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A novel epidermal gland type in lizards (α-gland): structural organization, histochemistry, protein profile and phylogenetic origins

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Chemical signalling is an essential component of the communication system of lizards, and epidermal glands are responsible for producing semiochemicals that regulate many behavioural interactions. Two types of epidermal glands have been previously described for lizards: follicular and generation glands. Generation glands are characterized by the aggregation of novel glandular cell types in the epithelium and the lack of a lumen or external pore. Despite the fact that several subtypes of generation glands have been recognized over the years, the morphology, taxonomic distribution, function and evolutionary origins of generation glands remain nearly unexplored in Neotropical clades. Here, we describe a novel escutcheon-type generation gland ('α-gland') for lizards of the South American family Tropiduridae, characterize its structural and ultrastructural organization, and study the homology of the constituent parts in a phylogenetic framework. The α-glands emerged in the ancestor of *Eurolophosaurus*, *Plica*, *Strobilurus*, *Tropidurus* and *Uracentron*, and are found in at least 39 species with diverse ecological habits. We preliminarily analysed the protein profile of α-glands and discovered differential expression of protein components between sexes. Our investigations change the general view about epidermal gland homology, leading us to argue that generation and follicular glands are possibly more closely related functionally and evolutionarily than previously thought.

ADDITIONAL KEYWORDS: chemical communication – chemical signalling – generation glands – homology – Iguania – South American collared lizards – squamate skin – Tropiduridae – Tropidurinae.

INTRODUCTION

Animal communication systems have evolved an extraordinarily varied set of structures and signals. Among squamate reptiles, communication is established through complex visual, acoustic and chemical signalling modes that allow for a diverse behavioural repertoire (Greenfield, 2002; Houck, 2009; Bro-Jørgensen, 2010; Apps *et al.*, 2015). In recent years, there has been a growing number of studies showing that chemical signalling is often a critical – yet underappreciated – component of the communication systems of lizards, worm lizards and snakes (Baeckens *et al.*, 2017a; García-Roa *et al.*, 2017a). Whereas squamates can release chemical cues to the exterior environment through simple mechanisms (e.g. faeces or skin), epidermal glands are exceptionally important for chemical signalling because the blends of semiochemicals they produce control essential behavioural interactions, including species and individual recognition (Cooper & Vitt, 1987; Alberts & Werner, 1993; Cooper *et al.*, 1999; Aragón *et al.*, 2001a, 2001b; Barbosa *et al.*, 2006; Carazo *et al.*, 2008; Gabirot *et al.*, 2010, 2012), establishment of social hierarchies (Mason, 1992; Mason & Parker, 2010; Martín & López, 2011), territoriality (Mason & Parker, 2010) and intersexual selection (Martín & López, 2006a, 2006b,

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2012, 2013a, 2013b; Johansson & Jones, 2007), not to mention their contribution to reproductive isolation and speciation (Gabirot *et al.*, 2012; Zozaya *et al.*, 2019).

To date, two major types of epidermal glands have been described in squamates, both with a holocrine mode of secretion (Maderson, 1972; Figs 1, 2): (1) follicular and (2) generation glands. Gabe & Saint-Girons (1965) includes a review of earlier works covering morphological and functional aspects of epidermal glands, and these authors explain that the epidermal glands treated here as 'follicular glands' (*sensu* Mayerl *et al.*, 2015) were originally noted as '*puncta callosa*' by Linnaeus (1758) but only characterized histologically more than a century later by Leydig (1872). Gabe & Saint-Girons (1965) also attributed the original description of generation glands to Tölg (1905), although thorough characterizations have only been provided more recently (e.g. Taylor & Leonard, 1956; Maderson, 1967a, 1967b; Van Wyk & Mouton, 1992; Dujsebayeva, 1998; Dujsebayeva *et al.*, 2007, 2009; Mouton *et al.*, 2010, 2014).

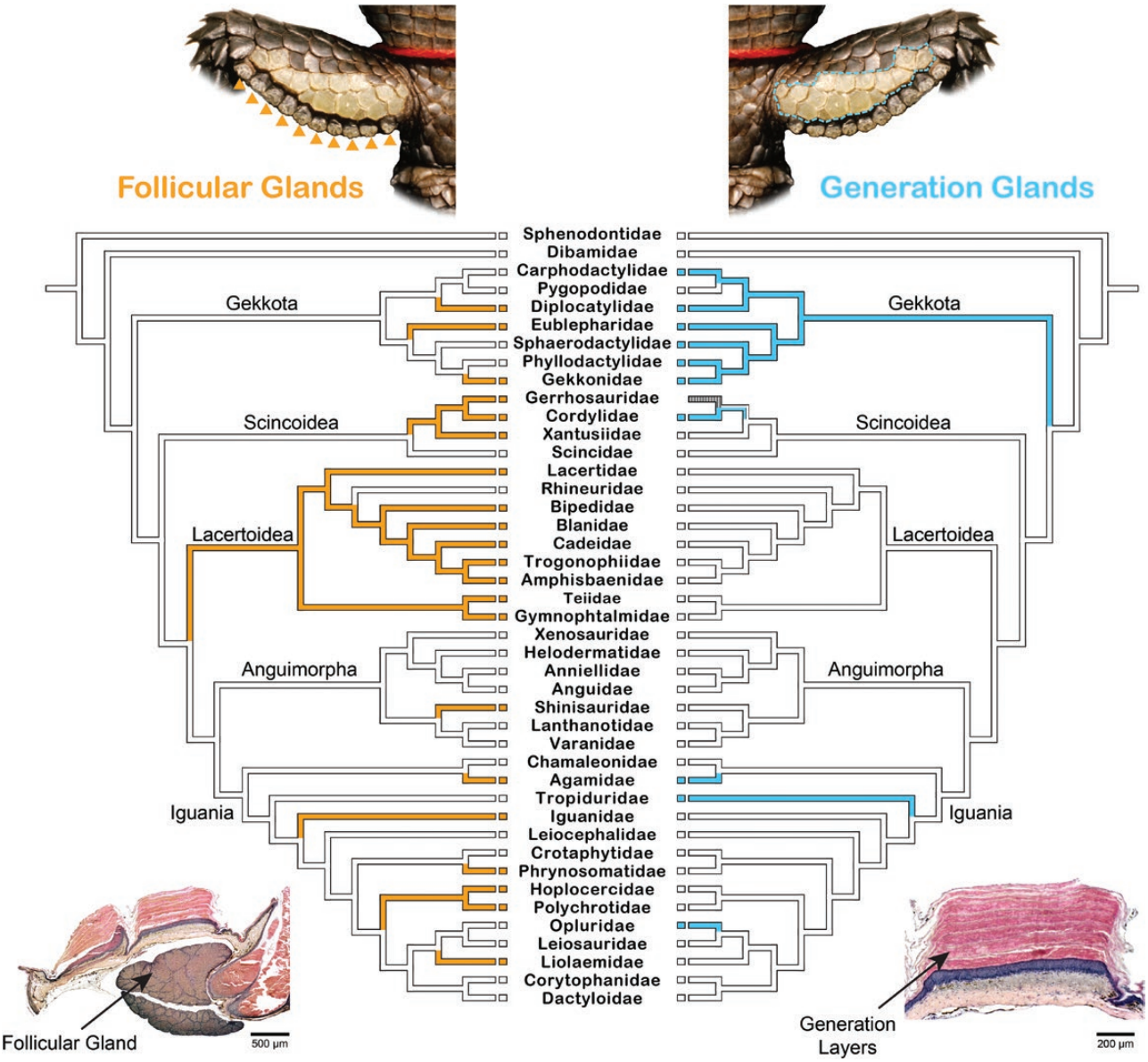

Figure 1. Phylogenetic distribution of major epidermal gland types in lizards. Multiple instances of independent evolution are supported for each gland type (i.e. follicular and generation glands). The topology shown is a pruned version of Pyron *et al.*'s (2013) squamate tree. Images of the external appearance and histological profile of the epidermal glands are from *Cordylus niger* Cuvier, 1829 and *Smaug giganteus* (Smith, 1844), respectively (images by J. H. van Wyk).

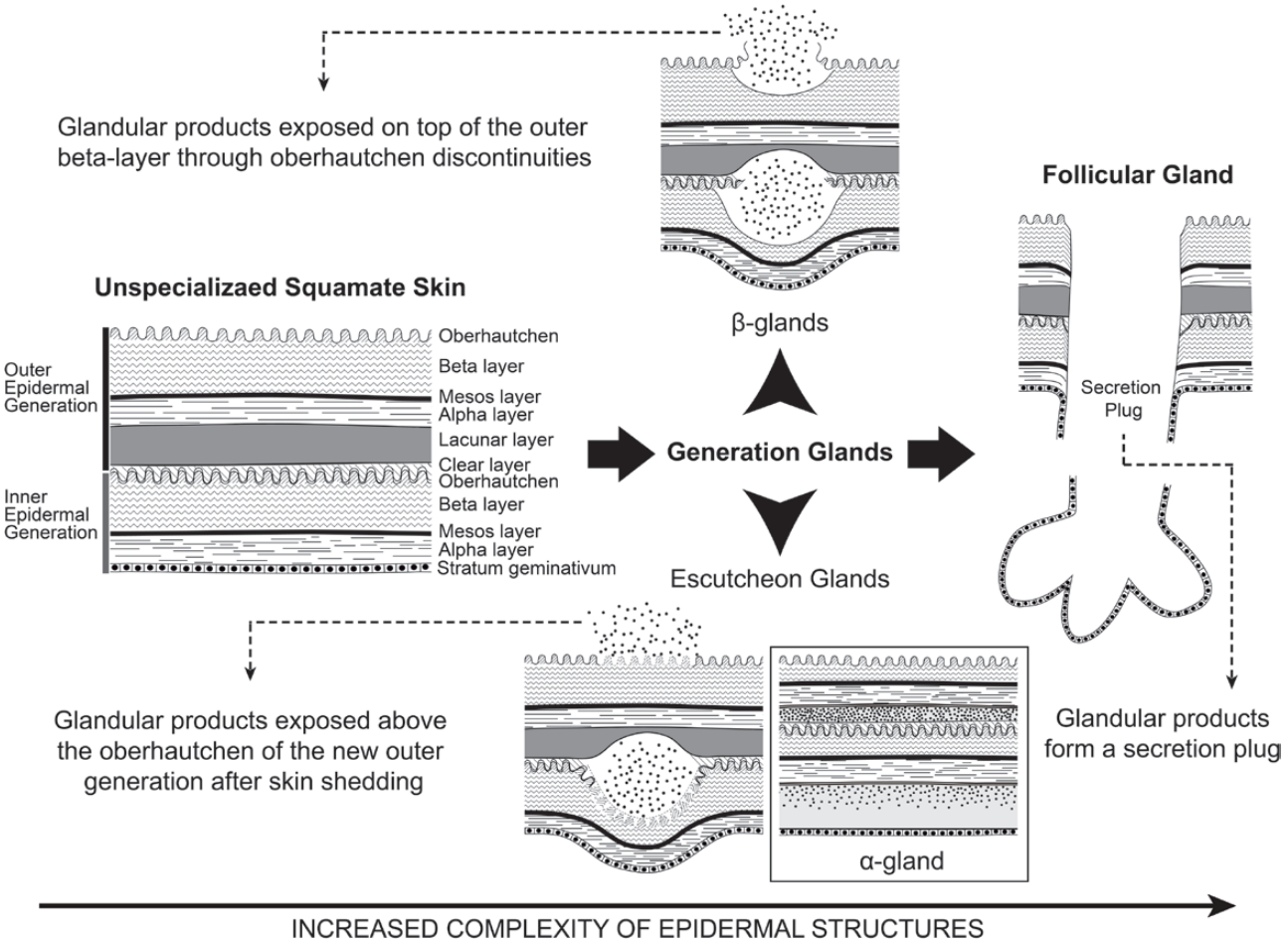

Figure 2. Histological structure of unspecialized skin and epidermal glands. Diagrammatic representation of the unspecialized squamate skin and major epidermal gland types, illustrating their respective secretion mechanisms as hypothesized by Maderson (1972).

Follicular glands are invaginated tubular or follicular units that are both morphologically and chronologically independent of the undifferentiated body epidermis around them. The protein- and lipid-rich, waxy products of these glands are stored extracellularly in a lumen as a solid secretion plug and released to the exterior through a pore located in the pre-cloacal, posterior abdominal and/or femoral region (Maderson, 1972).

Generation glands consist of aggregations of one or more novel cell types embedded in an otherwise undifferentiated six-layered epithelium. Unlike follicular glands, generation glands lack a lumen and are not associated with an external pore, and the differentiation of their products and subsequent exposure to the exterior environment are reliant upon a modification of the topography of the epidermal generation and/or a localized alteration of the shedding process (Maderson, 1967; 1972; Fig. 2). While follicular glands are found in ~25% of all non-ophidian squamates (Mayerl *et al.*, 2015; García-Roa *et al.*, 2017a), generation glands are restricted to fewer lizard groups [viz., geckos (Maderson, 1967; Maderson & Chiu, 1970), cordylids (Van Wyk & Mouton, 1992; Mouton *et al.*, 2010, 2014), agamids (Dujsebayeva, 1998; Dujsebayeva *et al.*, 2007) and oplurids (Dujsebayeva *et al.*, 2009); see Table 1 for more details]. However, we should not be surprised if, in the near future, novel generation gland types are discovered in other lizard clades, especially in the Neotropics. The results summarized in this paper provide clear evidence of that.

Although follicular glands have been considered to be more specialized and are assumed to have evolved from the more 'primitive' generation glands (Maderson & Chiu, 1970; Figs 2, 3), the evolutionary relationships between these gland types, if any, is uncertain. Thus far, only two types of generation

Table 1. Taxonomic distribution of generation glands among lizards. Lizard groups in which generation glands were indicated as absent in the published literature were also included

	Family	Genus	Generation gland				References
			Absent	Escutcheon	β-type	Callous scales	
Gekkota	Carphodactylidae	*Carphodactylus*	X				1
		Nephurus	X				1
		Phyllurus			X		1, 2
	Diplocatylidae	*Bavayia*			X		1, 2
		Diplodactylus			X		1, 2
		Hoplodactylus			X		1
		Oedura			X		1,2
		Pseudothecadactylus	X				1
		Rhacodactylus			X		1,2
		Rhynchoedura			X		1,2
	Eublepharidae	*Aeluroscalabotes*	X				1
		Coleonyx		X			3
		Eublepharis		X			2
		Hemitheconyx		X			4
	Sphaerodactylidae	*Chatogekko*		X			4
		Gonatodes		X			4, 5
		Lepidoblepharis		X			4
		Pseudogonatodes		X			4
		Sphaerodactylus		X			4
		Teratoscincus			X		2
	Gekkonidae	*Gehyra*			X		2
		Gekko			X		6, 7
		Hemidactylus			X		6, 7
		Heteronotia			X		2
		Lygodactylus			X		8, 9
Scincoidea	Gerrhosauridae	*Gerrhosaurus*					10*
	Cordylidae	*Chamaesaura*			X†		10
		Cordylus			X		11, 12, 13, 14, 15
		Hemicordylus			X‡		10, 11
		Karusasaurus			X		10
		Namazonurus			X		10
		Ninurta			X†		10
		Ouroborus			X		10
		Platysaurus			X†		10, 11
		Pseudocordylus			X†,‡		10

Table 1. Continued

	Family	Genus	Generation gland				References
			Absent	Escutcheon	β-type	Callous scales	
	Xantusiidae	*Xantusia*	X				1
Iguania	Agamidae	*Laudakia*				X	16
		Paralaudakia				X	16, 17, 18
	Iguanidae	*Dipsosaurus*	X				1
	Leiocephalidae	*Leiocephalus*			X		1
	Phrynosomatidae	*Uma*	X				1
	Opluridae	*Oplurus*		X [#]			19
	Dactyloidae	*Anolis*	X				1

References: 1, Maderson, 1970; 2, Maderson & Chiu, 1970; 3, Maderson, 1968b; 4, Maderson, 1972; 5, Maderson, 1967; 6, Chiu & Maderson, 1975; 7, Chiu *et al.*, 1975; 8, Maderson, 1968a; 9, Maderson, 1971; 10, Mouton *et al.*, 2014; 11, van Wyk & Mouton, 1992; 12, Louw *et al.*, 2011; 13, Mouton *et al.*, 2010; 14, Mouton *et al.*, 1998; 15, Toit *et al.*, 2005; 16, Baig & Böhme, 1991; 17, Dujsebayeva, 1998; 18, Dujsebayeva *et al.*, 2007; 19, Dujsebayeva *et al.*, 2009.
*Presumptive generation gland.
†Protruding single-layer generation gland.
‡Protruding two-layer generation gland.
#Callous-like in appearance, but histological investigation confirmed the presence of epidermal generation glands of the escutcheon type.

glands, escutcheon glands and β-glands, have been described in detail, and they differ essentially with regard to where the secretory material is synthesized and accumulated (Fig. 2). In geckos, escutcheon glands secrete material derived from a novel cell type that lies between the lacunar and the clear layer of the outer epidermal generation, whereas in β-glands, secretions derive from an extra cell type resting directly on cells of the β-layer of the inner generation (Maderson, 1967, 1972). After shedding is complete, gland products of escutcheon glands are exposed above the Oberhäutchen of the now outer epidermal generation. In β-glands, such products lie on top of the outer β-layer and are exposed through Oberhäutchen discontinuities (Fig. 2). The callous scales of agamid and oplurid lizards have been identified as a type of escutcheon gland, but their fine morphoanatomical organization and chemical secretions are still poorly understood (Baig & Böhme, 1991; Dujsebayeva, 1998; Dujsebayeva *et al.*, 2007, 2009).

Male lizards in the subfamily Tropidurinae (Tropiduridae) are known to possess flash marks (*sensu* Frost, 1992) on the underside of thighs, precloacal flap, mid-venter, posterior venter and/or base of tail, with single species bearing up to five different sections of their ventral surface covered with these marks (Frost, 1992; Fig. 4). Flash marks have been recognized as important systematic characters for classifying tropidurines (e.g. Etheridge, 1968; 1970; Rodrigues, 1987; Frost, 1992; Alvarez *et al.*, 1994; Ávila-Pires, 1995; Frost *et al.*, 1998, 2001; Harvey & Gutberlet, 1998, 2000; Carvalho, 2016; Carvalho *et al.*, 2016, 2018), yet their morphological structure and function remain uninvestigated. Alexander & Maderson (1972) suggested the occurrence of a glandular epithelium beneath the flash marks of tropidurines, but no histological evidence has been provided to support this claim. Here, we report on the discovery of a novel epidermal glandular tissue underneath the flash marks of tropidurine lizards.

Tropidurines are sedentary, territorial, visually oriented, sit-and-wait foragers with a complex system of visual signalling that take part in territoriality (Carpenter, 1977; Kohlsdorf *et al.*, 2006; Coelho *et al.*, 2018), dominance (Carpenter, 1977; Coelho *et al.*, 2018; Bruinjé *et al.*, 2019), mating displays (Carpenter, 1977; Pelegrin, 2019; Vasconcelos *et al.*, 2019) and both intra- and interspecific recognition (Watkins, 1997; Clark *et al.*, 2015; 2016; Bruinjé *et al.*, 2018). These traits, essentially plesiomorphic among iguanians (Huey & Pianka, 1981; Pianka & Vitt, 2003; Vitt *et al.*, 2003; Vitt & Pianka, 2005), contrast with the non-territorial, active, chemically oriented behaviour of non-iguanian clades that comprise species with either active or mixed foraging strategies (i.e. Anguimorpha,

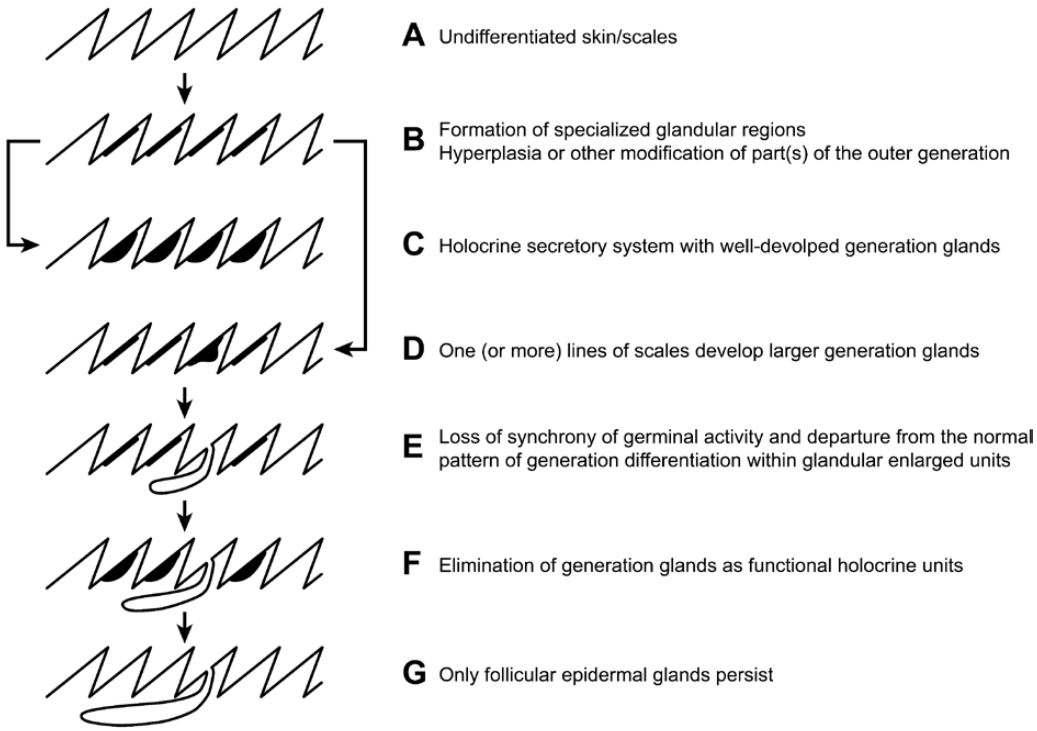

Figure 3. Maderson & Chiu's (1970) model of epidermal gland evolution. Steps of the model are briefly described in items A–G.

Lacertoidea, Scincoidea and Gekkota; Pianka & Vitt, 2003). It has been long assumed that lizards that adopt a sit-and-wait predatory feeding strategy rely primarily on visual cues, while active foragers would predominantly use chemical information (Baeckens *et al.*, 2017b). However, paradoxically, iguanians double non-iguanians in the frequency of epidermal glands, which challenges the longstanding view that iguanians are visually oriented while non-iguanian squamates are chemically oriented (García-Roa *et al.*, 2017a). This previous study (García-Roa *et al.*, 2017a) focused solely on follicular glands, and thus our understanding of chemical signalling in lizards is expected to grow considerably as more information on the nature and occurrence of generation glands across groups becomes available.

To help bridge this gap, we tested the hypothesis that the generation gland discovered among tropidurines represents a novel glandular organ. We characterized the structural and ultra-structural organization of the tropidurine gland, homologized its constituent parts and investigated its phylogenetic origin. We also investigated its potential secretory mechanisms and provided preliminary information about the protein profile of the gland. An exhaustive comparison of our results with published studies on epidermal glands of squamates, confirms that the skin patches delineated by colourful flash marks in tropidurines indeed correspond to a novel generation gland type. A critical discussion of this finding and its impacts on previous views about epidermal gland homology is included. We argue that generation and follicular glands are possibly more closely related functionally and evolutionarily than previously thought.

MATERIAL AND METHODS

STRUCTURAL AND HISTOCHEMICAL ANALYSES

We sampled 11 adult male and three adult female lizards representing six tropidurid species from three distinct genera that differ with respect to the presence, location and/or coloration of flash marks (Table 2). In addition to sampling epithelial fragments from areas delineated by flash marks, we collected tissue samples for histological and histochemical analyses from the dorsum and underside of the humeral region of most specimens as negative topological controls, since these regions lack flash marks. To allow for proper intersexual comparisons, we collected skin fragments from the same areas in both males and females of three out of the five species analysed. To test for the coincident occurrence of flash marks and specialized glandular epithelia, we compared the

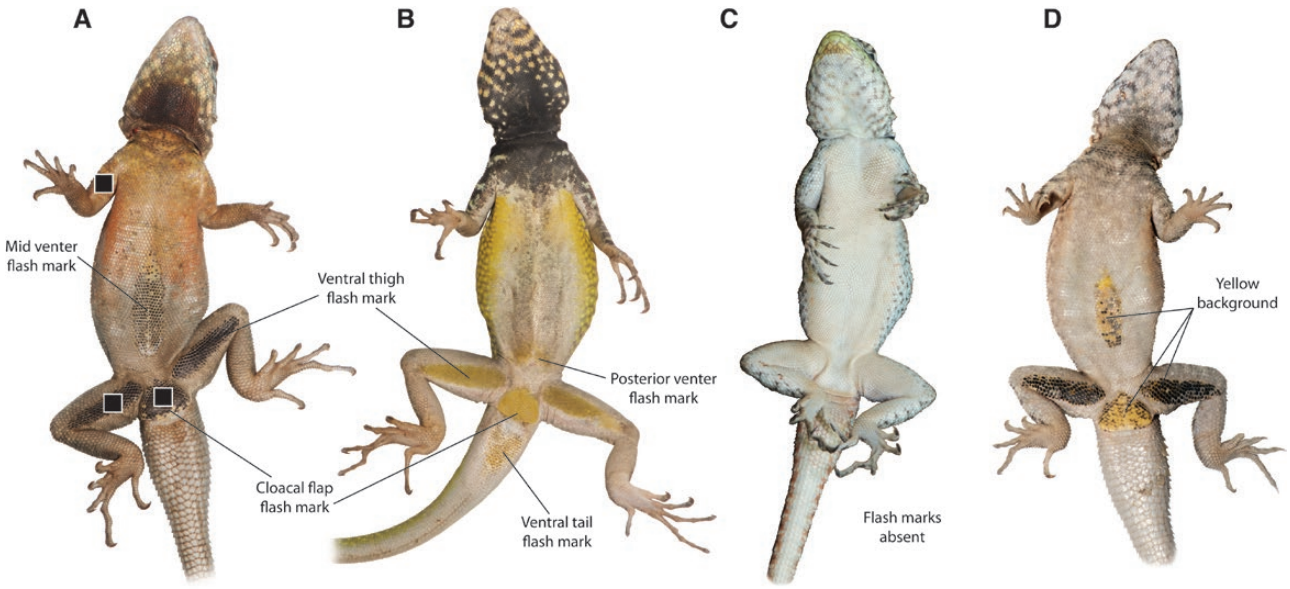

Figure 4. Ventral view of (A) a male *Tropidurus chromatops* Harvey & Gutberlet, 1998 (MHNC-R 3018) from ~30 km W Florida, Santa Cruz, Bolivia, (B) a male *T. melanopleurus* Boulenger, 1902 (IBIGEO-R 5331) from Aguas Blancas, Salta, Argentina, (C) a female *T. xanthochilus* Harvey & Gutberlet, 1998 (MZUSP-R 106321) from Santo Antônio do Leverger, Mato Grosso, Brazil, and (D) a male *T. etheridgei* Cei, 1982 (AMNH-R 176273) from Filadelfia, Boquerón, Paraguay, illustrating the location and coloration of flash marks observed (or not) on the ventral body of tropidurines. Black squares in (A) indicate body areas from which we collected skin samples for histological examination. In (D) the yellow coloration covering the background of the black flash-marks of *T. etheridgei* might either represent a transient ontogenetic state or an instance in which a yellow background persists throughout life.

histological structure of samples collected from species that possess flash marks to those of species that lack these structures. In addition to tropidurine species, interspecific comparisons included *Stenocercus caducus* (Cope, 1862), a member of the subfamily Stenocercinae, sister-group of Tropidurinae (Frost, 1992). In Stenocercinae, the absence of flash marks is the dominant and likely plesiomorphic condition. Patches of dark scales covering the underside of the thigh are rare among stenocercines. Out of the 69 species currently allocated in *Stenocercus* Duméril & Bibron, 1859 (Torres-Carvajal, 2007; Uetz, 2020), dark femoral patches have been observed in *S. chota* Torres-Carvajal (2000), *S. chrysopygus* Boulenger (1900), *S. ochoai* Fritts (1972) and *S. ornatus* (Gray, 1845), but it remains uninvestigated whether these species exhibit true flash marks similar to those found in tropidurines or simply pigmented zones that bear no association with a subjacent specialized glandular epithelium.

Histological samples were taken from recently collected specimens fixed in 10% unbuffered formalin and preserved in 70% ethanol. We removed skin fragments (~25 mm^2) from the underside of the humeral, femoral and pre-cloacal areas of the individuals. Samples were dehydrated in an ascending series of ethanol and embedded in historesin (Leica Microsystems Nussloch GmbH, Nussloch/Heidelberg, Germany), sectioned at 4.5 µm, and stained with toluidine blue + basic fuchsin for general structural description. We conducted histochemical staining techniques, including periodic acid-Schiff (PAS) (with haematoxylin used as a counterstain) (Bancroft & Stevens, 1982) + alcian blue pH 2.5 (Bancroft, 1975) for neutral and acid mucopolysaccharides, respectively, and naphthol yellow and bromophenol blue for peptides and proteins (Pearse, 1985). We analysed skin sections using microscopes Zeiss Axio Vert.A1 and Leica DM 1000, and cameras Canon EOS Rebel T7 and Leica DFC 295.

Transmission electron microscopy (TEM)

For transmission electron microscopy (TEM), samples were fixed overnight in a modified Karnovsky solution (2.0% paraformaldehyde and 2.5% glutaraldehyde in 0.1 mol/L sodium cacodylate buffer at pH 7.2; Karnovsky, 1965). Following fixation, the material was postfixed for 1 h in 1% osmium tetroxide (OsO_4) in buffer solution (0.1 mol/L sodium cacodylate at pH 7.2), en bloc-stained in 1% uranyl acetate ($UO_2(CH_3COO)_2$), dehydrated in an ascending series of ethanol and

Table 2. List of specimens, sex, collection localities, geographic coordinates, and analytical methods employed. Legend: H & H, histology and histochemistry; TEM, transmission electron microscopy; TPE, total protein electrophoresis, and stage of the shedding cycle (following Landmann, 1986) of each skin sample (H, humeral; F, femoral; P, pre-cloacal)

Species/Specimen	Sex	Locality / Collection Date	Latitude	Longitude	H & H	TEM	TPE	Shedding Stage		
								H	F	P
Tropidurus catalanensis										
MZUSP-R 106467	F	Parque Continental, Osasco, São Paulo, Brazil / 24.ii.2017	–23.5424	–46.7601	X		X	I	I	I
MZUSP-R 106468	M		–23.5424	–46.7601	X			I	V	V
MZUSP-R 106469	M		–23.5424	–46.7601	X	X	X	I	VI	VI
MZUSP-R 106470	M		–23.5424	–46.7601	X	X	X	I	V	V
Tropidurus chromatops										
MZUSP-R 106263	M	Vila Bela da Santíssima Trindade, Mato Grosso, Brazil / 23–24.vi.2016	–14.5663	–59.9541	X			I	VI	VI
MZUSP-R 106266	F		–15.0024	–59.9529	X			VI	I	I
MZUSP-R 106276	M		–14.5623	–59.9577	X			I	V	VI
MZUSP-R 106280	M		–14.5624	–59.9577	X			I	IV	IV
Tropidurus xanthochilus										
MZUSP-R 106321	F	Agrovila das Palmeiras, Santo Antônio do Lerveger, Mato Grosso, Brazil / 10.vii.2016	–16.1916	–55.9530	X			I	I	I
MZUSP-R 106331	M		–15.9624	–55.5313	X			I	VI	VI
MZUSP-R 106336	M		–15.9624	–55.5315	X			I	IV	V
MZUSP-R 106342	M		–15.9644	–55.5380	X			I	IV	VI
Plica plica										
MTR 18918	M	Lago Chaviana, Itapuru, right margin of the Purus River, Amazonas, Brazil / 23.x.2010	–4.3083	–61.8152	X			I	V	V
Stenocercus caducus										
MZUSP-R 82815	M	Vila Bela da Santíssima Trindade, Mato Grosso, Brazil / 7–22.ix.1997	–	–	X			I	I	I
Uranoscodon superciliosus										
MZUSP-R 91367	M	Lago do Cipotuba, Iguarapé Tapira, right margin of the Aripuanã River, Amazonas, Brazil / 23.v.2002	–5.8014	–60.2211	X			I	I	I

embedded in epoxy resin. Ultrathin sections (70–90 nm) were obtained using a Leica Ultracut UCT Microtome (Leica, Illinois, USA), mounted on copper slot-grids, contrasted with lead citrate per 10 min at room temperature and observed using Zeiss EM 900 (Karl Zeiss, Oberkochen, Germany) and JEOL JEM-1400 plus (JEOL, Tokyo, Japan) transmission electron microscopes.

Protein analysis

For protein composition analysis, we collected skin samples from areas delineated by dark-pigmented flash-marks on the femoral region and unpigmented skin from the humeral region of two adult males of *Tropidurus catalanensis* Gudynas & Skuk, 1983. Unpigmented skin fragments from both areas were also collected from one adult female of the same species (Table 2). Samples were frozen at –80 °C for 1 h and 30 min, macerated and kept in 1.5 mL of 80% acetronitrile 0.05% trifluoroacetic acid at –20 °C overnight. We vacuum-dried each sample (Speed-Vac Savant), resuspended them in 1 mL of ultrapure water and determined the amount of total protein, following the A205 custom method for protein and peptide quantification of the NanoDrop 2000C (Desjardins *et al.*, 2009). To determine the pattern, size and relative concentration of protein components, we employed a 15% sodium dodecyl sulphate (SDS) polyacrylamide gel (Laemmli, 1970). We added 100 µg of total protein to each well and employed two molecular weight standards (SeeBlue and a homemade one with lysozyme 14.2 KDa) to determine the size of the bands. Electrophoresis run was performed at a constant of 120 V for approximately 2 h. After finishing electrophoresis, the gel was stained with silver nitrate following a modified version of Bassam *et al.*'s (1991) method. We accelerated the reaction time at each step of the protocol by microwaving the gel in a buffer solution (each step = 90 s in total). The obtained banding patterns were compared qualitatively among and within individuals using the program PyElph v.1.4 (Pavel & Vasile, 2012).

Phylogenetic reconstruction of ancestral state

Ancestral state reconstructions were performed using a novel phylogenetic hypothesis produced for tropidurids. We followed the same laboratory and analytical procedures outlined in Carvalho *et al.* (2016, 2018) to generate, manipulate and phylogenetically analyse sequence data primarily produced and obtained from Genbank. In total, we analysed sequence data from 43 samples, representing species from all currently valid tropidurid genera (*sensu* Frost *et al.*, 2001), plus one sample of the agamid *Agama agama* (Linnaeus, 1758), chosen to root the phylogenetic trees produced. A full list of tissue samples analysed, respective voucher specimens and Genbank accession numbers are provided in Supporting Information, Appendix S1. We analysed sequence data from four mitochondrial (12S, 16S, *COI*, *Cytb*) and six nuclear loci (BACH1, kif24, NTF3, PRLR, PTPN, SNCAIP). Alignments were performed in MAFFT v.7 (Katoh & Toh, 2008) and concatenated in Sequence Matrix v.1.8 (Vaidya *et al.*, 2011). We employed PartitionFinder v.2.1.1 (Lanfear *et al.*, 2012) to select the best-fit nucleotide substitution models and data-partition schemes and performed tree searches in GARLI v.2.1 (Zwickl, 2006). Our best tree search was based on 100 replicates and the relative support of the clades was assessed through 1000 non-parametric bootstrap replicates (Felsenstein, 2004). We plotted bootstrap values over our best phylogenetic tree using SumTrees (Sukumaran & Holder, 2010). The general profile of our molecular dataset and the nucleotide models and partition schemes selected by PartitionFinder 2 are summarized in Supporting Information, Appendix S2. The molecular matrix analysed in this study (nexus format) and the novel phylogenetic tree produced for tropidurids (with bootstrap support values associated to nodes) are provided in Supporting Information, Appendices S3 and S4.

For reconstruction of ancestral states, ecological data on habitat use and morphological data relative to the presence, location and coloration of epidermal gland sites of tropidurids were either obtained from preserved specimens, the literature or based on field observations. Character states assigned to each species are detailed in Table 3. To infer the phylogenetic origins of α-glands, we scored the absence or presence of the glandular organ on the basis of its occurrence in at least one body site. Optimizations of the absence or presence of α-glands in different body sites were also implemented. We optimized flash-mark coloration using two colour categories, 'black' and 'yellow', since a finer definition of character states would require further investigation of the distribution and density of chromatophore types in the lizards' skin. In the few cases where information on α-glands or flash marks were unavailable for a given species, we applied '?' to that respective character (i.e. treated as missing data). We defined a multistate character to summarize the association of the lizards with up to four categories of substrate: ground (bare soil or leaf litter), tree (trunk, branches or hollows), rock (vertical rock surfaces or rock outcrops) and sand (dunes and open sand). We employed our novel phylogenetic hypothesis for tropidurids to perform all phylogeny-based analyses and adopted flash marks as a proxy for the presence

Table 3. Habitat and substrate used by tropidurid species, presence/absence and coloration flash marks, and source of morphological and ecological data

Species	Habitat	Substrate	Flash mark coloration	Flash mark / Generation gland location					Source Ecology / Morphology
				Mid-V	Post-V	Thigh	Cloaca	Tail	
TROPIDURIDAE									
TROPIDURINAE									
E.amathites	Open	Sand	Golden-yellow	–	–	X	X	–	Rodrigues, 1984a
E. divaricatus	Open	Sand	–	–	–	–	–	–	Rodrigues, 1986
E. nanuzae	Open	Rock	Golden-yellow	–	–	X	X	–	Rodrigues, 1981
Mi. albemarlensis	Open	Rock/Sand	–	–	–	–	–	–	Snell *et al.*, 1988; Jordan *et al.*, 2008
Mi. atacamensis	Open	Rock/Sand	–	–	–	–	–	–	Vidal *et al.*, 2002
Mi. barringtonensis	Open	Rock/Sand	–	–	–	–	–	–	Heller, 1903
Mi. bivittatus	Open	Rock/Sand	–	–	–	–	–	–	Rowe *et al.*, 2020
Mi. delanonis	Open	Rock/Sand	–	–	–	–	–	–	Werner, 1978
Mi. duncanensis	Open	Rock/Sand	–	–	–	–	–	–	O. Torres-Carvajal, pers. comm.
Mi. grayii	Open	Rock/Sand	–	–	–	–	–	–	O. Torres-Carvajal, pers. comm.
Mi. habelii	Open	Rock/Sand	–	–	–	–	–	–	O. Torres-Carvajal, pers. comm.
Mi. heterolepis	Open	Rock/Sand	–	–	–	–	–	–	Vidal *et al.*, 2017
Mi. indefatigabilis	Open	Rock/Sand	–	–	–	–	–	–	O. Torres-Carvajal, pers. comm.
Mi. jacobii	Open	Rock/Sand	–	–	–	–	–	–	O. Torres-Carvajal, pers. comm.
Mi. koepckeorum	Open	Rock	–	–	–	–	–	–	Mertens, 1956; Dixon & Wright, 1975
Mi. occipitalis	Open	Rock/Sand/ Tree trunk/ Branches	–	–	–	–	–	–	Mertens, 1956; Dixon & Wright, 1975
Mi. pacificus	Open	Rock/Sand	–	–	–	–	–	–	Schluter, 1984
Mi. peruvianus	Open	Rock/Sand	–	–	–	–	–	–	Mertens, 1956; Dixon & Wright, 1975; Pérez & Balta, 2007
Mi. p. peruvianus	Open	Rock/Sand	–	–	–	–	–	–	Mertens, 1956; Dixon & Wright, 1975
Mi. p. salinicola	Open	Rock/Sand	–	–	–	–	–	–	Mertens, 1956; Dixon & Wright, 1975
Mi. quadrivittatus	Open	Rock	–	–	–	–	–	–	Dixon & Wright, 1975; Báez & Cortes, 1990; Silva, 2014
Mi. stolzmanni	Open	Rock/Branches/ Fallen logs	–	–	–	–	–	–	Mertens, 1956; Dixon & Wright, 1975; Beuttner & Koch, 2019
Mi. tarapacensis	Open	Rock/Sand	–	–	–	–	–	–	Troncoso-Palacios, 2018
Mi. theresiae	Open	Rock/Sand	–	–	–	–	–	–	Mertens, 1956; Dixon & Wright, 1975; Pérez & Balta, 2007
Mi. theresioides	Open	?	–	–	–	–	–	–	–
Mi. thoracicus	Open	Sand	–	–	–	–	–	–	Mertens, 1956; Dixon & Wright, 1975
Mi. t. icae	Open	Sand	–	–	–	–	–	–	Dixon & Wright, 1975; Pérez & Balta, 2007

Table 3. Continued

Species	Habitat	Substrate	Flash mark coloration	Flash mark / Generation gland location					Source Ecology / Morphology
				Mid-V	Post-V	Thigh	Cloaca	Tail	
Mi. t. talarae	Open	Sand	–	–	–	–	–	–	Dixon & Wright, 1975
Mi. t. thoracicus	Open	Sand	–	–	–	–	–	–	Dixon & Wright, 1975
Mi. tigris	Open	Rock	–	–	–	–	–	–	Mertens, 1956; Dixon & Wright, 1975
Mi. yanezi	Open	Rock	–	–	–	–	–	–	Ortiz, 1980
P. caribeana	Forest	Tree trunk	?	?	?	?	?	?	Murphy & Jowers, 2013
P. kathleenae	Forest	Tree trunk	?	?	?	?	?	?	Murphy & Jowers, 2013
P. lumaria	Open	Rock	Yellow	–	–	X	X	–	Donnelly & Myers, 1991
P. medemi	Forest	Tree trunk	?	?	?	?	?	?	Murphy & Jowers, 2013
P. pansticta	Open	Rock	Yellow	–	–	X	X	–	Myers & Donnelly, 2001
P. plica	Forest	Tree trunk	Yellow	–	–	X	X	–	Etheridge, 1970; Ávila-Pires, 1995; Murphy & Jowers, 2013; T.C.S. Ávila-Pires, pers. comm.
P. rayi	Forest	Rock	?	?	?	?	?	?	Murphy & Jowers, 2013
P. umbra	Forest	Tree trunk	Yellow	–	–	X†	X†	–	Ávila-Pires, 1995; T.C.S. Ávila-Pires, pers. comm.
P. u. ochrocollaris	Forest	Tree trunk	Yellow	–	–	X†	X†	–	Ávila-Pires, 1995; T.C.S. Ávila-Pires, pers. comm.
P. u. umbra	Forest	Tree trunk	Yellow	–	–	X†	X†	–	Ávila-Pires, 1995; T.C.S. Ávila-Pires, pers. comm.
Strobilurus torquatus	Forest	Tree branches/ Tree trunk	Yellow	–	X	X	X	–	Etheridge, 1968; Ávila-Pires, 1995; Rodrigues *et al.*, 2013
T. gr. *bogerti*									
T. bogerti	Open	Rock	Black	X	–	X	X	X	Roze, 1958; Myers & Donnelly, 2008
T. gr. *semitaeniatus*									
T. helenae	Open	Rock	Sulphur yellow	–	–	X	X	–	Manzani & Abe, 1990; Pelegrin *et al.*, 2017
T. jaguaribanus	Open	Rock	Black	–	–	X	X	–	Passos *et al.*, 2011; Alcantara *et al.*, 2018
T. pinima	Open	Rock	Black	–	–	X	X	–	Rodrigues, 1984b; Xavier & Dias, 2017
T. semitaeniatus	Open	Sand	Black	–	–	X	X	–	Vanzolini *et al.*, 1980; Ribeiro *et al.*, 2010; Pelegrin *et al.*, 2017
T. gr. *spinulosus*									
T. callathelys	Open	Rock	Yellow	–	X	X	X	X	Harvey & Gutberlet, 1998; Morais *et al.*, 2014
T. guarani	Open	Rock	Yellow	–	–	X	X	–	Alvarez *et al.*, 1994; Carvalho, 2016
T. lagunablanca	Open	Tree trunk	Yellow	–	–	X	X	–	Carvalho, 2016

Table 3. Continued

Species	Habitat	Substrate	Flash mark coloration	Flash mark / Generation gland location					Source Ecology / Morphology
				Mid-V	Post-V	Thigh	Cloaca	Tail	
T. melanopleurus	Open	Rock	Greenish yellow	–	X	X	X	X	Laurent, 1980; Perez-Mellado & De la Riva, 1993; Schumacher & Barts, 2003; Rivadeneira, 2008
T. spinulosus	Open	Tree trunk	Cream	–	–	X	X	–	Alvarez *et al.*, 1994
T. tarara	Open	Tree trunk	Yellow	–	–	X	X	–	Carvalho, 2016
T. teyumirim	Open	Rock	Yellow	–	–	X	X	–	Carvalho, 2016
T. xanthochilus	Open	Tree trunk	Yellow	–	–	X	X	–	Harvey & Gutberlet, 1998
T. gr. *torquatus*									
T. azurduyae	Open	Rock	Black	X	–	X	X	–	Carvalho *et al.*, 2018
T. catalanensis	Open	Rock/Tree trunk	Black	X*	–	X	X	–	Gudynas & Skuk, 1983; Kunz & Borges-Martins, 2013
T. chromatops	Open	Rock	Black	X	–	X	X	–	Harvey & Gutberlet, 1998; Morais *et al.*, 2014
T. cocorobensis	Open	Sand	Black	X*	–	X	X	–	Rodrigues, 1987; Ribeiro *et al.*, 2012
T. erythrocephalus	Open	Rock	Black	–	–	X	X	–	Rodrigues, 1987
T. etheridgei	Open	Rock/Sand	Black	X	–	X	X	–	Rodrigues, 1987; Cruz *et al.*, 1998
T. hispidus	Open	Rock/Sand/Tree trunk	Black	–	–	X	X	–	Rodrigues, 1987; Albuquerque *et al.*, 2018
T. hygomi	Open	Sand	Black	–	–	X	X	–	Rodrigues, 1987; Martins *et al.*, 2010
T. imbituba	Open	Rock	Black	X*	–	X	X	–	Kunz & Borges-Martins, 2013
T. insulanus	Open	Rock	Black	–	–	X	X	–	Rodrigues, 1987; Ávila-Pires, 1995
T. itambere	Open	Rock	Black	X	–	X	X	X	Rodrigues, 1987; van Sluys, 1997
T. montanus	Open	Rock	Black	–	–	X	X	–	Rodrigues, 1987; Filogonio *et al.*, 2010
T. mucujensis	Open	Rock	Black	–	–	X	X	–	Rodrigues, 1987
T. oreadicus	Open	Rock	Black	–	–	X	X	–	Rodrigues, 1987; Meira *et al.*, 2007
T. psammonastes	Open	Sand	Black	–	–	X	X	X	Rodrigues, 1987; Rodrigues *et al.*, 1988
T. sertanejo	Open	Rock	Black	–	–	X	X	–	Carvalho *et al.*, 2016
T. torquatus	Open	Sand/ Rock/Tree trunk	Black	X*	–	X	X	–	Rodrigues, 1987; Kunz & Borges-Martins, 2013
Urac. azureum	Forest	Tree branches/ Tree trunk	–	–	–	–	–	–	Etheridge, 1968; Ávila-Pires, 1995
Urac. a. azureum	Forest	Tree branches/ Tree trunk	–	–	–	–	–	–	Etheridge, 1968; Ávila-Pires, 1995
Urac. a. guentheri	Forest	Tree branches	–	–	–	–	–	–	Etheridge, 1968; Ávila-Pires, 1995
Urac. a. werneri	Forest	Tree branches	–	–	–	–	–	–	Etheridge, 1968; Ávila-Pires, 1995
Urac. flaviceps	Forest	Tree branches	–	–	–	–	–	–	Zani & Vitt, 1995; Vitt & Zani, 1996

Table 3. Continued

Species	Habitat	Substrate	Flash mark coloration	Flash mark / Generation gland location				Source Ecology / Morphology
				Mid-V	Post-V	Thigh	Cloaca	Tail	
Uran. supercilliosus	Forest	Tree branches / Tree trunk	–	–	–	–	–	–	Ávila-Pires, 1995
STENOCERCINAE									
Stenocercus caducus	Semideciduous forest	Leaf litter	–	–	–	–	–	–	Torres-Carvajal, 2007; Teixeira *et al.*, 2016

* Mid-venter flash marks present in large adult males only.
† Flash marks may be present or absent, depending on the population.
Abbreviations: *E.*, *Eurolophosaurus*; *Mi.*, *Microlophus*; *P.*, *Plica*; Post-V, posterior venter; Mid-V, mid venter; *T.*, *Tropidurus*; *Urac.*, *Uracentron*; *Uran.*, *Uranoscodon*.

of epidermal glands. Reconstructions of ancestral character states were carried out under maximum parsimony, using the trace character history function in MESQUITE v.3.61 (Maddison & Maddison, 2019).

RESULTS

Flash marks

Our results confirm the occurrence of pigmented flash-marks in at least two locations of the ventral body of 39 species from four tropidurine genera (*sensu* Frost *et al.*, 2001), specifically the pre-cloacal and femoral areas (Fig. 4). Table 3 summarizes the taxonomic distribution of flash marks among tropidurines. Different genera and species exhibit flash marks with distinct colorations. The 'yellow type' comprises colour tones ranging from cream to yellow or orangey and the 'black type' varies from black to charcoal. Yellow flash-marks are observed in *Plica* Gray, 1831, *Tropidurus* Wied, 1825 [gr. *T. spinulosus* (Cope, 1862) and *T. helenae* (Manzani & Abe, 1990)] and *Strobilurus* Wiegmann, 1834, whereas black flash-marks are only found in *Tropidurus* [gr. *T. bogerti* Roze, 1958, gr. *T. semitaeniatus* (Spix, 1825) and gr. *T. torquatus* (Wied, 1820)]. Regardless of their topological distribution and coloration, flash marks are restricted to male lizards (Fig. 4). Subadult males approaching sexual maturity exhibit pale-yellow flash-marks that turn either yellow/orangey or black, depending on the genus (or species group), after reaching sexual maturity (the term 'subadult' is here used to refer to male individuals whose flash marks are not fully developed but that have a similar size to smaller adults with fully developed flash marks from the same population). Pale-yellow coloration can be present in the background of the black flash-marks of some species of the *T. semitaeniatus* and *T. torquatus* species groups (Fig. 4D), but it is unclear whether this coloration indicates a transient ontogenetic state or if the yellowish background persists throughout life in adult males of certain species with black flash-marks.

Histology

Samples collected from the pre-cloacal and femoral areas of tropidurine males with flash marks differ histologically from the rest of their body skin, the skin of females and the skin of male specimens from species that lack flash marks (Fig. 5A–C). Flash marks located in these areas delineate specialized skin patches characterized by the occurrence of a novel epidermal gland type, hereafter named 'α-gland' (Fig. 5C, E–I). No histological specialization is observed in skin areas that lack external evidence of pigmented flash-marks in tropidurids (Fig. 5A, B, D).

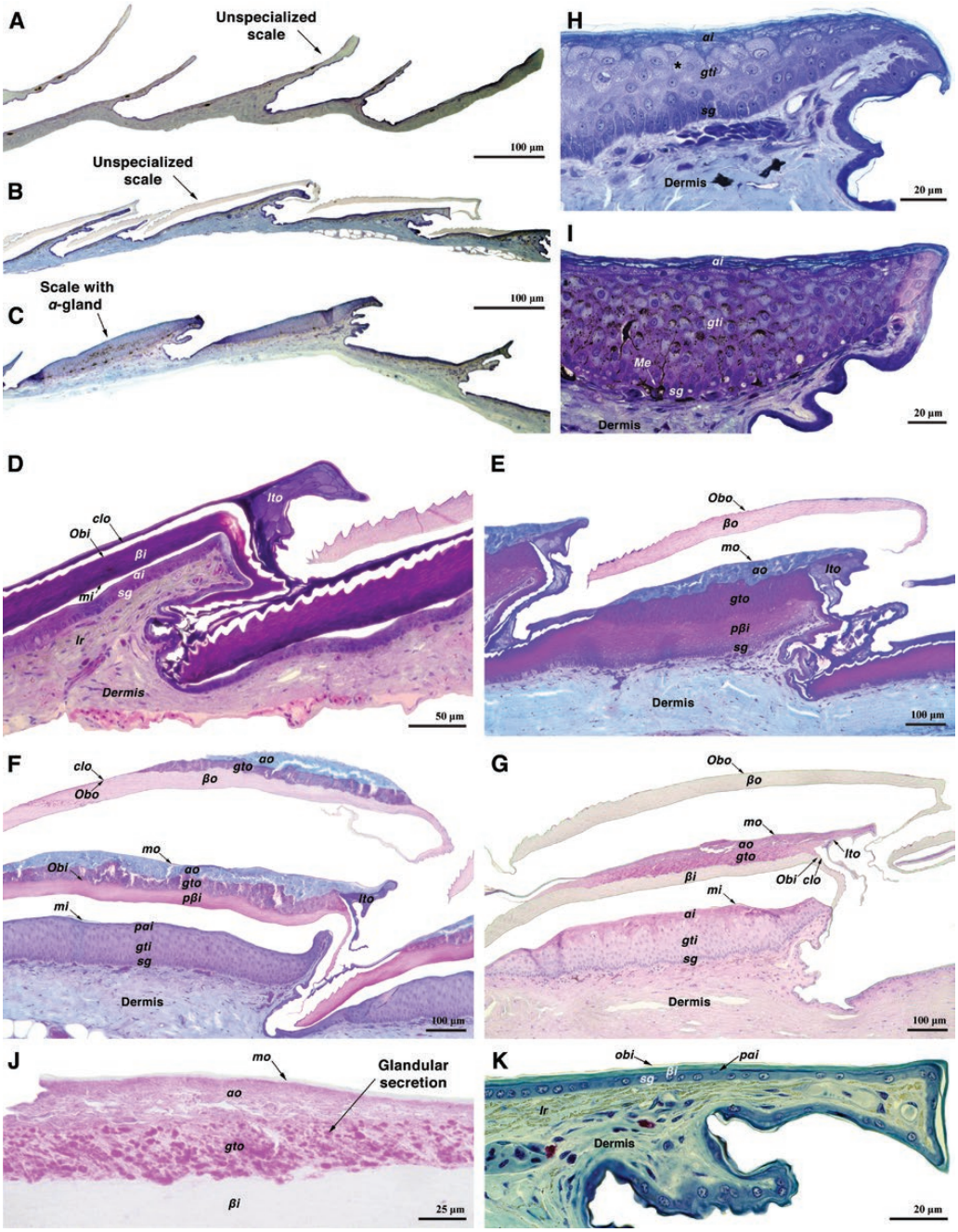

Figure 5. A, unspecialized scales from the pre-cloacal flap of a male *Stenocercus caducus* (MZUSP-R 82815). B, unspecialized scales from the femoral area of a female *Tropidurus chromatops* (MZUSP-R 106266). C, scales with α-glands from the femoral area of male *T. chromatops* (MZUSP-R 106263). Note that β-keratin layers are not present in A and C because they were lost during sample preparation. D, unspecialized scale (stage I) from the humeral region of a female *T. chromatops* (MZUSP-R 106266). E, detail of a scale with α-gland (stage IV) from the femoral area of a male *T. xanthochilus* (MZUSP-R 106342). F, detail of a scale with α-gland (stage V) from the pre-cloacal flap of a male *T. xanthochilus* (MZUSP-R 106342). G, detail of a scale with α-gland (stage VI) from the femoral areal of a male *T. xanthochilus* (MZUSP-R 106336). H, part of the inner generation of an α-gland from the femoral area of a male *Plica plica* (MTR 18918) showing the glandular stratum with a large number of secretory vesicles (indicated with an asterisk). I, part of the inner generation of the α-gland from the femoral area of a male *T. catalanensis* (MZUSP-R 106470) with melanophores transferring melanin granules into glandular cells and numerous melanin granules accumulated in their cytoplasm. J, glandular tissue of the outer generation

We are often able to recognize up to five strata in the unspecialized epidermis covering the humeral region of each species – from the inside out: the *stratum germinativum*, the α-keratin layer, the mesos layer, the β-keratin layer and the Oberhäutchen (Fig. 5D). We can also observe the presence of a clear layer and a lacunar layer lying right above the Oberhäutchen of the inner generation of cells. The lacunar tissue is especially conspicuous in the caudal hinge of the scales (Fig. 5D). In this area, lacunar cells are larger than any other skin cells forming the scales, irregular in shape, possess a peripheral, chromophilic nucleus and a homogeneous cytoplasm.

α-Glands are distinguished from the undifferentiated squamate epithelium by exhibiting an additional stratum of metabolically active columnar cells responsible for the production of glandular secretions. This unique glandular stratum is located between the *stratum germinativum* and the lacunar layer of the inner generation (Fig. 5H, I) and between the clear layer and the lacunar layer of the outer generation (Fig. 5E–G). It is composed of three to seven layers of cells with a central or subcentral euchromatic nucleus. Their cytoplasm is densely populated with secretory vesicles (Fig. 5H). The α-keratin layer of α-glands is formed by strata of progressively flattened cells with increasing degrees of keratinization towards the apical portion of the layer (Fig. 5H, I). A thin, translucent mesos layer formed by a single row of flat cells separates the α-keratin layer from the β-keratin layer (Fig. 5E–G). The latter is formed by several strata of dead cells that have lost their individual limits as the keratinization process progressed, giving them the appearance of a compact layer (Fig. 5E–G). The apical limit of the β-keratin layer is serrate in shape (especially towards the scale hinge) and covered with an apparently unornate Oberhäutchen (Fig. 5E–G). The inner and outer epidermal generations of α-glands have the same general histological organization. However, a conspicuous difference between them is that the glandular stratum of the outer generation accumulates large amounts of glandular products (Fig. 5J).

Chemical profile

The unspecialized epithelium of males and females of all examined species stain positively for naphthol yellow only in the β-keratin layer. In turn, α-gland samples of males show positive responses to dyes, especially in the glandular stratum and α-keratin layer. The degree of staining of the columnar cells of the glandular stratum increases considerably in more advanced stages of the sloughing cycle (Fig. 5G, J). The α-keratin layer of the inner generation shows a positive response for PAS, and both the glandular stratum and α-keratin layer stain positive for PAS in the outer generation. In the first case, the positive PAS signal marks α-keratin fibres, and in the second it highlights, respectively, α-keratin fibres in the α-keratin layer and neutral mucopolysaccharides secretions in the mature glandular stratum of the outer generation.

Transmission electron microscopy (TEM)

Ultrastructural observations of skin samples collected from the femoral area of *Tropidurus catalanensis* reveal a large number of intracellular vesicles in the glandular stratum of α-glands (Fig. 6). We are able to identify four different types of vesicles on the basis of the electron lucency and aspects of their internal material (Types V1–V4 in Fig. 6B–D). Type V1 is the most electron lucent (nearly translucent) and has a clear, smooth appearance. Type V2 shows a denser content than Type V1 and also exhibits a smooth appearance. Type V3 is the densest of all vesicles and apparently smooth. Type V4 varies in electron lucency, due to the presence of irregular granular structures in the vesicle lumen. Agglomerations of Golgi apparatuses exist in different parts of the cells (Fig. 6D, E), amid a large number of vesicles. Transmission electron microscopy observations alone do not allow us to determine whether different vesicle types store distinct secretory material or represent stages of a same secretory cycle. Figure 5A shows a density gradient of cellular material, with a gathering of vesicles in the bottom layer of the glandular stratum and a smooth transition to a less dense aggregation of cellular structures in the apical region of the glandular tissue. Autophagocytosis is observed, especially in the apical layers of the glandular stratum (Fig. 6D–F), promoting reorganization of the internal content of the cells. Lysis of organelles generates more internal space for accumulation of vesicles with secretory content.

of the scale shown in G, with glandular secretion accumulated in the mature layer. K, unspecialized scale (stage II) from the femoral region of a female *T. chromatops* (MZUSP-R 106276) showing iridophores in the apical portion of the dermis. Figures A–F and H–I were stained with toluidine blue + basic fuchsin, G and J with periodic acid-Schiff + alcian blue, and K with bromophenol blue. Legend: inner generation: *sg*, *stratum germinativum*; *pgt*, presumptive glandular tissue/stratum; *gti*, glandular tissue/stratum; *pαi*, presumptive α-layer; *αi*, α-layer; *mi*, mesos layer; *pβi*, presumptive β-keratin layer; *βi*, β-keratin layer; *Obi*, Oberhäutchen; outer generation: *clo*, clear layer; *gto*, glandular tissue/stratum; *lto*, lacunar tissue; *αo*, α-layer; *mo*, mesos layer; *βo*, β-keratin layer; *Obo*, Oberhäutchen; chromatophores: *Ir*, iridophores; *Me*, melanophores.

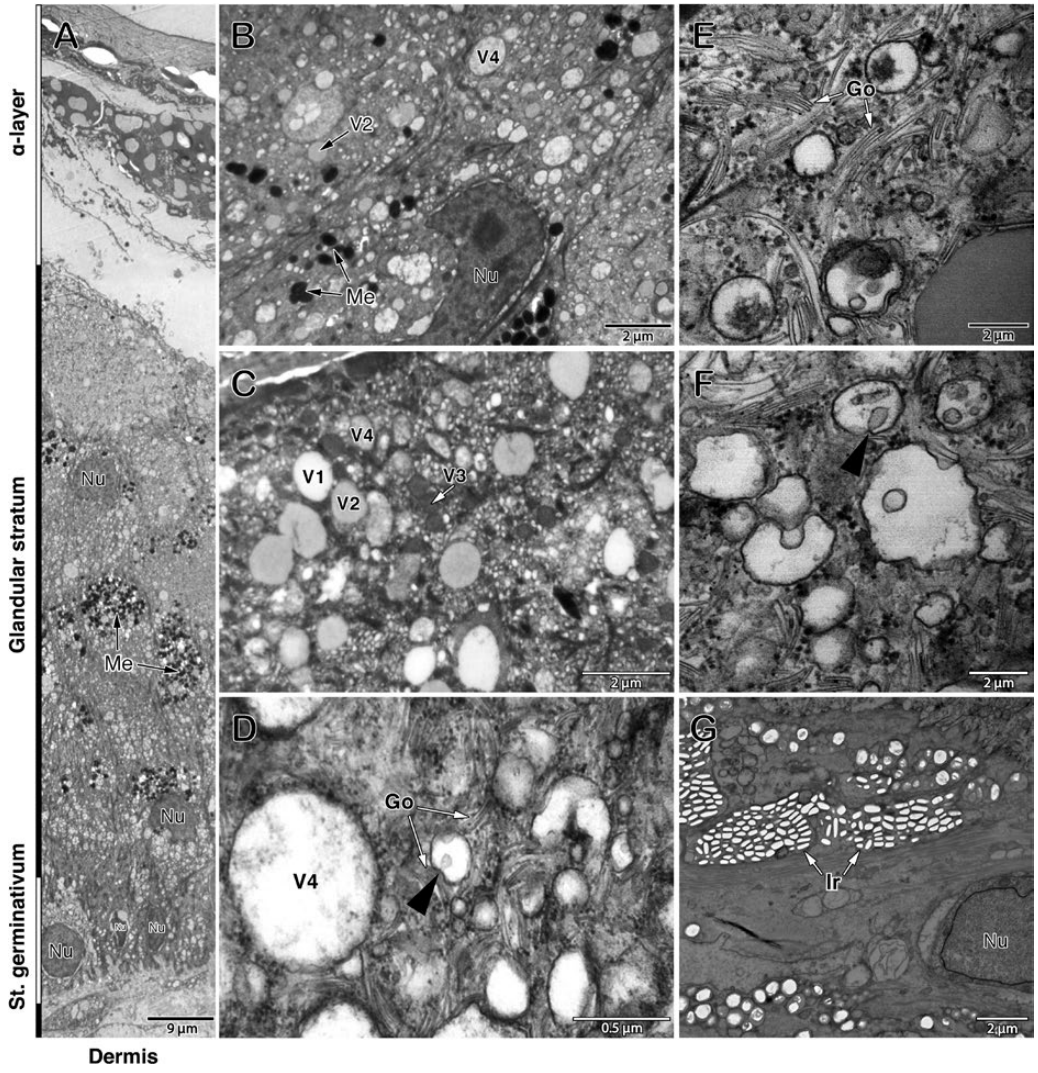

Figure 6. Transmission electron microscopy of the epithelium from the femoral area of *Tropidurus catalanensis*. A, general view of the glandular epithelium showing major skin layers and abundance of vesicles inside glandular cells. B–G, magnified view of glandular tissue showing: B, clusters of melanin granules and numerous vesicles; C, aggregations of four different types of vesicles (V1, V2, V3 and V4); D, E, Golgi apparatuses amid secretory vesicles; F, autophagocytic events (arrowheads indicate membrane projections); G, iridophores present in the apical portion of the dermis. Legend: Go, Golgi complex; Ir, iridophore; Me, melanin granule; Nu, nucleus; V1, vesicles type 1; V2, vesicles type 2; V3, vesicles type 3; V4, vesicles type 4.

Autophagocytic activity is associated with vesicles V4, whilst specific function and internal content of vesicles V1–V3 remain unknown. Transmission electron microscopy images confirm the lack of specialized lumina or ducts for storage and secretion of glandular products.

Protein composition

The total protein content and banding patterns (Fig. 7) vary between samples in relation to their topological origin and the sex of *Tropidurus catalanensis* individuals. Both males exhibit a higher number and also more intensely marked protein bands than the female specimen examined. Only a small difference in protein content is noticeable in the samples obtained from the humeral and femoral regions of the female specimen. In total, the female exhibits eight protein bands varying between ~14 KDa and ~198 KDa. Of those, six are present both in the humeral and femoral regions, and each of these areas has only one exclusive protein band with ~15 KDa and ~18 KDa, respectively. The most intense protein band registered in the female specimen has ~62 KDa and other bands are

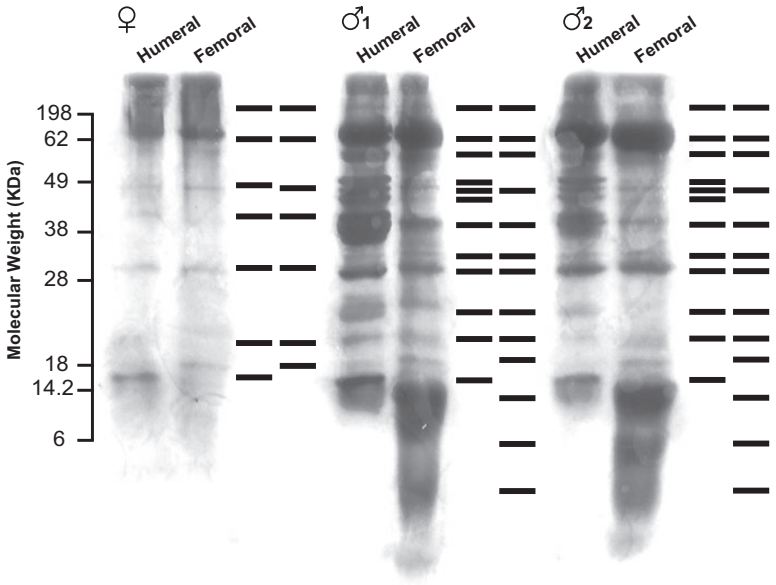

Figure 7. Protein banding pattern of humeral (unspecialized skin) and femoral (skin with α-glands in males; females lack these glands completely) samples of one adult female (MZUSP-106467) and two adult males (male 1: MZUSP-106469 and male 2: MZUSP-106470) of *Tropidurus catalanensis*.

not as conspicuously marked, indicating low amounts of proteins. The ~40 KDa protein band, present both in the humeral and femoral samples, is the only one exclusively found in the female.

No evident qualitative differences are found between samples collected from the same body site in males, indicating that both individuals analysed have similar, if not identical, protein profiles. The only noticeable distinction is due to specimen MZUSP-R 106469 showing bands slightly more intense in the humeral region and less intense bands in the femoral region, in comparison to specimen MZUSP-R 106470. Each male studied exhibits a total of 12 bands varying between ~3 KDa and ~198 KDa. In both of them, the two bands adjacent to the ~49 KDa band plus the ~15 KDa band are exclusively expressed in the humeral region, and three intense bands with molecular weight lower than ~14 KDa and one with ~18 KDa are exclusively found in the femoral (i.e. α-gland) area. Regardless of the topological origin of the sample, males and females exhibit six identical bands, meaning that most (six out of eight) bands present in the female are shared with males but only half of the bands present in males are shared with the female (six out of 12).

Phylogenetic origins and transformations

Phylogenetic reconstructions of ancestral states are shown in Figures 8 and 9. The origin of α-glands is inferred at the node grouping *Eurolophosaurus* Frost *et al.*, 2001, *Plica* Gray, 1831, *Strobilurus* Wiegmann, 1834, *Tropidurus* Wied, 1825 and *Uracentron* Kaup, 1826. Consequently, the absence of these epidermal organs in *E. divaricatus* (Rodrigues, 1986), *P. umbra* (Linnaeus, 1758) and *U. azureum* (Linnaeus, 1758) are reconstructed as a reversal event back to the ancestral tropidurid condition. Regarding coloration, ancestral state reconstructions reveal a relatively conserved phylogenetic distribution of the yellow and black flash-mark types. Parsimony reconstructions show no apparent association between shifts in ecological habit and the presence/absence of α-glands in tropidurines. However, all events involving the secondary loss of glandular structures are either associated to arboreal (i.e. *P. umbra* and *U. azureum*) or psammophilous (*E. divaricatus*) species, but never to rupicolous ones.

Femoral and pre-cloacal α-glands are the most widespread phylogenetically and the only ones to show fully congruent phylogenetic distribution, with secondary losses in *E. divaricatus*, *P. umbra* and *U. azureum*. α-Glands in other body regions are not only less common, but prove to be more labile phylogenetically. Mid-venter α-glands originated twice in the *T. torquatus* species group and another time in *T. bogerti*. Lower venter α-glands appear in the ancestor of the *Strobilurus* + *T. spinulosus* species group, with subsequent secondary loss in the later clade. The evolution of α-glands at the base of the tail

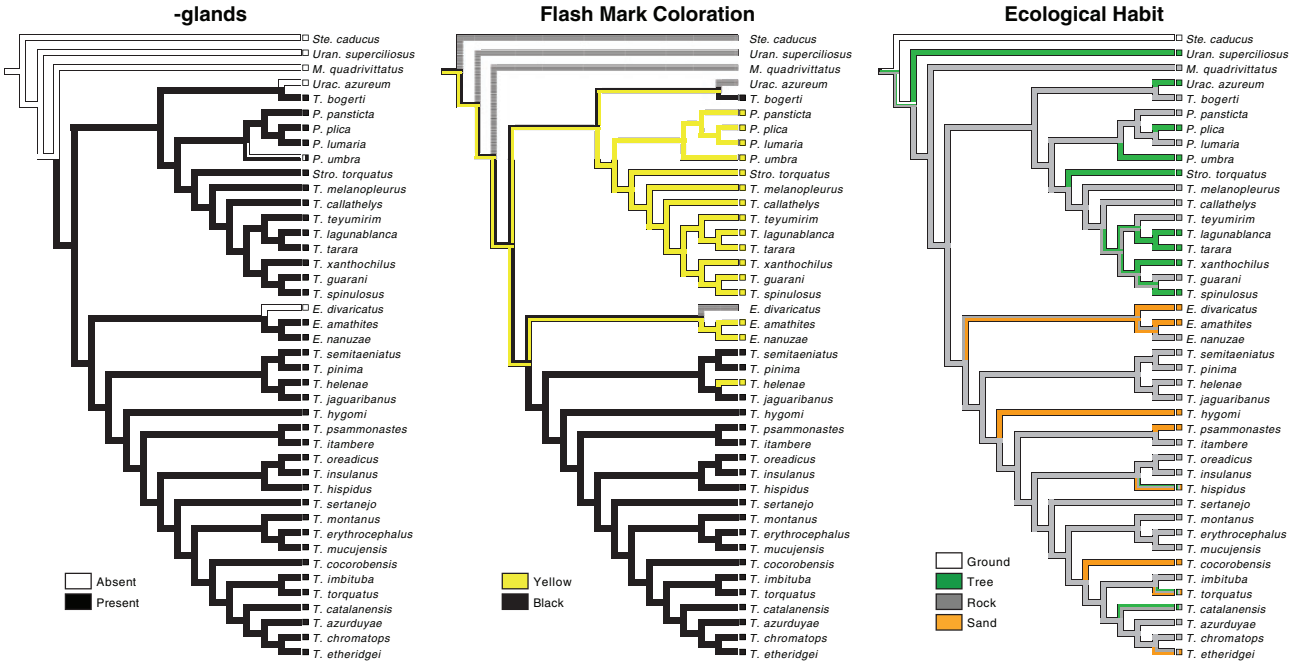

Figure 8. Ancestral state reconstructions of ecological and α-gland/flash mark related characters of tropidurid lizards.

occurred at least three times, appearing in *T. bogerti*, in the ancestor of *T. itambere* Rodrigues, 1987 + *T. psammonastes* Rodrigues *et al.*, 1988 and, possibly, in the ancestor of the *T. spinulosus* species group, with subsequent secondary loss in that clade. Nonetheless, parallel evolution of α-glands at the base of tail in *T. melanopleurus* Boulenger, 1902 and *T. callathelys* Harvey & Gutberlet, 1998 cannot be ruled out since these events imply an equally parsimonious scenario.

DISCUSSION

A NOVEL GENERATION GLAND TYPE

Comparative analyses of tropidurine samples revealed a novel escutcheon-type generation gland (*sensu* Maderson, 1972), herein denominated 'α-gland'. As observed in other lizard clades that possess similar gland forms, i.e. escutcheon glands of eublepharids and sphaerodactylids (Maderson, 1967, 1968b, 1972; Maderson & Chiu, 1970) and callous scales of agamids (Baig & Böhme, 1991; Dujsebayeva, 1998; Dujsebayeva *et al.*, 2007) and oplurids (Dujsebayeva *et al.*, 2009), the glandular secretion of α-glands is produced and stored in a multilayered stratum of cells that lies between the lacunar and clear layer. As such, the novel tropidurine gland is not to be confounded with the β-type generation gland of carphodactylids (Maderson, 1970; Maderson & Chiu, 1970), diplodactylids (Maderson & Chiu, 1970), sphaerodactylids (Maderson, 1970; Maderson & Chiu, 1970) and gekkonids (Maderson, 1968a, 1971; Maderson & Chiu, 1970; Chiu & Maderson, 1975; Chiu *et al.*, 1975), whose secretions derive from an extra cell type resting directly on cells of the β-layer of the inner generation (Maderson, 1967, 1972). The histological structure of α-glands is indeed remarkably similar to that of the callous scales of oplurids (Dujsebayeva *et al.*, 2009) and agamids (Dujsebayeva, 1998; Dujsebayeva *et al.*, 2007). However, in none of our samples have we identified the formation of a thick glandular deposit that resembles the so-called 'secretion plug' observed by Dujsebayeva, (1998) and Dujsebayeva *et al.*, (2007) in specimens of the agamid genus *Paralaudakia* Baig *et al.*, 2012.

The histological structure of α-glands is hardly comparable to the presumptive generation glands of gerrhosaurids, as no mature generations are observed in those lizards. Curiously, Mouton *et al.* (2014) even suggested that the epidermal glands of gerrhosaurids should not be regarded as true generation glands. These lizards possess a hypertrophied cellular mass comparable to the hypertrophied basal layer plus cellular mass containing presumptive and differential β-layer cells described for generation glands in cordylids (Van Wyk & Mouton, 1992; Van Wyk, 1997). In the same manner, despite cordylids possessing true generation glands, these are distinguished from α-glands by exhibiting either a single mature generation (single-layer stacked gland) or multiple glandular generations (multiple-layer stacked gland) in which the glandular

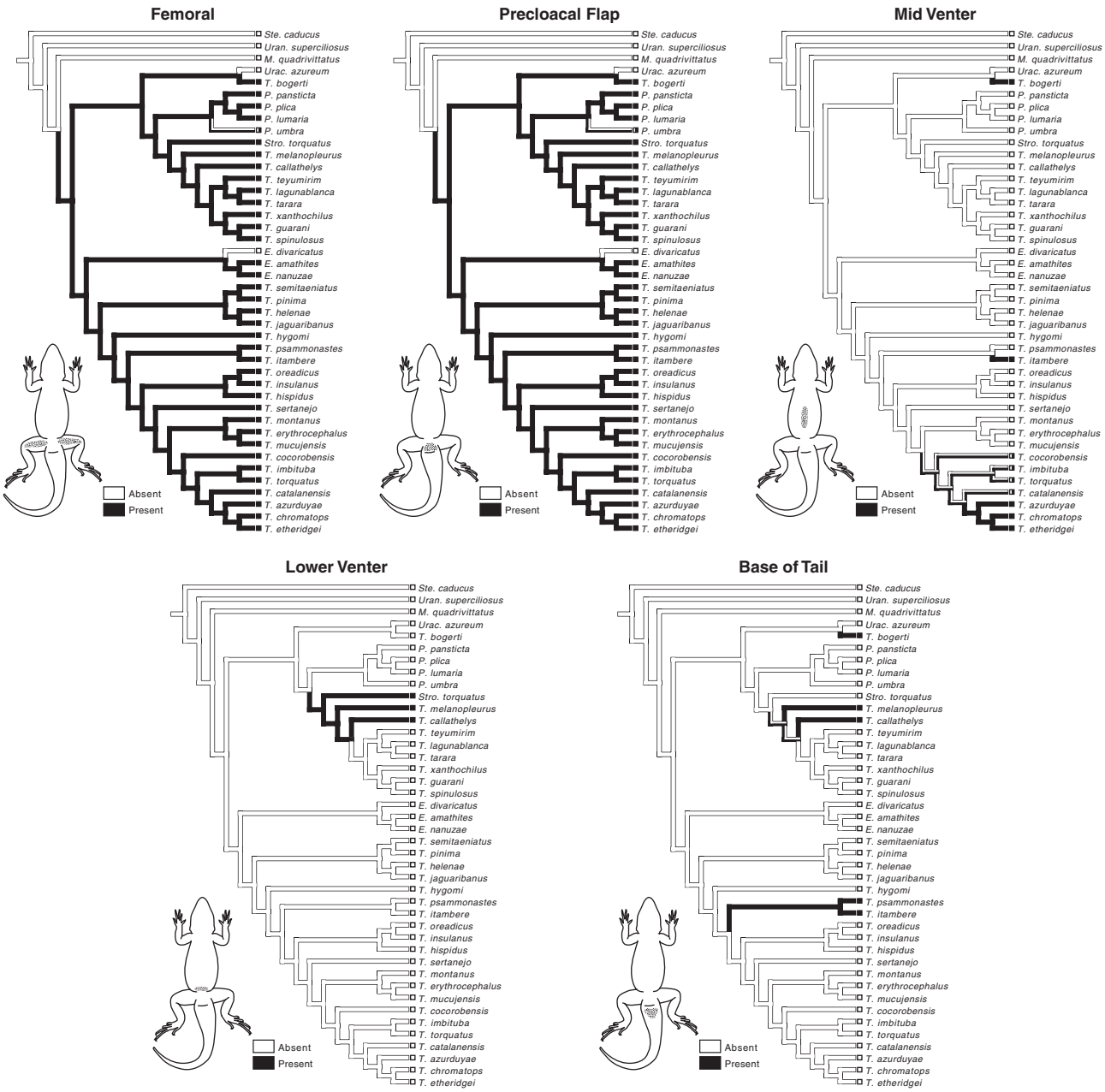

Figure 9. Ancestral state reconstructions of α-gland/flash mark related characters of tropidurid lizards.

secretion is produced in a modified β-layer of the inner epidermal generation (Searby, 2002). Therefore, it is clear that the glandular apparatus of α-glands is located in a distinct stratum of cells. Although developmental aspects of the glandular layer of tropidurines remain to be studied in detail, our analysis showed that it is unlikely to be related to β-layer cells and, as the skin goes through multiple shedding cycles, it neither forms a protruding single-layered gland nor accumulates multiple generations forming a pit-like (i.e. glands with sunken, multiple generations) or protruding multiple-layered gland characteristic of cordylids (Van Wyk & Mouton, 1992; Mouton *et al.*, 2010, 2014).

CELLULAR RELATIONSHIPS AND POSSIBLE DEVELOPMENTAL ORIGINS

Aside from distinguishing the tropidurine type from other escutcheon-type glands, we chose the term 'α-gland' to briefly convey our hypothesis that the glandular stratum that makes up this novel gland type is related to the lacunar and α-keratin layers,

more than to other epidermal cell types, in terms of both development and structure. Landmann (1986) explained that the cell layers of the lacunar tissue relate to immature α-keratin cells in at least three major ways: they secrete mucous granules into the intercellular space subjacent to the α-keratin layer; tight junctions interconnect their lateral plasma membranes; and these cells do not differentiate further during the sloughing cycle. The glandular stratum of α-glands proved to have similar characteristics, including the capacity to synthesize mucous compounds and to establish tight intercellular adhesion through desmosomes. The major distinctions in relation to the lacunar layer are twofold: the glandular stratum is composed of multiple layers of cells with fairly consistent appearance, whereas the lacunar tissue exhibits a variable number of cell layers and cells with irregular shapes and sizes in different section of the lizard scale, and perhaps more strikingly, the glandular stratum of α-glands differentiates into a storage layer during advanced stages of the sloughing cycle. Such a deposit layer is formed through the accumulation of large amounts of secretion products and intensification of cell keratinization (α-keratin) in the outer epidermal generation.

The differentiation of the glandular stratum of α-glands into a storage layer in the outer epidermal generation is particularly relevant if we take into consideration that Maderson *et al.* (1970) showed that the lacunar layer of desert-dwelling iguanian species, like *Dipsosaurus dorsalis* (Baird & Girard, 1852) (Iguanidae) and *Uma notata* Baird, 1858 (Phrynosomatidae), keratinize in a manner similar to the α-keratin layer. During the final stages of the 'renewal phase' (i.e. stages five to six of the sloughing cycle), all nuclei degenerate in the lacunar (and clear) layer cells, with the result that, at the time of skin shedding, these innermost layers of the outer generation become almost indistinguishable from the α-keratin layer. And as previously shown in Flaxman *et al.* (1968), complete keratinization of lacunar cells is similarly observed during *in vitro* cultivation of lizard epidermis. Conjointly, these observations indicate that the differentiation and maturation of the glandular stratum of α-glands relies on biochemical processes shared with related cell types (i.e. α-keratin cells, lacunar and clear layer cells) to form the secretion deposit layer. Further investigation of the hypothesized relationships among these cell types should shed light into the mechanisms of histodifferentiation responsible for the evolution of α-glands and their unexplored secretory machinery.

Epidermal gland homology in lizards

Our findings reject some previous statements concerning the homology of lizard epidermal glands. The two major types of epidermal glands found in lizards have been distinguished by the fact that generation glands produce their secreted material in association with periodic skin shedding, whereas follicular glands are morphologically and chronologically independent of the general body epidermis (Maderson, 1972). This hitherto well-established morphofunctional dichotomy (multilayered generation glands of cordylids representing a likely exception; Van Wyk & Mouton, 1992), coupled with the observation that the undifferentiated lepidosaurian epidermis exhibits characteristic synchrony of germinal activity over the entire body surface, led Maderson & Chiu (1970) to propose a transitional model that assumes that generation glands are per se less specialized structures and to hypothesize that follicular glands derive from generation glands (Figs 2, 3). Nevertheless, in a recent paper describing a formerly unreported glandular cloacal organ from phyllodactylid geckos of the genus *Gymnodactylus* Spix, 1825, De-Lima *et al.* (2018) proposed an expanded diagram summarizing the steps underlying the evolution of major epidermal gland types, including their novel posterior-proctodeal gland. According to them, generation glands originated from an ancestral dedifferentiation of the outer epidermal generation that later developed a holocrine secretory function, whereas follicular glands evolved independently from a hypertrophied region of the *stratum germinativum* and α-keratin layer, with posterior dedifferentiation in active holocrine secretion cells. Their model assumes that generation and follicular glands probably have independent evolutionary origins, driven by specific signalling of the epidermis on the inner and outer epidermal generation layers.

Our results offer no support for De-Lima *et al.*'s (2018) ideas. The production of glandular secretion in α-glands is initiated in the glandular stratum of the inner generation layer, and this glandular tissue develops into a mature secretion deposit layer in the outer generation layer. The secretory activity of the glandular cells of the inner epidermal generation of α-glands is confirmed by the presence of a massive number of vesicles containing secretion products in the cell cytoplasm (Figs 4, 5). We thus have no reason to assume that the signalling mechanisms controlling the secretory functions of glandular cells in the inner generation layer are distinct from those responsible for the secretory activities of the same glandular cells in the outer generation layer. De-Lima *et al.* (2018) considered all glandular scale-specific structures relating to different body regions derived from the outer epidermal generation layer as generation glands, and assumed that generation and follicular glands do not have the same tissue origin. Thereby, they

neglected the fact that the inner and outer epidermal generations of generation glands are nothing but homologous replicates of the same glandular unit at different developmental stages. Hence, generation and follicular glands might indeed share a common evolutionary origin, even though the structural organization of these gland types is undoubtedly distinct. Nevertheless, a critical issue that remains completely obscure is how β-type generation glands originated, since their secretory layer is different from that of other generation glands, i.e. not carried out by α-keratin-producing cells.

Secretion mechanisms

The topological co-occurrence of pigmented flash-marks and α-glands in tropidurines indicates that flash marks are a reliable proxy for the presence of glandular tissue. Although the location of glands varies among genera and species, these are restricted to the ventral side of the body. This observation leads us to presume that the release of secretory products is either facilitated by, or conditioned to, contact with the substrate traversed by the lizards. As α-glands are structurally comparable to the escutcheon gland-types found in geckos, oplurids and agamids, it is reasonable to hypothesize that their secretory mechanisms are at some degree similar, if not the same. Maderson (1967, 1972) proposed that in geckos, escutcheon gland products are exposed above the Oberhäutchen of the outer epidermal generation after shedding is complete. His model implied that the secretory activity of escutcheon glands is morphologically and chronologically linked to the shedding of the undifferentiated epidermis covering the lizard body (Fig. 2). Sloughing of the outer epidermal generation is seemingly indispensable for α-glands to release their products to the external environment, be it synchronized with the rest of the skin body or not. This is because the keratinized cell layers that lie right above the glandular deposit accumulated in their outer epidermal generation lack any sort of fissures, cracks or openings through which the liberation of gland products could be facilitated. Surprisingly, among our samples, all males that possessed α-glands exhibited unspecialized skin (humeral area) and glandular sites in different stages of the shedding cycle, indicating that they might release their glandular products in a timing independent of the body skin shedding, if not in a whole different fashion.

Yet, as suggested by Searby (2002) for cordylid generation glands, we considered the possibility that glandular material could be alternatively released via abrasion of the outer generation layer, in a similar manner of transfer assumed for femoral glands (Jared *et al.*, 1999; Chamut *et al.*, 2009; Martín & López, 2011). Nonetheless, the absence of significant wear and tear on the top histological layers of the glandular samples we have analysed seems to indicate otherwise. De Villiers *et al.* (2015) produced a fine histological study to determine the mechanism through which cordylids transfer generation gland secretion to the environment and, despite the fact that they found localized signs of abrasion in the outer gland surface of all species analysed, these appeared to be minor. These authors also insightfully noted that if abrasion were to be the main mechanism of secretion dispersal, cordylid species with single-layered glands would have to control abrasion rate in order to prevent exposure of the presumptive β-layer underneath. Although the actual glandular stratum of α-glands is localized more internally (i.e. between the lacunar and clear layers) in comparison to cordylids, this problem is likely to affect tropidurine species, especially those dwelling on hard, consolidated substrates (e.g. rupicolous species).

The fact that the mechanisms of secretion transfer of callous scales of agamid and oplurid lizards are also unknown (Baig & Böhme, 1991; Dujsebayeva, 1998; Dujsebayeva *et al.*, 2007; 2009), reinforces our belief that this is a truly challenging topic. Adding even more complexity to the investigation of mechanisms of epidermal gland secretion in lizards, Mouton *et al.* (2014) proposed that glandular layers of cordylid generation glands might function as reservoirs that transfer liquid semiochemicals to the substrate without the need of significant abrasion. They have also considered the possibility that generation glands do not transfer substances to the substrate at all, but instead provide chemical signals directly from the lizard's body. Despite the structural evidence we have gathered thus far indicating that sloughing, and not abrasion of the skin, is the most probable mechanism through which tropidurines transfer secretions to the environment, we are presently unable to rule out the alternative hypotheses raised by Mouton *et al.* (2014). Further commenting on them would be speculative, and for this reason we opt to resume our discussion of this topic when additional evidence becomes available.

Protein profile and functional aspects

Knowledge about the production and role of semiochemicals in social interactions of lizards is almost entirely based on analyses of follicular (i.e. femoral) gland products (Mayerl *et al.*, 2015). Several studies have revealed that follicular gland secretions are composed of both proteins and lipids (Alberts, 1990; Martín & López, 2000; Weldon *et al.*, 2008). However, as far as we are aware, information about the chemical composition of generation gland products is available for a single species, the South African sungazer, *Smaug giganteus* (Smith, 1844) (Cordylidae), albeit restricted to lipophilic compounds (Louw *et al.*, 2011).

Consequently, comparisons of the protein profile of α-glands can only be performed in reference to follicular gland products.

The handful of studies that characterized the spectrum of proteins produced by follicular glands of conspecifics of the same lizard population, identified unique chemical profiles that suggested their role as semiochemicals (Alberts, 1991; Alberts & Werner, 1993; Alberts *et al.*, 1993; Mangiacotti *et al.*, 2017, 2019a). In parallel, behavioural experiments have corroborated this idea by demonstrating that lizards are in fact able to detect the protein fraction of follicular gland secretions and use this information to recognize unfamiliar conspecifics (Alberts, 1992; Alberts & Werner, 1993; Alberts *et al.*, 1993; Mangiacotti *et al.*, 2019b), distinguish sexes (Alberts *et al.*, 1993), intraspecific communication (Mangiacotti *et al.*, 2020) and assess a plethora of social contexts (Alberts, 1992; Alberts & Werner, 1993). In agreement with these previous findings involving follicular gland products, the comparative analysis of the protein mass spectra of epidermal samples of *Tropidurus catalanensis* revealed conspicuous differential expression of protein components between sexes. Males of this species exhibited more intense protein bands, almost double the number of protein bands identified in the female, and showed nine exclusive bands (four of them only found in α-gland sites), whilst the female had only one that was not identified in males. These striking differences indicate more intense and diverse production of proteins in the male skin, and especially in α-glands sites, which are entirely absent in females. Coupled with the fact that male tropidurids are typically polygynous (Van Sluys, 1997; Wiederhecker *et al.*, 2003; Pinto *et al.*, 2005; Melo *et al.*, 2017), socially dominant (Carpenter, 1977; Coelho *et al.*, 2018; Bruinjé *et al.*, 2019) and maintain harems composed of several females (Van Sluys, 1997; Wiederhecker *et al.*, 2003; Kohlsdorf *et al.*, 2006; Melo *et al.*, 2017), makes us hypothesize that α-gland proteins might indeed play a role in intersexual recognition and perhaps contribute either directly or indirectly to the establishment of male dominance and territoriality.

Variability in the protein composition of follicular gland products of conspecifics is known to make them suitable to be used as chemical signals of individual identity (Alberts *et al.*, 1993; Mangiacotti *et al.*, 2017). Interestingly, recent studies have also demonstrated that the chemical composition of the follicular gland secretions is influenced by genetic (Gabirot *et al.*, 2012; Martín *et al.*, 2016) and ecological factors, such as environmental conditions (Martín & López, 2013b; Baeckens *et al.*, 2017b), diet (Martín & López, 2006a; García-Roa *et al.*, 2017b) and climate (Baeckens *et al.*, 2017b). It is actually uncommon to find two individuals in a given population with the same protein composition of their epidermal gland secretions (Alberts *et al.*, 1993; Weldon *et al.*, 2008; Mangiacotti *et al.*, 2017). For instance, proteins produced by follicular glands are, in general, so diverse that only two out of 29 male European wall lizards *Podarcis muralis* (Laurenti, 1768) (Lacertidae), sampled by Mangiacotti *et al.* (2017), possessed the same banding scheme. That being so, we noticed with great surprise that no apparent variability was present in the protein profile of male *T. catalanensis*, despite the fact that the total number of protein bands extracted from both individuals analysed fell within the range described for epidermal gland secretions of male lizards from distantly related families [i.e. seven to 15 bands reported by Alberts (1991) and Mangiacotti *et al.* (2017)]. However, we cannot disregard the possibility that the absence of variability could be artefactual, as protein bands with similar molecular weights may not be chemically identical or even homologous. As a consequence, certain proteins might have been masked during comparisons of traditional electrophoretic profiles. Furthermore, because our sample size is small, we cannot rule out the possibility that other proteins are present in the population. Additional sampling of specimens and employment of analytical methods capable of distinguishing 'hidden proteins' (e.g. two-dimensional protein electrophoresis and comparative transcriptomics) are expected to greatly enhance following assessments of the diversity and function of α-gland proteins.

Phylogenetic origins and transformations

Mapping the distribution of α-glands on to the tropidurid phylogenetic tree, we produced a hypothesis of character transformation that supports a single evolutionary origin for α-glands. However, α-glands are not inferred as a synapomorphy of tropidurines; they support a more inclusive group that contains *Eurolophosaurus*, *Plica*, *Strobilurus*, *Tropidurus* and *Uracentron*. These specialized epidermal organs emerged in a clade whose ancestor lacked epidermal glands of any kind, implying that their absence corresponds to the ancestral tropidurid condition. The inferred scheme of character transformation (Fig. 8) fits steps A through C of Maderson & Chiu's (1970) model of epidermal gland evolution, which suggests that generation glands derive from modifications of unspecialized scales (Fig. 3). Alas, because cordylids are the only other group investigated phylogenetically with respect to the origin and morphological transformation of epidermal glands [but see Kluge (1987) for information on the distribution of β- and escutcheon-type generation glands in Gekkonoidea], we are unable to determine whether the case of tropidurines (i.e. evolution of generation glands from

unspecialized scales) represents the general rule among lizards. All we know is that in what concerns the evolution of generation glands, tropidurines and cordylids followed utterly different paths. This is because the cordylid β-glands likely derived from a primitive gland-type similar in structure to the gland found in its sister family, the gerrhosaurids (Mouton *et al.*, 2014), rather than from unspecialized scales as observed in tropidurines.

Our results suggest a fairly conserved phylogenetic history for α-glands. Apparent phylogenetic inertia (*sensu* Shanahan, 2011) is first implied by the fact that only two secondary loss events were observed throughout the tropidurine phylogeny, and, second, by the fact that the morphology of the glands remained mostly unchanged at the histological level across all genera analysed. Most transformations recognized thus far seem to have affected the number and location of glandular patches, rather than the morphology of the glands themselves. More glandular sites can potentially expand the production and release of semiochemicals to the environment, and enlarging the total area of the body covered with α-glands should have an effect similar to increasing the number of femoral or cloacal pores in species that possess follicular glands. The factors driving the evolution of glandular patches in tropidurines are hypothesized to be, at least in part, the same as those responsible for driving the number of femoral pores in other lizard clades (e.g. substrate use, climatic conditions and genetic factors; Pincheira-Donoso *et al.*, 2008; Iraeta *et al.*, 2011; Baeckens *et al.*, 2015). Consequently, the signalling compensation hypothesis of Baeckens *et al.* (2015), which proposes that under harsh environmental conditions, species might either increase investment (within-channel hypothesis) or invest in additional or alternative signalling channels (between-channel hypothesis) that are likely to promote changes in the evolutionary direction of the existing sensory channel (leading to shifts in numbers, origins or losses of them), might also be key to explain the evolution of α-glands.

Although it is noteworthy that the cases of secondary loss of α-glands exclusively involved arboreal or psammophilous lizards, but never rupicolous species, which the majority of tropidurids are, these events do not appear to have a clear association with changes in their ecological habit. The same holds true regarding the coloration of the flash marks that delineate α-glands, which show a relatively well-structured phylogenetic distribution. On the other hand, it is possible that a positive correlation exists between the number or area of the glandular patches and the degree of territoriality and dominance displayed by male tropidurines. It is also conceivable that a relationship of some kind links the chemical nature of the glandular products, rather than the morphology and area of the glandular patches, to the environments occupied by the lizards. And, of course, these factors might even have interacted together to shape the evolution of α-glands. Although the degree of territoriality and dominance of males of most tropidurine species has never been measured, the fact that in several species that have mid-body patches of glandular scales, the development of these glandular areas only occurs in older, dominant males (Pinto *et al.*, 2005; A. L. G. Carvalho, pers. observ.), is interpreted as evidence of a possible relationship between social parameters and the evolution of α-glands. Nevertheless, the non-observation of an apparent relationship between the occurrence of the glands and the ecological habit of the lizards does not mean that social behaviour is to be interpreted beforehand as the most prominent factor underlying the evolution of α-glands. Further research targeting the contribution of extrinsic (i.e. environmental, ecological) vs. intrinsic (i.e. behavioural, social) factors to the morphological and biochemical evolution of α-glands is not only needed but deeply encouraged.

CONCLUSIONS

Tropidurines lack epidermal follicular glands and, consequently, α-glands are the main potential source of semiochemicals in this lizard group. Some similarities regarding functional aspects of these epidermal gland types are striking. First, both gland types show a marked sexual dimorphism, being more developed in males or even absent in females. Second, both appear in a ventral position, indicating that the secretion is likely passively deposited in the substrate. Although the homology of epidermal glands has not been investigated in depth yet, it is undisputable that under the traditional view of 'historical homology', implying descent with modification from an 'archetype' of a common ancestor (Butler & Saidel, 2000), they should not be treated as homologous at the level of all lizards (Fig. 1). In turn, under the 'deep homology' view, which considers the repeated use of highly conserved genetic circuits in the development of anatomical features that do not share homology in a strict historical or developmental sense (Shubin *et al.*, 1997), the identity and relationships between epidermal gland types can be approached more thoroughly in the near future. Hence, to disentangle the evolutionary and developmental origins of different epidermal gland types, instead of just exploring the anatomical development of epidermal glands, it would be salutary to focus on the genetic machinery responsible for function. If follicular epidermal glands indeed derive from a primitive generation gland-type, common

genetic machinery shared between them should be then identified. A comprehensive investigation of this issue in currently under way.

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REFERENCES

Alberts AC. 1990. Chemical properties of femoral gland secretions in the desert iguana, *Dipsosaurus dorsalis*. *Journal of Chemical Ecology* **16:** 13–25.

Alberts AC. 1991. Phylogenetic and adaptive variation in lizard femoral gland secretions. *Copeia* **1991:** 69–79.

Alberts AC. 1992. Pheromonal self-recognition in desert iguanas. *Copeia* **1992:** 229–232.

Alberts AC, **Werner DI. 1993.** Chemical recognition of unfamiliar conspecifics by green iguanas: functional significance of different signal components. *Animal Behaviour* **46:** 197–199.

Alberts AC, **Phillips JA**, **Werner DI. 1993.** Sources of intraspecific variability in the protein composition of lizard femoral gland secretions. *Copeia* **1993:** 775–781.

Albuquerque RL, **Protázio AS**, **Cavalcanti LBQ**, **Lopez LCS**, **Mesquita DO. 2018.** Geographical ecology of *Tropidurus hispidus* (Squamata: Tropiduridae) and *Cnemidophorus ocellifer* (Squamata: Teiidae) in a Neotropical region: a comparison among Atlantic Forest, Caatinga, and coastal populations. *Journal of Herpetology* **52:** 145–155.

Alcantara EP, **Ferreira-Silva C**, **Sousa JGG**, **Ávila RW**, **Morais DH. 2018.** Ecology and parasitism of the lizard *Tropidurus jaguaribanus* (Squamata: Tropiduridae) from northeastern Brazil. *Phyllomedusa* **17:** 195–210.

Alexander SA, **Maderson PFA. 1972.** Further observations on holocrine epidermal specializations in iguanid lizards. *American Zoologist* **12:** Abstract 728.

Alvarez BB, **Cei JM**, **Scolaro JA. 1994.** A new subspecies of *Tropidurus spinulosus* (Cope 1862) from the subtropical wet mesic Paraguayan region (Reptilia Squamata Tropiduridae). *Tropical Zoology* **7:** 161–179.

Apps PJ, **Weldon PJ**, **Kramer M. 2015.** Chemical signals in terrestrial vertebrates: search for design features. *Natural Product Reports* **32:** 1131–1153.

Aragón P, **López P**, **Martín J. 2001a.** Discrimination of femoral gland secretions from familiar and unfamiliar conspecifics by male Iberian rock-lizards, *Lacerta monticola*. *Journal of Herpetology* **35:** 346–350.

Aragón P, **López P**, **Martín J. 2001b.** Chemosensory discrimination of familiar and unfamiliar conspecifics by lizards: implications of field spatial relationships between males. *Behavioral Ecology and Sociobiology* **50:** 128–133.

Ávila-Pires TCS. 1995. Lizards of Brazilian Amazonia (Reptilia: Squamata). *Zoologische Verhandelingen* **299:** 1–706.

Baeckens S, **Edwards S**, **Huyghe K**, **Van Damme R. 2015.** Chemical signalling in lizards: an interspecific comparison of femoral pore numbers in Lacertidae. *Biological Journal of the Linnean Society* **114:** 44–57.

Baeckens S, **Herrel A**, **Broeckhoven C**, **Vasilopoulou-Kampitsi M**, **Huyghe K**, **Goyens J**, **Van Damme R. 2017a.** Author correction: evolutionary morphology of the lizard chemosensory system. *Scientific Reports* **7:** 17570.

Baeckens S, **Martín J**, **García-Roa R**, **Pafilis P**, **Huyghe K**, **Van Damme R. 2017b.** Environmental conditions shape the chemical signal design of lizards. *Functional Ecology* **32:** 566–580.

Báez C, **Cortes A. 1990.** Precisión de la termorregulación conductual del lagarto neotropical *Tropidurus quadrivittatus* (Lacertilia: Iguanidae). *Revista Chilena de Historia Natural* **63:** 203–209.

Baig KJ, **Böhme W. 1991.** Callous scalation in female agamid lizards (*Stellio* group of *Agama*) and its functional implications. *Bonner Zoologische Beiträge* **42:** 275–281.

Bancroft JD. 1975. *Histological techniques, 2nd edn*. London: Butterworths.

Bancroft JD, **Stevens A. 1982.** *Theory and practice of histological techniques*. London: Churchill Livingstone.

Barbosa D, **Font E**, **Desfilis E**, **Carretero MA. 2006.** Chemically mediated species recognition in closely related *Podarcis* wall lizards. *Journal of Chemical Ecology* **32:** 1587–1598.

Bassam BJ, **Caetano-Anollés G**, **Gresshoff PM. 1991.** Fast and sensitive silver staining of DNA in polyacrylamide gels. *Analytical Biochemistry* **196:** 80–83.

Beuttner A, **Koch C. 2019.** Analysis of diet composition and morphological characters of the Peruvian lizard *Microlophus stolzmanni* (Squamata: Tropiduridae). *Phyllomedusa* **18:** 47–62.

Bro-Jørgensen J. 2010. Dynamics of multiple signalling systems: animal communication in a world in flux. *Trends in Ecology & Evolution* **25:** 292–300.

Bruinjé AC, **Moura MO**, **Maggi BS**, **São Pedro VA**, **Pessoa DMA**, **Costa GC. 2018.** Conspecifics of the striped lava lizard are able to distinguish sex and male colour morphs in apparently homogeneous dull dorsal coloration. *Amphibia-Reptilia* **40:** 149–162.

Bruinjé AC, **Coelho FEA**, **Paiva TMA**, **Costa GC. 2019.** Aggression, color signaling, and performance of the male color morphs of a Brazilian lizard (*Tropidurus semitaeniatus*). *Behavioral Ecology and Sociobiology* **73:** 72.

Butler AB, **Saidel WM. 2000.** Defining sameness: historical, biological, and generative homology. *BioEssays: News and Reviews in Molecular, Cellular and Developmental Biology* **22:** 846–853.

Carazo P, **Font E**, **Desfilis E. 2008.** Beyond 'nasty neighbours' and 'dear enemies'? Individual recognition by scent marks in a lizard (*Podarcis hispanica*). *Animal Behaviour* **76:** 1953–1963.

Carpenter CC. 1977. The aggressive displays of three species of South American iguanid lizards of the genus *Tropidurus*. *Herpetologica* **33:** 285–289.

Carvalho ALG. 2016. Three new species of the *Tropidurus spinulosus* group (Squamata: Tropiduridae) from eastern Paraguay. *American Museum Novitates* **3853:** 1–44.

Carvalho ALG, **Sena MA**, **Peloso PLV**, **Machado FA**, **Montesinos R**, **Silva HR**, **Campbell G**, **Rodrigues MT. 2016.** A new *Tropidurus* (Tropiduridae) from the semiarid Brazilian Caatinga: evidence for conflicting signal between mitochondrial and nuclear loci affecting the phylogenetic reconstructions of South American collared lizards. *American Museum Novitates* **3852:** 1–66.

Carvalho ALG, **Rivas LR**, **Céspedes R**, **Rodrigues MT. 2018.** A new collared lizard (*Tropidurus*: Tropiduridae) endemic to the western Bolivian Andes and its implications for seasonally dry tropical forests. *American Museum Novitates* **3896:** 1–54.

Chamut S, **Valdez VG**, **Manes ME. 2009.** Functional morphology of femoral glands in the Tegu lizard, *Tupinambis merianae*. *Zoological Science* **26:** 289–293.

Chiu KW, **Maderson PF. 1975.** The microscopic anatomy of epidermal glands in two species of gekkonine lizards, with some observations on testicular activity. *Journal of Morphology* **147:** 23–39.

Chiu KW, **Maderson PF**, **Alexander SA**, **Wong KL. 1975.** Sex steroids and epidermal glands in two species of gekkonine lizards. *Journal of Morphology* **147:** 9–22.

Clark DL, **Macedonia JM**, **Rowe JW**, **Stuart MA**, **Kemp DJ**, **Ord TJ. 2015.** Evolution of displays in Galápagos lava lizards: comparative analyses of signallers and robot playbacks to receivers. *Animal Behaviour* **109:** 33–44.

Clark DL, **Macedonia JM**, **Gillingham JC**, **Rowe JW**, **Kane HJ**, **Valle CA. 2016.** Why does conspecific display recognition differ among species of Galápagos lava lizards? A test using lizard robots. *Herpetologica* **72:** 47–54.

Coelho FEA, **Bruinjé AC**, **Costa GC. 2018.** Ethogram with the description of a new behavioral display for the striped lava lizard, *Tropidurus semitaeniatus*. *South American Journal of Herpetology* **13:** 96–101.

Cooper WE Jr, **Vitt LJ. 1987.** Intraspecific and interspecific aggression in lizards of the scincid genus *Eumeces*: chemical detection of conspecific sexual competitors. *Herpetologica* **43:** 7–14.

Cooper WE, **Van Wyk JH**, **Mouton PLFN. 1999.** Discrimination between self-produced pheromones and those produced by individuals of the same sex in the lizard *Cordylus cordylus*. *Journal of Chemical Ecology* **25:** 197–208.

Cruz FB, **Silva S**, **Scrocchi GJ. 1998.** Ecology of the lizard *Tropidurus etheridgei* (Squamata: Tropiduridae) from the dry Chaco of Salta, Argentina. *Herpetological Natural History* **6:** 23–31.

De-Lima AKS, **Domingos FMCB**, **Chaves SB**, **Pic-Taylor A**, **Sebben A**, **Klaczko J. 2018.** A new scent organ for *Gymnodactylus* lizards (Squamata: Phyllodactylidae) and an updated evolutionary scenario for the origin of squamate epidermal glands. *Biological Journal of the Linnean Society* **125:** 561–575.

Desjardins P, **Hansen JB**, **Allen M. 2009.** Microvolume protein concentration determination using the NanoDrop 2000c spectrophotometer. *Journal of Visualized Experiments* **33:** 1–3.

De Villiers A, **Mouton PLFN**, **Flemming A. 2015.** Generation glands of cordylid lizards: mechanism of secretion transfer to the environment. *Amphibia-Reptilia* **36:** 351–360.

Dixon JR, **Wright JW. 1975.** A review of the lizards of the iguanid genus *Tropidurus* in Peru. *Natural History Museum of Los Angeles County, Contributions in Science* **271:** 1–39.

Donnelly MA, **Myers CW. 1991.** Herpetological results of the 1990 Venezuelan expedition to the summit of Cerro Guaiquinima, with new Tepui reptiles. *American Museum Novitates* **3017:** 1–54.

Dujsebayeva T. 1998. The histology of callous scales of the males of Asian rock agamas, *Laudakia caucasia* and *Laudakia himalayana* (Reptilia; Agamidae). *Russian Journal of Herpetology* **5:** 160–164.

Dujsebayeva TN, **Ananjeva NB**, **Miroschnichenko LV. 2007.** Studies on specialized epidermal derivatives in igianian lizards. I. Gross morphology, topography and

histology of callose scales in the Asian rock *Agama*, *Laudakia himalayana* (Steindachner, 1869) (Squamata: Agamidae). *Amphibia-Reptilia* **28:** 537–546.

Dujsebayeva T, **Ananjeva N**, **Böhme W**, **Wagner P. 2009.** Studies on specialized epidermal derivatives in iguanian lizards: II. New data on the scalation of the Malagasy iguanas of the genus *Oplurus* (Sauria: Iguanidae). *Amphibia-Reptilia* **30:** 89–97.

Etheridge R. 1968. A review of the iguanid lizard genera *Uracentron* and *Strobilurus*. *Bulletin of the British Museum Natural History* **17:** 47–64.

Etheridge R. 1970. A review of the South-American iguanid lizard genus *Plica*. *Bulletin of the British Museum (Natural History)* **19:** 237–256.

Felsenstein J. 2004. *Inferring phylogenies*. Sunderland: Sinauer Associates.

Filogonio R, **Del Lama FS**, **Machado LL**, **Drumond M**, **Zanon I**, **Mezzetti NA**, **Galdino CAB. 2010.** Daily activity and microhabitat use of sympatric lizards from Serra do Cipó, southeastern Brazil. *Iheringia, Série Zoologia* **100:** 336–340.

Flaxman BA, **Maderson PF**, **Szabó G**, **Roth SI. 1968.** Control of cell differentiation in lizard epidermis in vitro. *Developmental Biology* **18:** 354–374.

Frost DR. 1992. Phylogenetic analysis and taxonomy of the *Tropidurus* group of lizards (Iguania, Tropiduridae). *American Museum Novitates* **3033:** 1–68.

Frost DR, **Crafts HM**, **Fitzgerald LA**, **Titus TA. 1998.** Geographic variation, species recognition, and molecular evolution of cytochrome oxidase I in the *Tropidurus spinulosus* complex (Iguania: Tropiduridae). *Copeia* **1998:** 839–851.

Frost DR, **Rodrigues MT**, **Grant T**, **Titus TA. 2001.** Phylogenetics of the lizard genus Tropidurus (Squamata: *Tropiduridae*: Tropidurinae): direct optimization, descriptive efficiency, and sensitivity analysis of congruence between molecular data and morphology. *Molecular Phylogenetics and Evolution* **21:** 352–371.

Gabe M, **Saint-Girons H. 1965.** Contribution à la morphologie comparée du cloaque et des glandes épidermoïdes de la région cloacale chez les Lépidosauriens. *Mémoires du Muséum National d'Histoire Naturelle Série A - Zoologie* **33:** 149–292.

Gabirot M, **Castilla AM**, **López P**, **Martín J. 2010.** Differences in chemical signals may explain species recognition between an island lizard, *Podarcis atrata*, and related mainland lizards, *P. hispanica*. *Biochemical Systematics and Ecology* **38:** 521–528.

Gabirot M, **López P**, **Martín J. 2012.** Differences in chemical sexual signals may promote reproductive isolation and cryptic speciation between iberian wall lizard populations. *International Journal of Evolutionary Biology* **2012:** 698520.

García-Roa R, **Jara M**, **Baeckens S**, **López P**, **Van Damme R**, **Martín J**, **Pincheira-Donoso D. 2017a.** Macroevolutionary diversification of glands for chemical communication in squamate reptiles. *Scientific Reports* **7:** 1–10.

García-Roa R, **Sáiz J**, **Gómara B**, **López P**, **Martín J. 2017b.** Dietary constraints can preclude the expression of an honest chemical sexual signal. *Scientific Reports* **7:** 6073.

Greenfield MD. 2002. *Signalers and receivers. Mechanisms and evolution of arthropod communication*. New York: Oxford University Press.

Gudynas E, **Skuk G. 1983.** A new species of the iguanid lizard genus *Tropidurus* from temperate South America (Lacertilia: Iguanidae). *Centro Educativo Don Orione Contribuciones en Biologia* **10:** 1–10.

Harvey MB, **Gutberlet RL Jr**. **1998.** Lizards of the genus *Tropidurus* (Iguania: Tropiduridae) from the Serranía de Huanchaca, Bolivia: new species, natural history, and a key to the genus. *Herpetologica* **54:** 493–520.

Harvey MB, **Gutberlet RL Jr**. **2000.** A phylogenetic analysis of the tropidurine lizards (Squamata: Tropiduridae), including new characters of squamation and epidermal microstructure. *Zoological Journal of the Linnean Society* **128:** 189–233.

Heller E. 1903. Papers from the Kopkins Stanford Galapagos Expedition, 1898–1899. XIV. Reptiles. *Proceedings of the Washington Academy of Sciences* **5:** 39–98.

Houck LD. 2009. Pheromone communication in amphibians and reptiles. *Annual Review of Physiology* **71:** 161–176.

Huey RB, **Pianka ER. 1981.** Ecological consequences of foraging mode. *Ecology* **62:** 991–999.

Iraeta P, **Monasterio C**, **Salvador A**, **Díaz JA. 2011.** Sexual dimorphism and interpopulation differences in lizard hind limb length: locomotor performance or chemical signalling? *Biological Journal of the Linnean Society* **104:** 318–329.

Jared C, **Antoniazzi MM**, **Silva JR**, **Freymüller E. 1999.** Epidermal glands in Squamata: microscopical examination of precloacal glands in *Amphisbaena alba* (Amphisbaenia, Amphisbaenidae). *Journal of Morphology* **241:** 197–206.

Johansson BG, **Jones TM. 2007.** The role of chemical communication in mate choice. *Biological Reviews of the Cambridge Philosophical Society* **82:** 265–289.

Jordan MA, **Hollis JL**, **Stone PA**, **Snell HL. 2008.** Habitat as a source of intrapopulational variation of ornament size in Galápagos lava lizards (*Microlophus albemarlensis* complex). *Amphibia-Reptilia* **29:** 278–283.

Karnovsky MJ. 1965. A formaldehyde-glutaraldehyde fixative of high osmolality for use in electron microscopy. *Journal of Cell Biology* **27:** 137A–138A.

Katoh K, **Toh H. 2008.** Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics* **9:** 286–298.

Kluge AG. 1987. Cladistic relationships in the Gekkonoidea (Squamata, Sauria). *Miscellaneous Publications of the Museum of Zoology, University of Michigan* **173:** 1–54.

Kohlsdorf T, **Ribeiro JM**, **Navas CA. 2006.** Territory quality and male dominance in *Tropidurus torquatus* (Squamata, Tropiduridae). *Phyllomedusa* **5:** 109–118.

Kunz TS, **Borges-Martins M. 2013.** A new microendemic species of *Tropidurus* (Squamata: Tropiduridae) from southern Brazil and revalidation of *Tropidurus* catalanensis Gudynas & Skuk, 1983. *Zootaxa* **3681:** 413–439.

Laemmli UK. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227:** 680–685.

Landmann L. 1986. Epidermis and dermis. In: Bereiter-Hahn J, Matoltsy AG, Richards KS, eds. *Biology of the Integument. 2. Vertebrates*. Berlin: Heidelberg, 150–187.

Lanfear R, **Calcott B**, **Ho SY**, **Guindon S. 2012.** Partitionfinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* **29:** 1695–1701.

Laurent RF. 1980. Variacion y dimorfismo sexual en el complejo *Tropidurus melanopleurus–pictus* (Iguanidae). In: Salinas PJ, ed. *Actas del VIII Congreso Latinoamericano de Zoología.* Mérida: Venezuela, 621–626.

Leydig F. 1872. *Die in Deutschland lebenden Arten der Saurier*. Türbingen: Verlag der H. Laupp'schen Buchhandlung.

Linnaeus C. 1758. *Systema naturae*. Stockholm: L. Salvius.

Louw S, **Burger BV**, **Le Roux M**, **Van Wyk JH. 2011.** Lizard epidermal gland secretions. II. Chemical characterization of the generation gland secretion of the sungazer, *Cordylus giganteus*. *Journal of Natural Products* **74:** 1364–1369.

Maddison WP, **Maddison DR. 2019.** *Mesquite: a modular system for evolutionary analysis, v.3.61.* Available at: https://www.mesquiteproject.org/. Accessed March 2020.

Maderson PFA. 1967. The histology of the escutcheon scales of *Gonatodes* (Gekkonidae) with a comment on the squamate sloughing cycle. *Copeia* **1967:** 743–752.

Maderson PFA. 1968a. The epidermal glands of *Lygodactylus* (Gekkonidae, Lacertilia). *Breviora* **288:** 1–35.

Maderson PFA. 1968b. On the presence of 'escutcheon scales' in the eublepharine gekkonid *Coleonyx*. *Herpetologica* **24:** 99–103.

Maderson PFA. 1970. Lizard glands and lizard hands: models for evolutionary study. *Forma et Functio* **3:** 179–204.

Maderson PFA. 1971. The regeneration of caudal epidermal specializations in *Lygodactylus picturatus keniensis* (Gekkonidae, Lacertilia). *Journal of Morphology* **134:** 467–477.

Maderson PFA. 1972. The structure and evolution of holocrine epidermal glands in sphaerodactyline and eublepharine gekkonid lizards. *Copeia* **1972:** 559–571.

Maderson PFA, **Chiu KW. 1970.** Epidermal glands in gekkonid lizards: evolution and phylogeny. *Herpetologica* **26:** 233–238.

Maderson PFA, **Mayhew WW**, **Sprague G. 1970.** Observations on the epidermis of desert-living iguanids. *Journal of Morphology* **130:** 25–35.

Mangiacotti M, **Fumagalli M**, **Scali S**, **Zuffi MAL**, **Cagnone M**, **Salvini R**, **Sacchi R. 2017.** Inter- and intra-population variability of the protein content of femoral gland secretions from a lacertid lizard. *Current Zoology* **63:** 657–665.

Mangiacotti M, **Fumagalli M**, **Cagnone M**, **Viglio S**, **Bardoni AM**, **Scali S**, **Sacchi R. 2019a.** Morph-specific protein patterns in the femoral gland secretions of a colour polymorphic lizard. *Scientific Reports* **9:** 8412.

Mangiacotti M, **Gaggiani S**, **Coladonato AJ**, **Scali S**, **Zuffi MAL**, **Sacchi R. 2019b.** First experimental evidence that proteins from femoral glands convey identity-related information in a lizard. *Acta Ethologica* **22:** 57–65.

Mangiacotti M, **Martín J**, **López P**, **Reyes-Olivares CV**, **Rodríguez-Ruiz G**, **Coladonato AJ**, **Scali S**, **Zuffi MAL**, **Sacchi R. 2020.** Proteins from femoral gland secretions of male rock lizards *Iberolacerta cyreni* allow self – but not individual – recognition of unfamiliar males. *Behavioral Ecology and Sociobiology* **74:** 68.

Manzani PR, **Abe AS. 1990.** A new species of *Tapinurus* from the Caatinga of Piaui, northeastern Brazil (Squamata: Tropiduridae). *Herpetologica* **46:** 462–467.

Martín J, **López P. 2000.** Chemoreception, symmetry and mate choice in lizards. *Proceedings of the Royal Society B – Biological Sciences* **267:** 1265–1269.

Martín J, **López P. 2006a.** Vitamin D supplementation increases the attractiveness of males' scent for female Iberian rock lizards. *Proceedings of the Royal Society B – Biological Sciences* **273:** 2619–2624.

Martín J, **López P. 2006b.** Links between male quality, male chemical signals, and female mate choice in Iberian rock lizards. *Functional Ecology* **20:** 1087–1096.

Martín J, **López P. 2011.** Pheromones and reproduction in reptiles. In: Norris DO, Lopez KH, eds. *Hormones and reproduction of vertebrates. Reptiles.* San Diego: Academic Press, 141–167.

Martín J, **López P. 2012.** Supplementation of male pheromone on rock substrates attracts female rock lizards to the territories of males: a field experiment. *PLoS One* **7:** e30108.

Martín J, **López P. 2013a.** Responses of female rock lizards to multiple scent marks of males: effects of male age, male density and scent over-marking. *Behavioural Processes* **94:** 109–114.

Martín J, **López P. 2013b.** Effects of global warming on sensory ecology of rock lizards: increased temperatures alter the efficacy of sexual chemical signals. *Functional Ecology* **27:** 1332–1340.

Martín J, **López P**, **Iraeta P**, **Díaz JA**, **Salvador A. 2016.** Differences in males' chemical signals between genetic lineages of the lizard *Psammodromus algirus* promote male intrasexual recognition and aggression but not female mate preferences. *Behavioral Ecology and Sociobiology* **70:** 1657–1668.

Martins KV, **Dias EJR**, **Rocha CFD. 2010.** Ecologia e conservação do lagarto endêmico *Tropidurus hygomi* (Sauria: Tropiduridae) nas restingas do litoral norte da Bahia, Brasil. *Biotemas* **23:** 71–75.

Mason RT. 1992. Reptilian pheromones. In: Gans C, Crews D, eds. *Biology of the Reptilia. Hormones, brain, and behavior*. Chicago: University of Chicago Press, 114–228.

Mason RT, **Parker MR. 2010.** Social behavior and pheromonal communication in reptiles. *Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural, and Behavioral Physiology* **196:** 729–749.

Mayerl C, **Van Damme R**, **Baeckens S. 2015.** Evolution and role of the follicular epidermal gland system in non-ophidian squamates. *Amphibia-Reptilia* **36:** 185–206.

Meira KTR, **Faria RG**, **Silva MDDM**, **de Miranda VT**, **Zahn-Silva W. 2007.** História natural de *Tropidurus oreadicus*

em uma área de cerrado rupestre do Brasil Central. *Biota Neotropica* **7:** 155–163.

Melo GC, **Pinheiro LT**, **Passos DC**, **Galdino CAB. 2017.** Spatial organisation of the Neotropical lizard *Tropidurus hispidus* (Squamata: Tropiduridae). *Salamandra* **53:** 435–438.

Mertens R. 1956. Studien über die Herpetofauna Perus I. Zur Kenntniss der Iguaniden-Gattung *Tropidurus* in Peru. *Senckenbergiana Biologica* **37:** 101–136.

Morais CJS, **Barreto-Lima AF**, **Dantas PT**, **Domingos FMCB**, **Ledo RMD**, **Pantoja DL**, **Sousa HC**, **Colli GR. 2014.** First records of *Tropidurus callathelys* and *T. chromatops* (Reptilia: Squamata: Tropiduridae) in Brazil. *Check List* **10:** 1213–1217.

Mouton PLFN, **Flemming AF**, **Searby CA. 1998.** Active generation glands present in neonates of some Cordylid Lizards: A case study of *Cordylus macropholis* (Sauria: Cordylidae). *Journal of Morphology* **235:** 177–182.

Mouton PLFN, **Van Rensburg DAJ**, **Van Wyk JH. 2010.** Epidermal glands in cordylid lizards, with special reference to generation glands. *Zoological Journal of the Linnean Society* **158:** 312–324.

Mouton PlFN, **Flemming AF**, **Broeckhoven C. 2014.** Generation gland morphology in cordylid lizards: an evolutionary perspective. *Journal of Morphology* **275:** 456–464.

Murphy J, **Jowers M. 2013.** Treerunners, cryptic lizards of the *Plica plica* group (Squamata, Sauria, Tropiduridae) of northern South America. *ZooKeys* **355:** 49–77.

Myers CW, **Donnelly MA. 2001.** Herpetofauna of the Yutaje-Corocoro massif, Venezuela: second report from the Robert G. Goelet American Museum–Terramar Expedition to the northwestern tepuis. *Bulletin of the American Museum of Natural History* **261:** 1–85.

Myers CW, **Donnelly MA. 2008.** The summit herpetofauna of Auyantepui, Venezuela: report from the Robert G. Goelet American Museum–Terramar Expedition. *Bulletin of the American Museum of Natural History* **308:** 1–147.

Ortiz JC. 1980. Revisión taxonómica del género *Tropidurus* en Chile. *Reunionión Iberoamericana de Zoología de Vertebrados* **1:** 355–377.

Passos DC, **Lima DC**, **Borges-Nojosa DM. 2011.** A new species of *Tropidurus* (Squamata, Tropiduridae) of the semitaeniatus group from a semiarid area in northeastern Brazil. *Zootaxa* **2930:** 60–68.

Pavel AB, **Vasile CI. 2012.** PyElph – a software tool for gel images analysis and phylogenetics. *BMC Bioinformatics* **13:** 9.

Pearse AGE. 1985. *Histochemistry. Theoretical and applied, Vol. 2: analytical technology*. Edinburgh: Churchill Livingstone.

Pelegrin N. 2019. Reproductive behavior of *Tropidurus spinulosus* (Squamata: Tropiduridae) in captivity. *Phyllomedusa* **18:** 123–126.

Pelegrin N, **Mesquita DO**, **Albinati P**, **Caldas FLS**, **Cavalcanti LBQ**, **Costa TB**, **Falico DA**, **Galdino JYA**, **Tucker DB**, **Garda AA. 2017.** Extreme specialization to rocky habitats in *Tropidurus* lizards from Brazil: trade-offs between a fitted ecomorph and autoecology in a harsh environment. *Austral Ecology* **42:** 677–689.

Pérez ZJ, **Balta K. 2007.** Ecology of the diurnal lizards community in the Reserva Nacional de Paracas, Ica, Peru. *Revista Peruana de Biología* **13:** 169–176.

Perez-Mellado V, **De la Riva I. 1993.** Sexual size dimorphism and ecology: the case of a tropical lizard, *Tropidurus melanopleurus* (Sauria: Tropiduridae). *Copeia* **1993:** 969–976.

Pianka ER, **Vitt LJ. 2003.** *Lizards: windows to the evolution of diversity*. Berkley: University of California Press.

Pincheira-Donoso D, **Hodgson DJ**, **Tregenza T. 2008.** Comparative evidence for strong phylogenetic inertia in precloacal signalling glands in a species-rich lizard clade. *Evolutionary Ecology Research* **10:** 11–28.

Pinto A, **Wiederhecker H**, **Colli G. 2005.** Sexual dimorphism in the Neotropical lizard, *Tropidurus torquatus* (Squamata, Tropiduridae). *Amphibia-Reptilia* **26:** 127–137.

Pyron RA, **Burbrink FT**, **Wiens JJ. 2013.** A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. *BMC Evolutionary Biology* **13:** 93.

Ribeiro LB, **Kolodiuk MF**, **Freire EMX. 2010.** Ventral colored patches in *Tropidurus semitaeniatus* (Squamata, Tropiduridae): sexual dimorphism and association with reproductive cycle. *Journal of Herpetology* **44:** 177–182.

Ribeiro LB, **Brita MS**, **Barbosa LFS**, **Pereira LCM**, **Nicola PA. 2012.** *Tropidurus cocorobensis* Rodrigues, 1987 (Squamata, Tropiduridae): new record and geographic distribution map in northeastern Brazil. *Cuadernos de Herpetología* **26:** 63–65.

Rivadeneira EYD. 2008. *Variación geográfica del complejo* Tropidurus melanopleurus *(Sauria: Tropiduridae)*. Unpublished Undergraduate Thesis, Universidad Mayor de San Andrés.

Rodrigues MT. 1981. Uma nova espécie de *Tropidurus* do Brasil (Sauria, Iguanidae). *Papéis Avulsos de Zoologia* **34:** 145–149.

Rodrigues MT. 1984a. Uma nova espécie brasileira de *Tropidurus* com crista dorsal (Sauria, Iguanidae). *Papéis Avulsos de Zoologia* **35:** 169–175.

Rodrigues MT. 1984b. Sobre *Platynotus* Wagler, 1830, preocupado, substituido por *Tapinurus* Amaral, 1933, com a descrição de uma nova espécie (Sauria, Iguanidae). *Papéis Avulsos de Zoologia* **35:** 367–373.

Rodrigues MT. 1986. Um novo *Tropidurus* com crista dorsal do Brasil com comentários sobre suas relações, distribuição e origem (Sauria, Iguanidae). *Papéis Avulsos de Zoologia* **36:** 171–179.

Rodrigues MT. 1987. Sistemática, ecologia e zoogeografia dos *Tropidurus* do grupo *torquatus* ao sul do Rio Amazonas (Sauria, Iguanidae). *Arquivos de Zoologia* **31:** 105–230.

Rodrigues MT, **Kasahara S**, **Yonenaga-Yassuda Y. 1988.** *Tropidurus psammonastes*: uma nova espécie do grupo torquatus com notas sobre seu cariótipo e distribuição (Sauria, Iguanidae). *Papéis Avulsos de Zoologia* **36:** 307–313.

Rodrigues KC, **Delfim FR**, **Castro CSS**, **França FGR**, **Filho EL**, **Mesquita DO**, **Oliveira FA**, **Santos ACA**,

Ferrari SF, **Valença-Montenegro MM. 2013.** *Strobilurus torquatus* Wiegmann, 1834 (Squamata: Tropiduridae): new records from the Brazilian state of Paraíba and a geographic distribution map. *Check List* **9:** 614–617.

Rowe JW, **Clark DL**, **Martin CE**, **Valle C. 2020.** Diel and seasonal variations in the thermal biology of San Cristobal Lava Lizards (*Microlophus bivittatus*). *Journal of Thermal Biology* **88:** 102518.

Roze JA. 1958. Resultados zoológicos de la expedición de la Universidad Central da Venezuela a la región del Auyantepui en la Guayana venezolana, Abril de 1956. 5. Los reptiles del Auyantepui, Venezuela, basándose en las colecciones de las expediciones de Phelps-Tate, del American Museum of Natural History, 1937–1938, y de la Universidad Central de Venezuela, 1956. *Acta Biologica Venezuelica* **2:** 243–270.

Schluter D. 1984. Body size, prey size and herbivory in the Galapagos lava lizard, *Tropidurus*. *Oikos* **43:** 291–300.

Schumacher R, **Barts M. 2003.** *Tropidurus melanopleurus* Boulenger. *Sauria (Suppl.)* **25:** 591–596.

Searby CA. 2002. *Histological description of generation glands and their functional relationship to the shedding cycle in cordylid lizards.* Unpublished M.Sc. Thesis, University of Stellenbosch.

Shanahan T. 2011. Phylogenetic inertia and Darwin's higher law. *Studies in History and Philosophy of Biological and Biomedical Sciences* **42:** 60–68.

Shubin N, **Tabin C**, **Carroll S. 1997.** Fossils, genes and the evolution of animal limbs. *Nature* **388:** 639–648.

Silva BS. 2014. Observaciones sobre la alimentación de *Microlophus quadrivittatus* (Tschudi 1845). *Boletin Chileno de Herpetología* **1:** 18.

Snell HL, **Jennings RD**, **Snell HM**, **Harcourt S. 1988.** Intrapopulation variation in predator-avoidance performance of Galápagos lava lizards: the interaction of sexual and natural selection. *Evolutionary Ecology* **2:** 353–369.

Sukumaran J, **Holder MT. 2010.** DendroPy: a Python library for phylogenetic computing. *Bioinformatics (Oxford, England)* **26:** 1569–1571.

Taylor EH, **Leonard AB. 1956.** Concerning the relationship of certain Neotropical gekkonid lizard genera, with comments on the microscopic structure of their glandular scales. *University of Kansas Science Bulletin* **38:** 1019–1029.

Teixeira M Jr, **Prates I**, **Nisa C**, **Silva-Martins NS**, **Strüssmann C**, **Rodrigues MT. 2016.** Molecular data reveal spatial and temporal patterns of diversification and a cryptic new species of lowland *Stenocercus* Duméril & Bibron, 1837 (Squamata: Tropiduridae). *Molecular Phylogenetics and Evolution* **94:** 410–423.

du Toit DA, **Mouton PFN**, **Flemming AF**, **Van Niekerk A. 2005.** Climate and the presence of generation glands in female girdled lizards: A case study of the *cordylus-niger-oelofseni* complex. *Journal of Herpetology* **39:** 384–388.

Tölg F. 1905. Beiträge zur Kenntnis drüsenartiger Epidermoidalorgane der Eidechsen. *Arbeiten aus dem Zoologischen Instituten der Universität Wien und der Zoologischen Station in Triest* **15:** 119–154.

Torres-Carvajal O. 2007. A taxonomic revision of South American *Stenocercus* (Squamata: Iguania) lizards. *Herpetological Monographs* **21:** 76–178.

Troncoso-Palacios J. 2018. Propuesta de restricción de la localidad tipo de *Microlophus tarapacensis* (Donoso-Barros, 1966) (Squamata: Tropiduridae) en base a evidencias históricas. *Cuadernos de Herpetología* **32:** 123–127.

Uetz P. 2020. *The reptile database.* Available at: http://www.reptile-database.org (accessed 4 March 2020).

Vaidya G, **Lohman DJ**, **Meier R. 2011.** SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. *Cladistics* **27:** 171–180.

Van Sluys M. 1997. Home range of the saxicolous lizard *Tropidurus itambere* (Tropiduridae) in Southeastern Brazil. *Copeia* **1997:** 623–628.

Van Wyk JH. 1997. Comparative histology of skin glands in girdled lizards. *Microscopy Society of South Africa - Proceedings* **27:** 110.

Van Wyk JH, **Mouton PLF. 1992.** Glandular epidermal structures in cordylid lizards. *Amphibia-Reptilia* **13:** 1–12.

Vanzolini PE, **Ramos-Costa A**, **Vitt LJ. 1980.** *Répteis das Caatingas*. Rio de Janeiro: Academia Brasileira de Ciências.

Vasconcelos L, **Bruinjé AC**, **Coelho FEA**, **Tinôco MS**, **Marques R. 2019.** Full mating repertoire of *Tropidurus hygomi* Reinhardt and Lütken, 1861 on a coastal sand dune habitat in Bahia, Brazil (Squamata: Tropiduridae). *Herpetology Notes* **12:** 353–357.

Vidal M, **Ortiz JC**, **Labra A. 2002.** Sexual and age differences in ecological variables of the lizard *Microlophus atacamensis* (Tropiduridae) from northern Chile. *Revista Chilena de Historia Natural* **75:** 283–292.

Vidal M, **Ortiz JC**, **Escobar G**, **Torres-Pérez F. 2017.** Identidad taxonómica de *Microlophus heterolepis* (Wiegmann, 1835) y *Microlophus yanezi* (Ortiz, 1980): dos buenas especies del desierto interior de Chile y Perú. *Gayana (Concepción)* **81:** 100–110.

Vitt LJ, **Pianka ER. 2005.** Deep history impacts present-day ecology and biodiversity. *Proceedings of the National Academy of Sciences of the United States of America* **102:** 7877–7881.

Vitt LJ, **Zani PA. 1996.** Ecology of the elusive tropical lizard *Tropidurus* [= *Uracentron*] *flaviceps* (Tropiduridae) in lowland rain forest of Ecuador. *Herpetologica* **52:** 121–132.

Vitt LJ, **Pianka ER**, **Cooper WE Jr**, **Schwenk K. 2003.** History and the global ecology of squamate reptiles. *The American Naturalist* **162:** 44–60.

Watkins GG. 1997. Inter-sexual signalling and the functions of female coloration in the tropidurid lizard *Microlophus occipitalis*. *Animal Behaviour* **53:** 843–852.

Weldon PJ, **Flachsbarth B**, **Schulz S. 2008.** Natural products from the integument of nonavian reptiles. *Natural Product Reports* **25:** 738–756.

Werner DI. 1978. On the biology of *Tropidurus delanonis*, Baur (Iguanidae). *Ethology* **47:** 337–395.

Wiederhecker HC, **Pinto ACS**, **Paiva MS**, **Colli GR. 2003.** The demography of the lizard *Tropidurus torquatus*

(Squamata, Tropiduridae) in a highly seasonal Neotropical savanna. *Phyllomedusa* **2:** 9–19.

Xavier MA, **Dias EJR. 2017.** Ecology of endemic and saxicolous lizard *Tropidurus pinima* (Rodrigues, 1984) from Caatinga domain, Brazil. *Herpetology Notes* **10:** 491–492.

Zani PA, **Vitt LJ. 1995.** Techniques for capturing arboreal lizards. *Herpetological Review* **26:** 136–137.

Zozaya SM, **Higgie M**, **Moritz C**, **Hoskin CJ. 2019.** Are pheromones key to unlocking cryptic lizard diversity? *The American Naturalist* **194:** 168–182.

Zwickl DJ. 2006. *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. Unpublished D.Phil. Thesis, University of Texas at Austin.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Appendix S1. Full list of tissue samples analysed molecularly, respective collection data and GenBank accession numbers to sequence data.
Appendix S2. Molecular matrix (nexus format) used to reconstruct the phylogenetic relationships of tropidurids.
Appendix S3. Profile of the molecular dataset analysed (Table S1) and summary of the best-fit nucleotide substitution models and partition schemes identified by PartitionFinder 2 (Table S2).
Appendix S4. Best molecular phylogenetic tree (newick format) inferred for tropidurids under maximum likelihood. Nonparametric bootstrap values (1000 replicates) are associated to nodes.