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**Evolution and role of the follicular epidermal gland system in non-ophidian squamates**

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Review

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22 **Abstract.** Many lizard and amphisbaenian lineages possess follicular glands in the  
23 dermis of the inner thighs and/or the area anterior to the cloaca. These tubular glands  
24 produce a holocrine secretion that finds its way to the external world through pore-  
25 bearing scales (femoral and/or pre-anal pores). Secretions are composed of proteins and  
26 many lipophilic compounds that may function as chemosignals in lizard and  
27 amphisbaenian communication. In recent years, we have begun to develop an  
28 understanding of the adaptive significance of these secretions, and they are currently  
29 thought to play an important role in a variety of processes in these animals. While it  
30 appears that epidermal gland secretions function in intra- and interspecific recognition  
31 and territoriality, research has focused largely on their role in mate assessment. Despite  
32 these recent studies, our knowledge of the true role of the chemicals found in epidermal  
33 secretions remains poorly studied, and there are many possible avenues for future  
34 research on this topic. Here, we review the literature on the follicular epidermal glands  
35 of non-ophidian squamates and provide a first taxon-wide overview of their distribution.

36

37 *Keywords:* amphisbaenians, ancestral state reconstruction, chemical communication,  
38 chemosignals, lizards, pheromones, pores, secretions.

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## 44 **Introduction**

45 Nearly half a century ago, Charles J. Cole (1966a) wrote a seminal review on epidermal  
46 glands in lizards, tallying what was known on their functional significance, and pointing  
47 out promising avenues for new research. In the years that followed, and increasingly so  
48 in the past decade, studies along these lines have revealed the prominent roles epidermal  
49 gland secretions play the biology of lizards and amphisbaenians. The present review  
50 acts as an update to Cole's work, discussing the extensive scientific progress made since  
51 his initial review, and provides a framework for the numerous possible areas of future  
52 research in the field.

53         Initially, we focus on the chemical composition of epidermal gland secretions  
54 and how these vary among individuals, sexes, taxa and habitats. We then discuss the  
55 presence of epidermal glands in extant non-ophidian squamates, and provide hypotheses  
56 for the evolution of the epidermal gland system based on an ancestral state  
57 reconstruction. Based on the literature available, we address the potential roles that  
58 different chemosignals play in non-ophidian squamate social communication, and  
59 discuss possible patterns in the overall investment and structural composition of the  
60 "epidermal gland system": a collective name for follicular epidermal glands, pores and  
61 secretions. We proceed to detail the possible functions of epidermal glands, focusing on  
62 territoriality and dominance as well as their role in mate assessment. Finally, we provide  
63 some ideas for future research in the field.

64

## 65 **Terminology and classification**

66 Two broad types of holocrine secretory structures occur in the dermis of lizards and  
67 worm lizards: generation and follicular glands. Follicular glands are often considered to  
68 be more specialized structures that have evolved from the more primitive generation  
69 glands (Maderson and Chiu, 1970; Baig and Böhme, 1991). Generation glands can be  
70 found in Cordylidae (Van Wyk and Mouton, 1992) and Gekkonidae (Maderson, 1967;  
71 Maderson and Chiu, 1970), and are modified skin scales which display the typical  
72 sequence of epidermal cell layers and occur as patches of glandular scales in the  
73 femoral, precloacal, antebrachial, inguinal, or dorsal epidermal regions of the body (Fig.  
74 1) (Maderson, 1967, 1968; Van Wyk and Mouton, 1992). Within cordylids, the  
75 glandular material is produced in a modified epidermal  $\beta$ -layer and each time the scale  
76 goes through the shedding cycle, new glandular material is produced in the  $\beta$ -layer of  
77 the new epidermal generation (Van Wyk and Mouton, 1992). In contrast to generation  
78 glands, the follicular exocrine glands are typically tubular and embedded in the dermis,  
79 and the produced secretions protrude through pores as solid plugs (Fig. 2) (Cole,  
80 1966a). Based on their exact anatomical position on the lizards' or amphisbaenians'  
81 body, follicular glands are often referred to in the literature as preanal, precloacal, or  
82 femoral glands. Because of confusing terminology encountered in the literature (e.g.,  
83 "preano-femoral pores" by Loveridge, 1947; "femoro-anal pores" by Gerlach and  
84 Canning, 1996; "femoro-precloacal pores" by Grismer, 2002), we decided not to  
85 discriminate between specific follicular glands, but to include them all in this study  
86 within the same category: epidermal glands.

87

## 88 **Gland morphology**

89 Many non-ophidian squamates possess a number of epidermal glands that secrete a  
90 waxy substance through pores (Antoniazzi et al., 1993; Imparato et al., 2007). These  
91 epidermal structures are predominantly located on the preanal abdominal area (preanal  
92 pores), or on the ventral surface of each thigh (femoral pores) (Fig. 1c) (Mason, 1992;  
93 Arnold and Oviden, 2004). In most lizards, pores open in the centre of a modified  
94 scale (Imparato et al., 2007), although in some agamid species pores open between  
95 scales (Cole, 1966b; Witten, 1982). The number and morphology of pores, as well as  
96 their distribution on the body (Kluge, 1976; Witten, 1993), varies extensively across  
97 different groups (Pincheira-Donoso, Hodgson and Tregenza, 2008; Mouton, Van  
98 Rensburg and Van Wyk, 2010; Baeckens et al., 2015). There is also variation between  
99 sexes, as females may either not have epidermal pores at all, or may have pores with  
100 reduced sizes (Cole, 1966b). As such, they have been used extensively as taxonomic  
101 characters in lizards and amphisbaenians (Linnaeus, 1758; Duméril and Bibron, 1834;  
102 Arnold and Oviden, 2004).

103         The epidermal glands form into a follicular unit after an invagination of the  
104 stratum germinativum, and are continuous with the generalized body epidermis  
105 (Maderson, 1972). Epidermal glands can be tubulo-acinar structured as in  
106 *Acanthodactylus scutellatus*, *Acanthodactylus boskianus* (Khannoon, Dollahon and  
107 Bauer, 2013), *Gekko gecko* (Chiu and Maderson, 1975), and *Hemidactylus falviridis*  
108 (Chauhan, 1986); tubular as in *Hemidactylus bowringii* (Chiu and Maderson, 1975),  
109 *Uromastix hardwickii* (Athavale et al., 1977); tubule-alveolar as in *Liolaemus* sp.  
110 (Valdecantos, Martinez and Labra, 2014); or branched tubular as in *Crotaphytus*  
111 *collaris* (Cole, 1966b). The glands of most species are embedded in the dermis of the  
112 scales anterior to their pores connecting the pores by elongated ducts (Blasco, 1975;

113 Chiu and Maderson, 1975; Chauhan, 1986; Imparato et al., 2007; Khannoon, Dollahon  
114 and Bauer, 2013), or can be situated immediately deep to the pores as observed in *C.*  
115 *collaris* (Cole, 1966b). Secretory cells in the glands undergo four different stages of  
116 differentiation: (1) formation of the secretory granules, (2) the accumulation of these  
117 granules, (3) disintegration and (4) formation of the solid secretory plug, which  
118 protrudes externally (Khannoon, Dollahon and Bauer, 2013). The secretions of the  
119 epidermal glands in lizards and amphisbaenians are under the control of androgenic  
120 hormones and start to differentiate at the onset of sexual maturity (Chiu and Maderson,  
121 1975; Díaz, Alonso-Gómez and Delgado, 1994; Chamut, Valdez and Manes, 2009;  
122 Mouton et al., 2010). Van Wyk (1990) observed an increase in the tubule diameter of  
123 the follicular epidermal glands of male *Cordylus polyzonus* during the reproductive  
124 season, and found a significant correlation with seasonal variation in testis volume.

125

## 126 **Chemical composition**

127 At the time of Cole's publication little was known on the chemical nature of the  
128 secretion produced by epidermal glands, other than that it "contained a fatlike  
129 substance" – which was based on observations on but a handful lizard species. With this  
130 limited knowledge, it is not surprising that hypotheses on the possible functions of these  
131 secretions were difficult to test. Now, we know that the gland secretions are made up of  
132 a variety of both proteins and lipids (Fergusson, Bradshaw and Cannon, 1985; Alberts,  
133 1991; Weldon, Flachsbarth and Schulz, 2008). Alberts (1990) found that proteins were  
134 four times more prevalent in the secretions of the desert iguana (*Dipsalis dorsalis*) than  
135 were lipids. It has been postulated that these proteins may transmit chemical

136 information (Alberts, Phillips and Werner, 1993; Martín and López, 2000), function in  
137 individual recognition (Glinsky and Krekorian, 1985; Alberts, 1992a), serve as a matrix  
138 to retard the evaporation of the secretion similar to the major urinary proteins (MUPs)  
139 in the urine of *Mus domesticus* (Humphries et al., 1999), or be involved in the  
140 cornification of the secretion (Cole, 1966a). Despite the presence of proteins in gland  
141 secretions and their importance in other groups such as amphibians and mammals  
142 (Toyoda et al., 2004; Wyatt, 2014), it is thought that lipids play a greater role in  
143 reptilian chemical communication, and they have thus been studied in much greater  
144 detail (Mason, 1992; Martín and López, 2011). Indeed, most research on chemicals  
145 from epidermal gland secretions does not even attempt to analyse the composition of  
146 proteins within the secretion (Escobar, Labra and Niemeyer, 2001; Louw et al., 2007,  
147 2011; Font et al., 2012). We postulate three reasons for this: (1) shared ancestry, not  
148 ecological factors, appears to account for much of the variability in the protein secretion  
149 composition in studied species, as proteins are inheritable traits (Alberts, 1991).  
150 However, we believe that this assumption is what makes the study of proteinaceous  
151 chemosignals interesting. It is much more straightforward to perform evolutionary  
152 studies on proteins than lipid mixtures, because proteins likely evolve to species-  
153 specificity during or after speciation, contributing to reproductive isolation and  
154 avoidance of hybridization. This species-specificity can be easily studied by inspecting  
155 the amino acid sequence of the involved proteins. (2) Proteins have a lower degree of  
156 molecular diversity than lipids, which decreases the potential information content of a  
157 chemosignal (Martín and López, 2000). However, this can be argued as well, since the  
158 concentration of the secreted proteins could change with features like dominance or  
159 health status. Furthermore, in mice MUPs make up a very diverse set of proteins with a

160 high level of polymorphism, which allows for individual recognition (Hurst et al.,  
161 2001). So proteins could allow for kin recognition, individual recognition and even  
162 more temporary characteristics, since protein expression is a versatile mechanism that  
163 can experience shifts in what proteins are expressed as well as shifts in concentration.  
164 Moreover, only a few amino acids have to be different to influence the binding of a  
165 protein to a receptor. Although protein compounds could be lesser candidates to transfer  
166 information on age, health status, dominance and territoriality, we should be cautious  
167 stating that proteins have lower potential information content. (3) In contrast to the  
168 protein fraction of epidermal secretions, which are non-volatile by nature, only volatile  
169 lipids are likely to be detected by tongue-flicks into the air (Alberts and Werner, 1993),  
170 which facilitates opportunities for comparative behavioural studies. We believe this  
171 reason to be the most convincing, although many species can detect proteins (Alberts  
172 and Werner, 1993; Weldon, Flachsbarth and Schulz, 2008). We therefore believe that  
173 the study of proteins as potential sources of chemical communication should be  
174 considered more fully in the future.

175         The standard procedure for analysing the lipophilic fraction of the gland  
176 secretion is gas chromatography-mass spectrometry (GC-MS). The general composition  
177 of chemicals across different groups is often quite similar, with steroids and carboxylic  
178 acids being found in every family of lizards studied (Weldon, Flachsbarth and Schulz,  
179 2008), usually as major components in the secretion. Often, cholesterol is the  
180 predominant molecule found in the lipophilic fraction, and it has been hypothesized to  
181 provide information on individual identity, and to stabilize the other molecules in the  
182 secretion (Escobar et al., 2003; Louw et al., 2007, 2011; Khannoon et al., 2011b). Table  
183 1 illustrates an updated account of the lipophilic fraction of epidermal gland secretions

184 in lizards and amphisbaenians, including those species covered by Weldon and  
185 colleagues (2008) and those studied following their review. Most of our knowledge  
186 comes from lacertid lizards, while some research has evaluated other families (Table 1).  
187 As we begin to amass a more detailed knowledge on the secretions of epidermal glands  
188 in other families, we can assess the degree to which certain chemicals are  
189 phylogenetically constrained, and the degree to which others may be environmentally  
190 dependent. For example, while alcohols are present, and even comprise a major portion  
191 of the secretion in many families, they have not been reported at all in Liolaemidae  
192 (Escobar, Labra and Niemeyer, 2001; Escobar et al., 2003). Only with more studies  
193 which take into account phylogenetic relationships and ecological conditions can we  
194 begin to piece together a coherent explanation for patterns such as this.

195

#### 196 **What are epidermal glands and their secretions for?**

197 Historically, five hypotheses have been proposed concerning the function of epidermal  
198 gland secretions (Cole, 1966a): (1) the secretion securely fastens males to females  
199 during copulation, (2) the secretion stimulates or quiets females, facilitating mating  
200 opportunities, (3) the glands are vestigial and serve no present function, (4) the secretion  
201 has a signalling function and is used by males to mark their territory (e.g., Aragón,  
202 López and Martín, 2003; Moreira, López and Martín, 2006; Carazo, Font and Desfilis,  
203 2007), and (5) the secretion is used for self-, sex and species recognition and orientation  
204 (e.g., Alberts, 1992a; Alberts and Werner, 1993; Barbosa et al., 2006). Of all five  
205 hypotheses, the first three have not been substantiated by any scientific claim, and can  
206 probably be disregarded. The latter two hypotheses have received support, and the

207 secretion is now considered to be an important component of the non-ophidian  
208 squamate chemical signalling system (Alberts, 1993; Imparato et al., 2007; Mason and  
209 Parker, 2010; López and Martín, 2012; Khannoon, Dollahon and Bauer, 2013).

210         Epidermal gland secretions have been shown to function in a variety of  
211 signalling processes: from providing social cues (Aragón et al., 2006; Martín and  
212 López, 2014 Martín and López, 2015) and assisting in habitat selection (Aragón, López  
213 and Martín, 2001a,b; Le Galliard, Ferrière and Clobert, 2005; Font et al., 2012), to  
214 allowing intra- and interspecific recognition and providing territorial signals (Moreira,  
215 López and Martín, 2006; Khannoon et al., 2011b). These secretions can be thought of as  
216 a signature mixture (Wyatt, 2014), in which a variable subset of molecules of an  
217 animals' chemical profile are detected by others, allowing them to distinguish  
218 individuals.

219

## 220 **Variation in secretion composition and pore characteristics**

221 Although there was little knowledge on epidermal gland secretions, and what  
222 knowledge existed was present for only a few species, Cole (1966a) hypothesized that  
223 there must be interspecific variation in secretion composition, solely based on  
224 differences in secretion colour. We now know that extensive variation exists both  
225 between and within species. Variation in different aspects of the epidermal gland system  
226 has been observed in individuals of different age and sex (e.g., Cole, 1966b; Martins et  
227 al., 2006; Khannoon et al., 2011a,b; Martín and López, 2006a), different morphological  
228 characteristics (e.g., López, Moreira and Martín, 2009a; Pellitteri-Rosa et al., 2014),

229 different populations (e.g., Escobar et al., 2003; Gabirot, López and Martín, 2012a,b;  
230 Khannoon et al., 2013; Martín et al., 2013a) and different species (Table 1).

231           Researchers have also observed seasonal variation in the epidermal gland system  
232 (Chiu and Maderson, 1975; Van Wyk, 1990; Alberts et al., 1992), with an increase in  
233 the quantity of secretion and gland size during the breeding season, which is expected if  
234 the chemicals within the secretion play a role in reproduction (Alberts et al., 1992;  
235 Hews, Knapp and Moore, 1994). Surprisingly, pore size seems not to vary seasonally  
236 (Van Wyk, 1990). Changes in the chemical composition of the secretion during the  
237 mating season have also been observed and possibly serve to increase the secretion's  
238 volatility and detectability (Alberts et al., 1992).

239           If natural selection acts to maximize efficiency of information transfer, then  
240 animal signals should exhibit structural features optimal for specific functions under  
241 particular environmental conditions. Variation in the epidermal gland system can  
242 therefore be partly explained by adaptations to specific extant ecological conditions  
243 (Alberts, 1990, 1991). A variety of abiotic variables seem to potentially affect the  
244 number of epidermal pores present on each thigh (Escobar, Labra and Niemeyer, 2001;  
245 Pincheira-Donoso, Hodgson and Tregenza, 2008; Baeckens et al., 2015). In *Liolaemus*  
246 lizards, Escobar and colleagues (2001) observed an increase in pore number with  
247 altitude, suggesting that lizards and amphisbaenians may produce more secretions under  
248 harsh environmental conditions. However, a subsequent paper by Pincheira-Donoso et  
249 al. (2008) on the same genus, but with additional phylogenetic input, failed to find any  
250 effect of environmental conditions on pore number. They explain the assessed variation  
251 in pore numbers in *Liolaemus* as a result of shared ancestry rather than a result of  
252 phylogenetic independent adaptive events. A decrease of pore number with altitude has

253 also been found, which is explained by the hypothesis that low elevation individuals  
254 might be subjected to more intense sexual selection than individuals at high elevation  
255 (Iraeta et al., 2011).

256         Using phylogenetic informed analyses, Baeckens and colleagues (2015)  
257 investigated the role of the physical environment on the variation in epidermal pore  
258 number in 162 lacertid species, including all genera of the family. They found no effect  
259 of climate conditions or latitude on species pore number, but did find an effect of  
260 substrate use: shrub-climbing species tended to have fewer femoral pores than species  
261 inhabiting other substrates, possibly because of the lower scent mark persistence on  
262 such substrates. A similar explanation regarding substrate use has been offered for  
263 lizards living in meadows or grasslands, such as *Psammodromus hispanicus*, which has  
264 much less epidermal pores and compounds in its secretions when compared with other  
265 closely related species (López and Martín, 2009). It has also been observed that in cool  
266 or dry environments, individuals of the same species produce more secretion than in  
267 hotter and more humid climates (Gabirot et al., 2008, 2010; Mouton, Van Rensburg and  
268 Van Wyk, 2010), probably because in the latter environmental conditions, chemical  
269 components of the secretion volatilize more rapidly and subsequently have decreased  
270 efficiency (Alberts, 1992b).

271         In addition to variation in the chemoreceptive qualities or quantities of the  
272 secretion, variation in spectroscopic characteristics of the secretion in lizards has also  
273 been observed. Desert iguanas (*D. dorsalis*) inhabit hot, arid deserts, whereas green  
274 iguanas (*Iguana iguana*) live in humid tropical forest, suggesting that signal  
275 transmission is subjected to different environmental constraints (Alberts, 1990). Due to  
276 the protein-rich character of the epidermal secretions of desert iguanas, the secretions

277 are relatively non-volatile, and therefore difficult to detect by chemoreception at long  
278 distance. Spectroscopic analyses of the gland secretions revealed strong absorption in  
279 the ultraviolet light spectra (Alberts, 1989a). These findings argue that secretion  
280 deposits of the desert iguana also act as an ultraviolet visual marker, providing a  
281 possible mechanism for locating these low volatility chemosignals at far range (Alberts,  
282 1989a). In contrast, green iguanas secrete a more lipid-rich (and thus) volatile secretion  
283 with no strong light absorption in the long wave light spectra (Alberts, 1990). Because  
284 green iguana secretions transmit well under humid conditions, there is no need for a  
285 visual cue (Alberts, 1990). As far as we know, no spectrometric information is available  
286 on the epidermal secretions of other lizard or amphisbaenian species.

287         Differences in pore-bearing scale morphology among species can also be linked  
288 to environmental factors. The desert species *Acanthodactylus boskianus* and *A.*  
289 *scutellatus* are equipped with pore-bearing scales divided into two pieces: a large  
290 convex piece and a small dome-shaped piece (Khannoon, Dollahon and Bauer, 2013).  
291 This is different from other lizards studied, which have rosette-like pore bearing scales  
292 (Cole, 1966b; Blasco, 1975). The convex part of the pore-carrier differentiated scales of  
293 both *Acanthodactylus* species may be useful in ‘cutting’ the secretory plug in pieces  
294 with relatively a small surface area to volume ratio when deposited on the substrate.  
295 This ratio allows the low molecular weight compounds of the secretion to slowly release  
296 from the plug pieces as they degrade (Khannoon, Dollahon and Bauer, 2013). As such,  
297 the deposited secretion will remain on the substrate for enough time to convey their  
298 chemical message in a desert-like environment (Khannoon et al., 2010; Khannoon, El-  
299 Gendy and Hardege, 2011).

300 As differences in chemical composition play a key role in determining mating  
301 success, we can say that the epidermal gland system is subjected to both sexual and  
302 natural selection, and displays extensive intra- and interspecific variation. It is a  
303 complex system that remains, for the most part, poorly understood.

304

### 305 **The phylogenetic distribution of epidermal pores in non-ophidian squamates**

306 Epidermal gland secretions thus seem to play an important role in many species.  
307 However, glands are absent in many other species. This disparity may offer unique  
308 opportunities for testing evolutionary and ecological hypotheses on the significance of a  
309 chemical signalling system. Because epidermal glands function as an essential  
310 component of the non-ophidian squamate chemical signalling system, their absence will  
311 subsequently affect a species' ability to communicate in this way. By reconstructing  
312 ancestral character states, we can attempt to comprehend this variation in epidermal  
313 gland expression.

314 We extracted data on the presence or absence of epidermal pores in all 42 non-  
315 ophidian squamate families from the literature. Because direct histological information  
316 on the presence or absence of epidermal glands is available for only a handful of  
317 species, we infer epidermal gland presence based on the absence or presence of male  
318 pores. If a taxon was said to not have pores, we did not investigate beyond the family  
319 level. However, when a family had at least some genera or species with epidermal  
320 pores, we obtained data at a higher resolution. In total, we gathered information on  
321 epidermal pores of 2899 species, which corresponds to approximately 46% of all  
322 described Lacertilia and Amphisbaenia species (Uetz and Hošek, 2014). We followed

323 the nomenclature according to Pyron, Burbrink and Wiens (2013). To evaluate  
324 evolutionary changes, we performed an ancestral-state reconstruction in Mesquite  
325 v2.75, using a MK1 maximum likelihood approach (Mooers and Schluter, 1999;  
326 Maddison and Maddison, 2011). Taxa were scored with binary coding: 0 (epidermal  
327 pores absent) or 1 (epidermal pores present), and mapped onto a recent nuclear and  
328 mitochondrial DNA-based phylogeny of Squamata by Pyron, Burbrink and Wiens  
329 (2013). A difference of 2 log likelihood units (lnL) between character states for a given  
330 node was considered as statistically significant (Pagel, 1994, 1999). This corresponds to  
331  $\sim 0.89$  proportional likelihood (Calley and Linder, 2006).

332 The results of the reconstruction are visualized in Fig. 3 and Fig. 4 (A–G;  
333 supplemental material) representing a large-scale phylogeny of the non-ophidian  
334 squamates on a family level and on a species level, respectively. Of all sampled species,  
335 1695 were reported without pores ( $\sim 58\%$ ) and 1204 with pores ( $\sim 42\%$ ) (Table 2). We  
336 found that epidermal pores were most likely absent in the common ancestor of non-  
337 ophidian squamates, but are widely distributed in extant taxa. In 11 families, all species  
338 possessed pores, in 18 families, all species lack pores, while in the remaining 13  
339 families, pores were present in some species, but absent in others (Fig. 3). There  
340 appears to be extensive lability in the acquisition and loss of pores, as many groups  
341 appear to have gone through multiple gain/loss events independently, both between  
342 families and within a genus (Fig. 4, supplemental material).

343 Although we find that pores were most likely absent in the common ancestor of  
344 non-ophidian squamates, we do not make any assumption on the presence or absence of  
345 secretory glands in general in the common ancestor. Our reconstruction only focuses on  
346 those epidermal glands that secrete their secretion through pores and does not include

347 other glands, such as urodeal, gular, cloacal, or generation glands. Our data are  
348 insufficient to hypothesize on where and when chemical signalling through epidermal  
349 glands evolved or vanished in squamates, but allow us to think about the drivers behind  
350 the evolution of the epidermal gland system (Imparato et al., 2007; Baeckens et al.,  
351 2015). Based on the results of this study, one main conclusion can be drawn: the  
352 frequency of character shifts varies considerably between clades. For example, there are  
353 20 character shifts within Gekkonidae, two of which are secondary follicular epidermal  
354 gland gains. In contrast, within Scincidae – the family containing most species within  
355 Lacertilia – not a single character-shift is observed. There are at least two possible  
356 explanations for this disparity: (1) the number of character state shifts could be higher in  
357 a clade inhabiting a wide variety of habitats due to adaptations to their local  
358 environment, as chemical signalling efficiency through epidermal glands is affected by  
359 environmental conditions (Pincheira-Donoso, Hodgson and Tregenza, 2008; Escobar,  
360 Labra and Niemeyer, 2011; Baeckens et al., 2015) or (2) morphological constraints  
361 could inhibit (or allow) for the gain or loss of epidermal glands. These two explanations  
362 are by no means exclusive, as in some instances one may play a greater role than the  
363 other.

364         We do not have a complete understanding of what drives the evolutionary  
365 acquisition of these glands, nor do we understand what drives different groups to lose  
366 them. For example, what prompted the species *Meroles anchietae* – the only lacertid  
367 lizard without glands – to lose epidermal glands? By approaching the evolution of  
368 epidermal glands within a phylogenetic framework, we can now begin to tease apart  
369 how environment, ecology, and morphology impact the evolution of epidermal glands.  
370

371 **Functions of epidermal gland secretions**

372 *Territoriality and dominance*

373 Many species are territorial. This often results in a variety of physical displays and  
374 agonistic behaviour in which males will actively defend their territory, and where the  
375 outcome of these encounters helps to establish dominance hierarchies (e.g., Baird and  
376 Timanus, 1998; Sheldahl and Martins, 2000; Peters and Ord, 2003). The establishment  
377 of dominance hierarchies often determines the size and quality of an individual's  
378 territory. There are many obvious morphological factors such as body mass and head  
379 width that help to establish these hierarchies (Olsson and Madsen, 1998). However,  
380 dominance status can also be assessed solely on the basis of epidermal secretions (Font  
381 et al., 2012; Heathcote et al., 2014). In Iberian rock lizards (*Iberolacerta monticola*),  
382 males with higher dominance status have higher concentrations of hexadecanol and  
383 octadecanol in their secretions (Martín, Moreira and López, 2007a). In addition, males  
384 tend to respond aggressively toward hexadecanol while responding neutrally towards  
385 other chemicals, and show differential chemosensory and aggressive behaviour  
386 according to their own dominance status. Male *A. boskianus* are also known to respond  
387 more aggressively towards cotton swabs confronted with male secretions than towards  
388 control swabs (Khannoon et al., 2010). The ability to determine dominance status of  
389 conspecifics by epidermal secretions has been hypothesized to minimize the cost of  
390 agonistic encounters, lending credence to the idea that secretion can function as territory  
391 markers (Aragón, López and Martín, 2001a; Khannoon, El-Gendy and Hardege, 2011).  
392 These functions have been tested experimentally, and it has been found that some lizard  
393 species are able to identify the competitive ability of conspecific males based on scent  
394 marks alone. Additionally, it has been found that they are able to consistently identify

395 specific individuals based on these cues, which allows resident males to change their  
396 behaviour accordingly (Carazo, Font and Desfilis, 2008).

397       Because density dependent interactions powerfully affect post-natal growth,  
398 colonization of habitats can easily be related to intraspecific competition. Unsettled  
399 individuals will often stay longer in initially empty habitats than they do in occupied  
400 patches (Le Galliard, Ferrière and Clobert, 2005). Resident males of *Iberolacerta*  
401 *monticola* can discriminate neighbours from unfamiliar males based on chemical cues  
402 left on the substrate, and intruders have been shown to display significantly more escape  
403 behaviour in response to unfamiliar male cues (Aragón, López and Martín, 2001a,b,c,  
404 2003).

405

#### 406 *Mate assessment*

407 As with many animals, lizards and amphisbaenians utilize elaborate sexual signals in  
408 order to attract potential mates (Andersson, 1994). Attraction of females to sexual  
409 signals of males can evolve if these signals provide honest information on certain  
410 favourable characteristics of males (Grafen, 1990; Johansson and Jonas, 2007), or if the  
411 signal exploits the sensory system of females that have a sensory bias for certain traits  
412 (Fuller, Houle and Travis, 2005; Macías-García and Ramirez, 2005). A plethora of  
413 studies have shown that non-ophidian squamates rely strongly on chemical sexual  
414 signals to attract mates (Martín and López, 2011), and that secretion from the epidermal  
415 glands bears valuable information in relation to mate choice (Martín and López, 2000;  
416 López, Munoz and Martín, 2002; López, Aragón and Martín 2003; López and Martín,  
417 2006c). Females have been shown to discriminate between males based on the scent

418 from these secretions alone, and there is strong evidence that, at least in some species,  
419 mate choice plays an important role in reproduction (Olsson et al., 2003; Font et al.,  
420 2012; Swierk et al., 2012).

421 For example, female lizards have been found to be able to determine the health  
422 status of males based only on the secretion of epidermal glands (e.g., López and Martín  
423 2005a; Martín and López 2006b; López, Gabirot and Martín, 2009; Martín et al. 2007),  
424 suggesting that there is a discernible signal found within the secretion. As a general  
425 rule, signals can only be evolutionary stable if they are honest and condition dependent,  
426 or costly to the sender, and if the cost is correlated with the sender's quality (Grafen,  
427 1990; Zahavi and Zahavi, 1997). Because chemicals are costly to produce, as only  
428 males in good health can afford to allocate chemicals to femoral secretions instead of to  
429 other bodily functions, they are considered to be honest signals which display accurate  
430 information about a male's health state (Martín, Amo and López, 2008). Male Iberian  
431 Rock Lizards with a high immune response have been shown to have higher proportions  
432 of various alcohols and carboxylic acids within their secretions than individuals with a  
433 low immune response (López, Amo and Martín, 2006). This may be important, as when  
434 presented with cues from only femoral secretions, López and colleagues (2006) found  
435 that females preferentially choose male scents which signal a higher immune response.  
436 Female *Psammodromus algirus* also have been found to show more chemoreceptive  
437 behaviour in response to secretions of males with low parasite infections and high T-  
438 cell-mediated immune responses (Martín et al., 2007). Female lizards have also been  
439 shown to prefer males with secretions having high proportions of compounds that have  
440 important metabolic functions for organisms. Martín and López (2012) found that  
441 females prefer to associate with areas marked by males with experimentally increased

442 provitamin D. A similar conclusion was reached by Kopena et al. (2011) when  
443 investigating increases in Vitamin E. Oleic acid also plays a role in female mate choice  
444 (Martín and López 2010a).

445         When determining which male to reside with, females have been shown to  
446 evaluate and react to the symmetry of males based on scent alone. Symmetry indicates  
447 the developmental stability of an individual and thus, its ability to cope with genetic and  
448 environmental disturbances during development (Van Valen, 1962). Fluctuating  
449 asymmetry (FA), small random deviations in the development of both sides of a  
450 bilateral symmetric character is therefore often used as a measure for developmental  
451 instability (Martín and López, 2000; Lens et al., 2002; Klingenberg, 2003). FA can be  
452 quantified using many metrics, including by comparing the number of femoral pores on  
453 each thigh of a lizard. It is generally assumed that animals, which exhibit a high FA are  
454 of a low fitness (Van Valen, 1962). For example, in *Iberolacerta monticola* (López and  
455 Martín, 2002) and *Psammodromus algirus* (Martín and López, 2001), males with more  
456 asymmetrical femur length have lower escape speeds, and *Sceloporus occidentalis*  
457 males with high FA have an increased susceptibility to parasitism (Schall, 1995). Lazic  
458 et al. (2013) found that *Podarcis muralis* individuals inhabiting urban areas (associated  
459 with high levels of environmental stress) are more asymmetric than those in more rural  
460 areas. Based on only the chemicals found within femoral secretions, female Iberian rock  
461 lizards have been shown to prefer to associate with the scents of males with low FA,  
462 and with individuals with more femoral pores on each leg (López, Munoz and Martín,  
463 2002). Although social dominance is traditionally thought to demonstrate the quality  
464 and mating potential of a male, the dominance status of males has not been shown to  
465 correlate with FA, and females seem to prefer areas marked by low FA individuals

466 rather than dominant males. As such, it is postulated that in some species, symmetry  
467 potentially plays a greater role in mate choice than does dominance (López, Muñoz and  
468 Martín, 2002). It should be noted that the results of some studies investigating FA are  
469 somewhat ambiguous, and their use as a metric of male quality has recently declined  
470 (Van Dongen, 2006).

471         In many vertebrate groups, females prefer to mate with older males, possibly  
472 because their continued survival displays high quality (Osada et al., 2003). Females are  
473 able to discriminate between young and old males based on chemical cues alone (López,  
474 Aragón and Martín, 2003; Martins et al., 2006), and subsequently choose habitats in  
475 which older males reside. Older *Psammodromus algirus* males secrete proportionally  
476 less carboxylic acids and more steroids than younger lizards, and the ability of females  
477 to detect and react to these differences implies that age related differences of chemicals  
478 in femoral secretions play an important role in the organization of this lizard's social  
479 hierarchy (Martín and López, 2006a). Similarly, in *Podarcis muralis*, younger males  
480 show significantly higher proportions of aldehydes, alcohols and ketones and  
481 significantly lower proportions of tocopherols than older males (Pellitteri-Rosa et al.,  
482 2014).

483         As a general rule, other than advertising indirect information on male quality  
484 (e.g., body size, FA, maximum sprint speed), chemical signals also contain honest  
485 information on direct benefits (e.g., access to high quality food or thermal resources),  
486 which are often argued to be more important (Dussourd et al., 1991; Hill, 1991;  
487 Candolin, 2000). As access to high-quality thermal resources is crucial for reproductive  
488 success in female squamates, females may be able to detect thermally-induced variation  
489 in chemical composition of male scent marks when assessing the quality of his territory.

490 A study by Heathcote et al. (2014) showed that the amount of time male wall lizards  
491 (*Podarcis muralis*) are allowed to bask significantly alters the chemical composition of  
492 their femoral secretion, and that females can discriminate between scent marks of males  
493 which experience different basking conditions.

494 The results of these studies indicate that chemical compounds in the epidermal  
495 secretions of lizards and amphisbaenians may provide reliable information on  
496 morphological traits, health status and territory quality of males (Table 2). Certain  
497 chemosignal compounds of the epidermal secretions have the possibility to therefore act  
498 as a chemical ornament and to serve as a basis for adaptive female choice (Martín and  
499 López, 2000; López, Amo and Martín, 2006).

500

### 501 **Prospects on future research**

502 Despite the recent surge in research, a significant gap in our knowledge on the true role  
503 of the chemicals found in epidermal secretions remains, and there are many possible  
504 avenues for future research on the topic.

505

#### 506 *Understanding the function of the components in epidermal secretions*

507 We now know that many non-ophidian squamates use secretions from epidermal glands  
508 in a variety of processes, but little to no work has been done to determine what the  
509 function of the various chemicals in the secretion are. Field and laboratory studies by  
510 Mason et al. (1989, 1990) have unequivocally identified, characterized and synthesized  
511 a sex attractiveness pheromone of the red-sided garter snake (*Thamnophis sirtalis*

512 *parietalis*), which was subsequently identified as the first pheromone in reptiles. Future  
513 work should investigate this possibility in the epidermal gland secretions of lizards and  
514 amphisbaenians, and research should be expanded to include the proteins found in those  
515 secretions, because they could also function as chemosignals (Alberts, Phillips and  
516 Werner, 1993), as they do in many other groups (Toyoda et al., 2004; Janssenswillen et  
517 al., 2015). As far as we are aware, A.C. Alberts is the only researcher who has studied  
518 the protein fraction of epidermal gland secretion in lizards (1990, 1991, 1993). As we  
519 begin to discover which proteins in secretions function as chemosignals, it may be  
520 possible to reconstruct the evolution of the genes producing these molecules. Doing this  
521 would allow us to understand how the evolution of epidermal gland secretions occurred,  
522 and would assist in our understanding of squamate evolution in general.

523         Currently, there is a heavy bias towards studying the femoral gland secretions of  
524 West-European lacertids (Table 1), but without knowledge of other groups, it is  
525 impossible to fully understand how the chemosignals within epidermal glands have  
526 evolved. Furthermore, we know far more about the male epidermal gland system than  
527 we do about female glands, resulting in a gender bias. The tendency for researchers to  
528 avoid studying female animals is a worldwide phenomenon in science (Zucker and  
529 Beery, 2010; Clayton and Collins, 2014). This trend is also apparent in chemical  
530 ecology, as most research has focused on the epidermal gland system of male lizards  
531 and amphisbaenians, despite the knowledge that females of many species both possess  
532 and make use of this system (e.g., Alberts, 1991). In studies where the content of female  
533 epidermal secretions has been analysed, researchers find differences between both sexes  
534 (López and Martín, 2005b; Louw et al., 2007; Khannoon et al., 2011b). In desert  
535 iguanas, Alberts (1989b) found that while all males possess active epidermal glands

536 during the breeding season, only unmated females show glandular activity. Alberts  
537 (1990) thereby posited that female secretions may facilitate pairing by allowing  
538 unmated females to make their presence known to potential mates using epidermal  
539 deposits. Little has been done to investigate this hypothesis, but Kelso and Martins  
540 (2008) found that in *S. graciosus* females secrete more and larger secretion deposits  
541 when initially presented with males using specific courtship behaviour. Thus, although  
542 female epidermal secretions may not be as prevalent as they are in males, they still may  
543 serve an important role.

544         In our search to unveil the functions of the various chemical compounds, we  
545 have to understand that many signals in nature tend to be very complex (Partan and  
546 Marler, 2005; Bro-Jørgensen, 2009), and acknowledge that epidermal chemosignals  
547 seem to be too. This complexity brings along many possibilities. Do different  
548 components convey the same information (i.e., redundant signal hypothesis), or are  
549 there several bits of information on different aspects of quality (i.e., multiple message  
550 hypothesis), or are some of the signals obsolete? No research has tackled these standing  
551 questions using only chemical cues. However, more and more studies in communication  
552 biology are focusing on the relationships among and the integration of components from  
553 different sensory channels (Partan and Marler, 2005). Still, studies investigating  
554 multimodal communication signals in squamates are provisionally scarce (but see Hews  
555 and Benard, 2001; Whiting, Webb and Keogh, 2009; Ossip-Klein et al., 2013).

556

557 *Predation costs of chemical communication*

558 Individuals are at risk when communicating because conspicuous signals attract both  
559 conspecifics and eavesdropping predators (Magnahagen, 1991; Zuk and Kolluru, 1998;  
560 Huyghes, Kelley and Banks, 2012). Canids that track their prey eavesdrop on  
561 intraspecific signals, such as deposits from interdigital glands in cervids and bovids, or  
562 urine marks of rodents (Müller-Schwarze, 2006). The Texas blind snake (*Leptotyphlops*  
563 *dulcis*) feeds on termites and ants and finds its prey by following their pheromone trails  
564 (Gehlbach, Watkins and Kroll, 1971). No studies have attempted to investigate the  
565 potential interspecific costs of the use of scent marks in lizards and amphisbaenians  
566 with regards to chemical eavesdropping e.g., would they invest less in epidermal  
567 secretions when hunted by chemically-oriented predators, such as snakes?

568

#### 569 *Tongue flicking*

570 While there are many potential future topics regarding the epidermal gland secretions  
571 themselves, how non-ophidian squamates analyse these secretions is another avenue for  
572 future research. Tongue flicking in lizards functions to sample chemicals for  
573 vomerolfactory analyses, and is assumed to indicate chemosensory exploration (Cooper,  
574 1994; Cooper, Deperno and Arnett, 1994). To investigate how tongue flicking is used,  
575 researchers have presented lizards with chemical stimuli on either a cotton-tipped  
576 applicator or on a substrate in a test cage (Cooper, 1998), and then documented the  
577 lizards' response. One aspect of this behaviour that remains poorly explored is the  
578 direction of the tongue flicks. Alberts and Werner (1993) have shown that in male  
579 Green Iguanas, lizards tongue-flick the air proportionally more when presented with the  
580 lipid fraction of femoral gland secretions, and the chemical stimuli itself proportionally

581 more when presented with proteins from the secretion, a behaviour explained by the  
582 more volatile character of lipids than proteins. Future studies should account for this  
583 variation in behaviour (as in e.g. Cooper, Van Wyk and Mouton, 1996, 1999), as  
584 different types of tongue flicks can capture different aspects of the chemosignal content  
585 of epidermal gland secretions. Ultimately, we lack information on the neurological basis  
586 of chemical discrimination and the specific neural responses to specific compounds  
587 (e.g., MRI). This has been studied in other animal groups, such as starlings (Gugliemetti  
588 et al, 2012) and mice (De Groof et al., 2010), but information for squamates is still very  
589 incomplete.

590

#### 591 *Follicular glands vs. generation glands*

592 As previously mentioned, gekkonids and cordylids are the only lizard taxa where two  
593 types of holocrine epidermal glands have been described. Where follicular glands and  
594 generation glands co-exist, it is hard to accept that generation glands are merely under-  
595 evolved follicular glands, as advocated by Maderson and Chiu (1970). In cordylids,  
596 while both types of epidermal glands are present in males of all species, females display  
597 considerable variation: there are species with females having (1) both follicular and  
598 generation glands, (2) only follicular glands, or (3) no glands at all (Mouton, Van  
599 Rensburg and Van Wyk, 2010). Moreover, gland number in female cordylids appears  
600 strongly affected by climate, with a lower amount of glands in cooler compared with  
601 warmer environments (Mouton, Van Rensburg and Van Wyk, 2010). The role of the  
602 physical environment on gland number, and the apparent sexual dimorphism in the  
603 glandular system in Cordylidae (Mouton and Van Wyk, 1993), reflects the complexity

604 of the chemical communication system in lizards having both epidermal gland types.  
605 More research on the association of both glandular systems should be encouraged.

606

607 *Scope*

608 Our goal has been to try to make evolutionary sense of the mass of data that  
609 accumulated on the role of follicular epidermal glands and their secretion. While our  
610 knowledge of lizard and amphisbaenian biology has vastly increased in recent years,  
611 and although some major patterns in the epidermal gland system seem clear, much  
612 uncertainty remains, resulting in both a daunting and exciting future for biologists in the  
613 field.

614

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619

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1076 **Table 1.** Composition of the major lipophilic chemicals reported in lizards and amphisbaenians. Bolded entries are those not included in  
 1077 Weldon, Flachsbarth and Schulz (2008).

Family	Species	Steroids	Carboxylic acids	Alcohols	Alkanes	Ketones	Squalene	Tocopherol	Reference(s)	Intraspecific variation
Agamidae	<b><i>Acanthocercus atricollis</i></b>	66.9	17.7	x	x	6.4	2.6	2.8	Martín, Ortega and Lopez, 2013a	
Agamidae	<b><i>Uromastix aegyptia microlepis</i></b>	58.6	16.5	0.5	x	1.6	9.5	3.4	Martín et al., 2012	Martín et al., 2012*
Amphisbaenidae	<i>Blanus cinereus</i>	86.8	4.2	x	x	x	4.6	x	López and Martín, 2005b	López and Martín, 2005b*
Cordylidae	<i>Cordylus giganteus</i>	42.53	23.34	0.06	0.02	0.81	26.56	1.5	Louw et al., 2007	Louw et al., 2007*



Lacertidae	<i>Acanthodactylus erythrurus</i>	19.6	6.8	72.2	x	0.04	0.3	0.04	López and Martín 2005c	López and Martín 2005c
Lacertidae	<i>Iberolacerta cyreni</i>	84.6	14.6	0.4	x	x	0.3	x	López and Martín 2005d	Aragón et al., 2008
Lacertidae	<b><i>Ibertolacerta monticola</i></b>	89.3	8.4	1.7	x	x	0.28	x	López et al., 2009a	López et al. 2006; Martín and López 2006b; López, Moreira and Martín, 2009
Lacertidae	<i>Lacerta schreiberi</i>	66.7	10.9	2.5	x	0.22	0.2	18.3	López and Martín, 2006	
Lacertidae	<b><i>Lacerta viridis</i></b>	59.6	4.6	2.6	x	0.2	2.5	28.5	Kopena,	

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									López and Martín, 2009
Lacertidae	<i>Podarcis atrata</i>	78.47	9.89	2.75	x	0.13	0.35	x	Gabirot et al., 2010
Lacertidae	<i>Podarcis hispanicus Type 1</i>	84.18	10.45	2.24	x	0.08	0.44	x	Martín and López 2006d†
									Martín and López 2006d; Gabirot et al., 2010; Gabirot, López and Martín, 2012a,b, 2013
	<i>Podarcis hispanicus Type 2</i>	84.88	12.07	0.88	x	0.04	0.42	x	Martín and López 2006d†
									Martín and López 2006d; Gabirot et al., 2010; Gabirot,

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										López and Martín, 2012a,b, 2013
Lacertidae	<i>Podarcis lilfordi</i>	94.4	0.6	0.5	1.4	0.8	0.7	x	Martín et al., 2013	Martín et al., 2013
Lacertidae	<i>Podarcis muralis</i>	83.7	12.8	0.29	x	0.11	0.97	1.95	Martín and López, 2006d	Pellitteri-Rosa et al., 2014
Lacertidae	<i>Psammodromus algiurus</i>	48.1	48.5	2.3	x	0.12	0.3	0.17	Martín and López, 2006a	
Lacertidae	<i>Psammodromus hispanicus</i>	72.6	23.5	3.8	x	x	0.1	x	López and Martín, 2009	
Lacertidae	<i>Timon lepida</i>	59	12.2	0.7	x	0.02	0.15	27.9	Martín and López,	

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Lacertidae	<i>Zootoca vivipara</i>	92.14	6.2	x	x	x	1.68	1.01	2010b	Gabirot et al., 2008	Gabirot et al., 2008
Liolaemidae	<i>Liolaemus bellii</i>	45.5	48.7	x	5.8	x	x	x	Escobar, Labra, Niemeyer, 2001		
Liolaemidae	<i>Liolaemus fabiani</i>	14.4	72.6	x	12.9	x	x	x	Escobar et al., 2003	Escobar et al., 2003	
Liolaemidae	<i>Liolaemus sp.</i>	19.9	61.5	x	18.6	x	x	x	Escobar, Labra, Niemeyer, 2001		

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1078 “x” - There have been no reported chemicals of the specified type for that species. “\*” – Female secretions were evaluated. “†” - This species has since  
 1079 been split into two species (Geniz et al., 2014), however we followed the two types listed in Martín and López 2006 to avoid confusion.

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1081 **Table 2.** An overview of the different social contexts that epidermal gland secretions are known to be used in lizards and  
 1082 amphisbaenians.

General function	Information content	Species	Reference(s)
Interspecific recognition		<i>Liolaemus sp.</i>	Labra, 2011
General intraspecific recognition	Familiar vs. unfamiliar	<i>Iguana iguana; Iberolacerta monticola</i>	Alberts and Werner, 1993; Aragón, López and Martín, 2001b,c
	Population recognition	<i>Podarcis hispanica</i>	Gabirot, López and Martín, 2012a,b
	Colour morph	<i>Iberolacerta monticola; Podarcis muralis</i>	López, Moreira and Martín, 2009; Pellitteri-Rosa et al., 2014
	Sex recognition	<i>Acanthodactylus boskianus</i>	Khannoon et al., 2010
Self-recognition		<i>Dipsosaurus dorsalis</i>	Alberts, 1992

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Social dominance		<i>Amphibolurus fordi</i> ; <i>Cordylus polyzonus</i> ; <i>Iberolacerta monticola</i> ; <i>Iguana iguana</i>	Fergusson et al., 1985; Van Wyk, 1990; Alberts et al., 1992; Moreira, López and Martín, 2006
Territoriality		<i>Acanthodactylus boskianus</i> ; <i>Dipsosaurus dorsalis</i> ; <i>Iberolacerta monticola</i>	Alberts, 1993; Martín, Moreira and López, 2007; Khannoon et al., 2010; Martín and López, 2012
Mate assessment	FA	<i>Iberolacerta monticola</i>	Martín and López, 2000
	Age	<i>Iberolacerta monticola</i>	López et al., 2003; Martín and López, 2006a
	Accessibility to quality thermal resources	<i>Podarcis muralis</i>	Heathcote et al., 2014
	Immune response level	<i>Iberolacerta cyreni</i> ;	López and Martín, 2005a;

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	<i>Iberolacerta monticola</i> ;	Martín et al., 2007; López,
	<i>Psammodromus algirus</i>	Gabirot and Martín, 2009
Parasite load	<i>Psammodromus algirus</i>	Martín et al., 2007
Vitamin E concentration	<i>Lacerta viridis</i>	Kopena, López and Martín, 2011; Kopena et al., 2014

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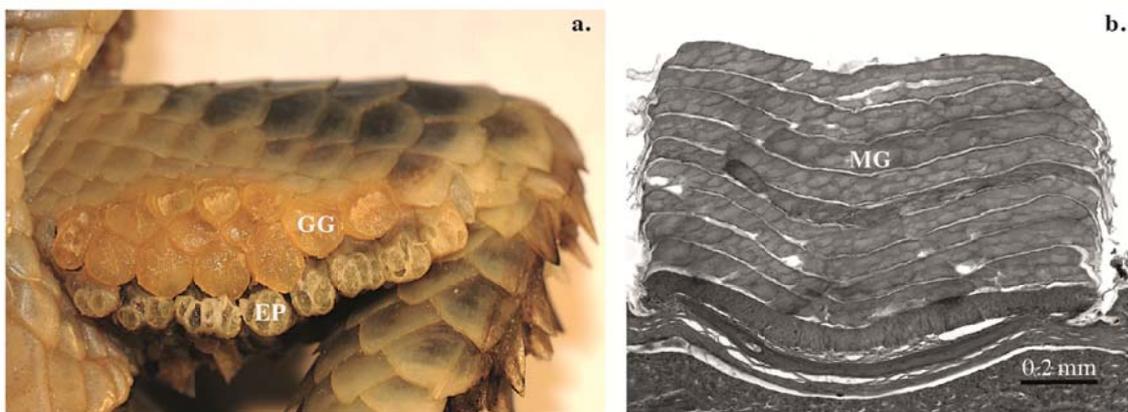
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1095 **Figure 1.** Photomicrographs of (a) the ventral region of the thigh of a cordylid lizard,  
1096 showing the patches of generation glands and a row of epidermal pores, and (b) a cross-  
1097 section through a protruding multiple-layer type generation gland of *Cordylus minor* (both  
1098 pictures obtained from Mouton, Flemming and Broeckhoven, 2014 with permission) (EP,  
1099 epidermal pore; GG, generation gland; MG, mature generation layer).

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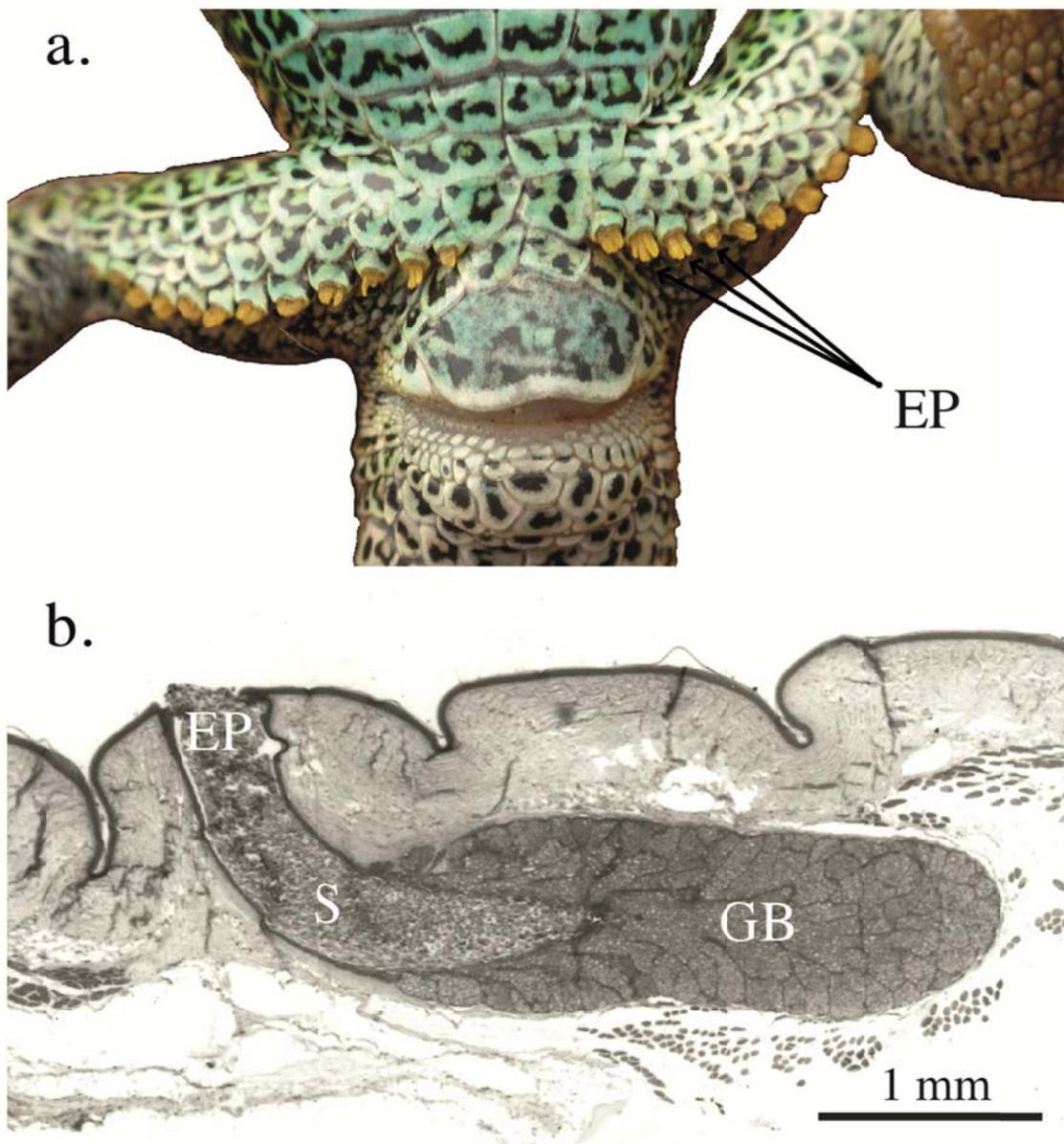
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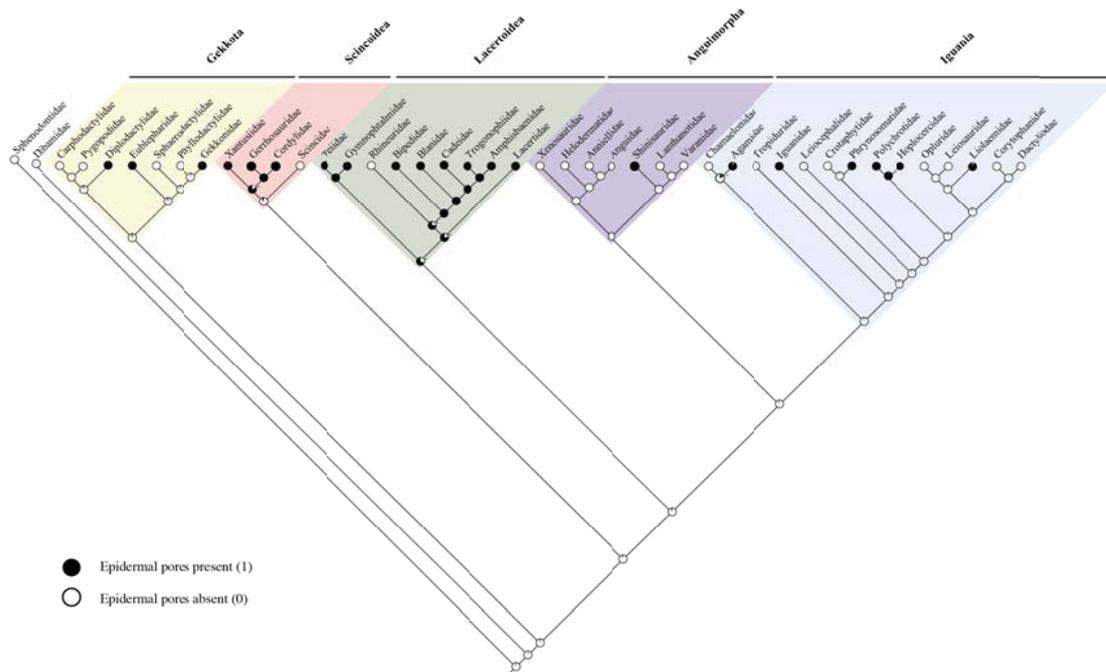
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1107 **Figure 2.** Picture of (a) the cloacal region of a male *Lacerta agilis* adult, showing  
1108 epidermal pores with protruding secretion, and (b) a longitudinal section of a follicular  
1109 epidermal gland of *Amphisbaena alba* (latter picture obtained from Antoniazzi et al. 1993  
1110 with permission) (EP, epidermal pore; GB, glandular body; S, secretion).



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1114 **Figure 3.** Evolution of epidermal pores in non-ophidian squamates: a family overview.  
 1115 Phylogeny proposed by Pyron, Burbrink and Wiens (2013). Pie charts from the maximum  
 1116 likelihood analyses are shown for ancestors, with the proportion of black representing the  
 1117 likelihood of epidermal pores being present in this ancestor. Pie charts are marked with a  
 1118 minus symbol “-“ (upper left side) when optimization is statistically not significant, all  
 1119 other nodes are significant.



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