two distantly related caenophidians. We consider the spontaneous atavism hypothesis previously suggested by Miralles and Ineich (2006) in light of our additional data and also suggest that it may be more appropriate to regard PPs as a particular manifestation of a more general underlying developmental mechanism that produces a wide variety of integumentary sensory structures in snakes.

Keywords. Elapoidea, Psammophiidae, cephalic pits, iterative homology, atavism

Introduction

Pits or pit-like depressions have been documented in the scales of a variety of snake lineages. Apical pits on scales of the body dorsum have been documented in at least 250 “colubrid” and viperid species and often serve as useful diagnostic features, yet their functional role remains ambiguous and has received only little attention (Marx and Rabb, 1972; Smith, 1982; Ball, 1996). Superficially similar cephalic pits have been noted in a number of head shields in a diversity of caenophidian lineages (Underwood, 1967; Malnate and Underwood, 1988; de Haan 2003; 2006). Among these, novel pit-like structures have been signalled on the parietal shields of snakes of the monophyletic elapoid snake radiation Psammophiidae (Kelly et al., 2008; 2009; Zaher et al., 2009; or Psammophiinae — Vidal et al., 2007; 2008; Pyron et al., 2011), where they occur in four of eight genera in the clade (de Haan 2003, 2006). In addition, Miralles and Ineich (2006) have reported that morphologically similar parietal pits also occur on a natricid, *Atretium schistosum*. Such pits are variably present within individual taxa, but when present in an individual are stable across ontogeny (Miralles and Ineich, 2006). The function of these parietal pits (PPs)

has been discussed (de Haan 2003; 2006; Miralles and Ineich, 2006), but remains speculative because of a lack of definitive histological or ultrastructural data. Likewise, the phylogenetic distribution of these structures remains poorly known because previous investigations have been neither taxonomically comprehensive nor robust with respect to intra-taxon sampling.

As part of a broader study investigating the biology of psammophiids, we examined the parietal shields of preserved specimens and recorded the occurrence patterns of these pits. A substantial increase in sample sizes over previous studies and the addition of data from five additional psammophiid species (including a representative of a previously uninvestigated genus, *Psammophylax*) permit the evaluation of earlier generalizations about PP distribution and provide a more robust basis for assessing possible explanations for the presence of these enigmatic structures.

Materials and Methods

We examined 842 preserved museum specimens (representing seven psammophiid species, Table 1) with intact parietal shields for the presence of pits. Specimens were obtained from the U.S. National Museum of Natural History (USNM), California Academy of Sciences (CAS), Field Museum of Natural History (FMNH), Museum of Vertebrate Zoology (MVZ), Ditsong (formerly Transvaal Museum; TM), and Bayworld (formerly Port Elizabeth Museum; PEM). When possible, sex and age class (juvenile versus adult) were recorded for specimens.

When parietal pits were present, their condition of symmetry (i.e., presence of equal number of pits on both parietal shields)

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Table 1. *Psammophis* and *Psammophylax* species examined for presence of parietal pits (PPs). For some specimens we were unable to determine sex. All five species displaying PP below had yet to be documented exhibiting PPs in previous studies.

Species	Total N	N with PPs	N ♀ with PPs	N ♂ with PPs
<i>Psammophis schokari</i>	208	31 (14,9%)	13 (48%)	14 (52%)
<i>P. crucifer</i>	226	19 (8,4%)	8 (50%)	8 (50%)
<i>P. biseriatus</i>	25	2 (8,0%)	-	2 (100%)
<i>P. punctulatus</i>	14	-	-	-
<i>P. condanarus</i>	29	-	-	-
<i>P. brevirostris</i>	1	1 (100%)	1 (100%)	-
<i>Psammophylax rhombeatus</i>	339	39 (11,5%)	13 (45%)	16 (65%)

and total number of pits were recorded. If pits were symmetrical, the regularity of pit positioning was assessed. Pits were considered regular when PPs on one shield were mirror images of those on the other. We regard deviation from symmetry or regularity as evidence of a lack of constraint on pit placement. Specimens in which one parietal shield was obscured or damaged were scored for presence only and not for symmetry. For 15 specimens regularity was not assessed.

All markings and/or pits on the parietal shield were scrutinized under a dissecting microscope to assure that they were not artefacts of injury or post-mortem damage and were consistent with the structures identified and described by de Haan (2003; 2006). In cases of uncertainty specimens were excluded from analysis, thereby rendering the frequencies of occurrence reported here conservative. Chi-square tests were performed using JMP 4.0.2 (SAS).

Results

Of seven species examined in the genera *Psammophis* and *Psammophylax*, five are newly reported here to exhibit parietal pits (PPs) at some frequency (Table 1). Only 11% (N=82) of all the psammophiids examined contained PPs and the frequency of occurrence within each of the well-sampled species (N>200) was also low (Table 1). Where samples sizes permitted evaluation, there was no evidence of a gender bias in PP presence (*P. schokari*: $\chi^2=0.0323$, df =1, P=0.858; *P. crucifer*: $\chi^2=0.527$, df =1, P=0.819; *P. r. rhombeatus*: $\chi^2=0.360$, df =1, P=0.548).

PPs occurred more often in an asymmetrical condition (71%, N=58) than symmetrical, and this difference was significant ($\chi^2= 14.532$, df =1, P <0.0001). PP presence varied across psammophiids in various combinations. Representatives of all species with PPs (with the exception of *P. brevirostris*) most commonly displayed a single PP (60% of all specimens recorded with PPs) on either the left or right parietal shield. Twenty-eight percent of all specimens with PPs possessed a single

pit in each shield, with 55% of these (N=13) exhibiting irregular placement. Two *P. schokari* specimens (2% of all specimens recorded with PPs) displayed two PPs in their left shield and none in their right. Of all specimens recorded with PPs, 6% (N=2 for both *P. r. rhombeatus* and *P. crucifer*, N=1 for *P. brevirostris*) displayed one pit in the left shield and two in the right. For the *P. brevirostris* specimen two of its three PPs were positioned regularly. The opposite combination (two PPs in the left shield and one PP in the right shield) of PPs occurred in 2% of the 82 individuals displaying PPs (N=1 for both *P. crucifer* and *P. schokari*). The maximum number of pits occurring on an individual was four (two on each shield in one *P. r. rhombeatus* specimen). Finally, 35% of all specimens containing PPs were juveniles. This frequency of occurrence was not different than expected given the proportions of adults and juveniles comprising the entire sample ($\chi^2=1.090$, df =1; P=0.2964).

Discussion

Previous studies have reported PP occurrence in seven species representing four psammophiid genera: *Psammophis*, *Dromophis*, *Rhamphiophis*, and *Malpolon* (de Haan, 2003; 2006). Here five additional species, including a representative of a fifth psammophiid genus (*Psammophylax*), are confirmed to contain PPs (Table 1). Thus, almost one third of the ~40 species in this clade have been recorded to exhibit these novel structures. As shown here and consistent with previous reports (de Haan 2003; 2006; Miralles and Ineich, 2006), PPs mostly occur at low frequencies. For *Psammophis condanarus* and *P. punctulatus* (where no PPs were found), the sample sizes were too small (14 and 29, respectively) to reasonably exclude their actual occurrence. The significance of adequate sampling is exemplified by the case of *P. schokari*. De Haan (2003) examined 21

specimens of this species and found no PPs, whereas we found this species to have the highest PP frequency in the study (14.9%) based on our sample of 208. Two species, *Psammophis phillipsi* and *P. elegans*, reported on by de Haan (2003) deviate from this low frequency pattern (61% with PPs, N=74 and 39% with PPs, N=36, respectively), but whether this represents a real species-specific difference or a sampling artefact remains unclear. For example, in de Haan's study (2003), 67 *P. phillipsi* were the offspring of seven captive parents, five of which exhibited PPs. If PPs are heritable at any level, one might reasonably expect that frequency of occurrence in this sample would be artificially high due to the proportion of parents with pits.

Our data confirm that there is no distinction in PP presence based on age or sex. The lack of an ontogenetic effect on PP presence is also supported by de Haan (2003) whose data were largely derived from captive animals and was, therefore, able to ascertain that PPs were permanently present within an individual throughout its life. Equal ratios of males and females with PPs suggest that pit presence is also not sex-linked (Table 1) and this is also consistent with previous findings (de Haan 2003; 2006). A marked difference exists, however, between our observations and those of previous with respect to the frequency of asymmetrically occurring PPs. De Haan (2003; 2006) and Miralles and Ineich (2006) reported an almost equal number of asymmetrical and symmetrical PPs in their samples, whereas the majority of PPs in this sample (71%) occurred asymmetrically. Finally, previous authors did not assess regularity within symmetrically distributed pits, and our evaluation of this aspect (only 45% of symmetrical pits positioned regularly) further demonstrates the probable lack of constraints on pit positioning.

Our data partly support the atavistic hypothesis suggested by Miralles and Ineich (2006), which suggests that PPs represent an ancestral condition that is expressed in only some individuals of a species. In order to be considered atavistic, a character should 1) be present throughout the life of an individual, 2) be absent (not expressed) in closely related individuals, 3) occur at a low frequency within a species, and 4) be demonstrably homologous with an ancestrally expressed character (Hall, 1984). De Haan (2003; 2006) demonstrated that the first condition was met by direct observation and this is corroborated statistically in our sample. Likewise, the second condition appears to be met (de Haan, 2003), although the expression of the trait may, nonetheless, have some heritable basis (see

above). The addition of our data, with high numbers of individuals sampled in several species, emphasizes that PPs do indeed occur at low frequencies across the species displaying them, validating the third condition. However, the fourth condition remains unconfirmed. Miralles and Ineich (2006) considered the PPs of *Atretium schistosum*, currently considered a natricine or natricid (Miralles and Ineich, 2006; Zaher et al., 2009), to be homologous with those of psammophiids. If this is indeed the case, the atavistic expression of the same character in representatives of two different caenophidian superfamilies, Colubroidea and Elapoidea (Vidal et al., 2007; Pyron et al., 2011), would imply their ancestral occurrence among derived snakes. We would, therefore, expect to find them (at least occasionally) in other lineages, and particularly in basal members of the Caenophidia. This may be the case, but as the presence of these easily overlooked structures (de Haan, 2003; 2006) has not been systematically assessed across all snakes, no conclusions can currently be drawn.

The lack of evidence bearing on the fine structure of PPs leaves open the interpretation of these structures as putative homologues of similar structures in other snakes and on other parts of the body. De Haan (2003; 2006) considered that cephalic pits reported in a variety of caenophidian lineages (e.g. Underwood, 1967; Malnate and Underwood, 1988) were not structurally or functionally comparable with the PPs of psammophiids. However, such non-congruence of structure may not be relevant to the question of homology, and the various cephalic pits heretofore described, despite their differences, might reflect the variable expression of the same ancestral trait. Indeed, the apparent current lack of functionality of these structures, their low frequency of occurrence, and their largely asymmetrical and irregular distribution suggests that their expression in other lineages might likewise be under little constraint and details of structure might be only grossly similar. Further, parietal and other cephalic pits may be more broadly related to the widespread apical pits of many caenophidians. The function of these structures has long been a point of controversy (Underwood, 1967; Smith, 1982; Ball, 1996; de Haan, 2003), but some interpretations view them as sensory structures. If this is true, then PPs, other cephalic pit structures, and apical pits could be considered iterative homologues (Ghiselin, 1976; Roth, 1984; Wagner, 1989; Haszprunar, 1992) of one another and their appearance in individuals of taxa in multiple lineages might be viewed more generally as the convergent expression of a widespread

and homologous developmental program to form sensory structures in the integument. This may be viewed as analogous to the differential expression of the spinulate Oberhäutchen as scansorial setae on the digits or tail tips of gekkotan lizards (Maderson, 1971; 1985; Bauer, 1998; Nussbaum, Raxworthy and Pronk, 1998) or, perhaps more appropriately, to the condition of hypertrichosis, in which abnormal expression of hair follicle morphology and position occurs at low frequency in human populations (Bondeson and Miles, 1996; McElwee and Sinclair, 2008; Mendiratta, Hariai and Gupta, 2008) and can be seen as one of many alternative modes of expression of the fundamental mammalian homology of a hairy integument. Such an interpretation is fundamentally compatible with the atavistic hypothesis, but predicts only a very general level of homology among integumentary sense organs and thus obviates the need to explain away the apparent absence of PPs in most other caenophidians. Morphological and developmental data, as well as experimental genetic studies, may ultimately be used to test the homology of PPs with cephalic and apical pits and thereby assess this interpretation.

The evidence presented here highlights the importance of using a robust sample size of individuals to confirm the existence and estimate frequency of occurrence of variably or sporadically occurring features, such as parietal pits in snakes. Our data confirm that PPs are more widespread (at least within psammophiids) than previously thought and that their frequency of occurrence is often so low that without a large sample their presence could easily go unnoticed. In addition, our data show that PPs are often asymmetric and irregular in their distribution. Together this suggests that these structures are probably non-functional and that their expression is not highly constrained. We support the hypothesis that the occurrence of PPs is an expression of a more fundamental (homologous) ancestral trait (de Haan, 2006; Miralles and Ineich, 2006) but suggest that it is more appropriate to regard this homology as the general ability of snake scales to form sensory organs, rather than as the possession of functional dorsal cephalic pit organs, the presence of which has yet to be confirmed among the majority of caenophidians.

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