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Diversity and evolution of sexually dimorphic mental and lateral glands in Cophomantini treefrogs (Anura: Hylidae: Hylinae)

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We describe the structure and histochemistry of mental and lateral glands in a representative array of 28 species of five genera of the Neotropical hylid frog tribe Cophomantini. Structural diversity was coded in 15 characters that were optimized on the most recent phylogenetic hypothesis. Mental and lateral glands occur in 17 species and 10 species, respectively, whereas nine species have both. Each glandular concentration may have two types of sexually dimorphic skin glands (SDSGs), specialized mucous and specialized serous glands, which occur independently or may co-occur. Distinctive characteristics related to these glands are shape, aspect of the secretion, disposition, and distribution. The occurrences of mental and lateral glands, and the characters derived from macroscopic and microscopic examinations, have an intricate taxonomic distribution, with differing levels of homoplasy. The function of SDSGs in Cophomantini is currently unknown. However, based on structural and histochemical similarities to SDSGs from other species of amphibians where experimental evidence exists, we infer they might be involved in the secretion of chemical signals during courtship behaviour. The distribution pattern of these glands, along with the existence of different signals (i.e. acoustic, visual, tactile), suggests the presence of multimodal signalling for some species of the tribe.

ADDITIONAL KEYWORDS: amphibian – breeding – chemical communication – courtship – multimodal communication – SDSGs – skin.

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INTRODUCTION

The skin of amphibians is characterized by the presence of multicellular exocrine glands distributed throughout the whole body (Fox, 1986; Toledo & Jared, 1995). In some species, these glands may be distributed in limited skin regions forming voluminous clusters, or macroglands (Toledo & Jared, 1995). They are defined as 'specialized' glands because of their limited location, in order to differentiate them from 'ordinary' glands, which are homogeneously distributed throughout the body (Brizzi, Delfino & Jantra, 2003). Besides this topographical criterion, both gland types are distinguished by histological structure and histochemical properties (Brizzi, Delfino & Pellegrini, 2002). The specialization of these glands is mainly related to defence against predators, social communication, and reproduction (Brizzi *et al.*, 2003). In particular, glands associated with reproductive function are known as breeding glands or sexually dimorphic skin glands (SDSGs; Thomas, Tsang & Licht, 1993; Brizzi *et al.*, 2003; Brunetti, Faivovich & Hermida, 2012).

Across Caudata, SDSGs have been subject of numerous studies (Noble, 1929; Lanza, 1959; Sever, 1976, 1989; Houck & Verrel, 1993), which have contributed to a general understanding of their diversity, function (Houck & Sever, 1994), and also to advances in the study of the secreted molecules (Houck, 1998; Watts *et al.*, 2004). By contrast, published accounts of SDSGs in anurans have been limited to comments detailing their occurrence, with few histological descriptions (Brizzi *et al.*, 2003). Even though there is an increasing interest in the structure and ultrastructure of these glands (Vences *et al.*, 2007; Gonçalves & Brito-Gitirana, 2008; Siegel *et al.*, 2008; Brunetti *et al.*, 2012), knowledge of their diversity, taxonomic distribution, and evolution remains poor.

In Hylidae, the most species-rich family of anurans (Frost, 2014), SDSGs have been described in mental, lateral and ventrolateral regions, as well as in nuptial pads. Mental glands were reported in two subfamilies: in the *Litoria citropa* species group (Pelodryadinae; Tyler & Anstis, 1975; Mahony *et al.*, 2001) and in species of the tribes Cophomantini and Hylini (Hylinae; Duellman, 1961, 1972; Trueb & Tyler, 1974; Hoogmoed, 1979; Campbell & Duellman, 2000). In particular, recent studies in Cophomantini show that mental glands are more frequent than previously reported (Faivovich *et al.*, 2005, 2006, 2009; Faivovich & De la Riva, 2006; Faivovich, McDiarmid & Myers, 2013), whereas Brunetti *et al.* (2012) have also demonstrated the presence of lateral glands co-occurring with mental glands in *Hypsiboas punctatus*, a species of the *H. punctatus* group.

Despite the occurrence of SDSGs in several species of Hylidae across different body regions, only three studies to date have characterized the structure of SDSGs in this family (Thomas *et al.*, 1993; Romero de Perez & Ruiz Carranza, 1996; Brunetti *et al.*, 2012). The results show a complex scenario of character distribution: the ventrolateral glands of *Duellmanohyla schmidtorum* consist of specialized serous glands (SSGs; Thomas *et al.*, 1993), the mental gland of two species of the *Hyloscirtus bogotensis* group [i.e. *H. alytolylax*, and *H. piceigularis* (as *Hyla* sp. 'C'); Romero de Perez & Ruiz Carranza, 1996] is formed by specialized mucous glands (SMGs; Romero de Perez & Ruiz Carranza, 1996), whereas the mental and lateral glands in *Hypsiboas punctatus* have both types of glands, SMGs and SSGs (Brunetti *et al.*, 2012). This intricate pattern suggests a complexity that defies any generalization regarding taxonomic distribution and inferences about its biological function, and illustrates the need for a more thorough analysis in order to understand the structure and variation of SDSGs in other species within the family.

Because of their sexually dimorphic nature, SDSGs have been suggested to have critical roles during reproductive activities (Brizzi *et al.*, 2003). Unfortunately, despite such suggestions and inferences, the function of these glands in most anurans remains unknown, with a few exceptions found in the adhesive abdominal glands of the microhylid *Gastrophryne carolinensis* (Conaway & Metter, 1967) and in the pheromone-producing femoral glands of mantellids (Poth *et al.*, 2012). One possible reason for this deficiency is that studies of reproductive biology and communication in anurans have historically been focused on acoustic signals. However, some studies have shown that several species may use other signal modalities for communication (i.e. visual, tactile, chemical) and, in most cases, these interact with each other as multimodal signals (Haddad & Giaretta, 1999; Hödl & Amézquita, 2001; Stephenson & Verrell, 2003; Giasson & Haddad, 2006; Toledo *et al.*, 2007; Taylor *et al.*, 2011; Grafe *et al.*, 2012; Preininger *et al.*, 2013). In particular, there is increasing behavioural (Pearl *et al.*, 2000; Poth *et al.*, 2012) and chemical evidence (Poth *et al.*, 2012; Willaert *et al.*, 2013) for the hypothesis that SDSGs of anurans release chemical signals associated with reproduction. Although the field of anuran chemical communication is on its very early stages of development, knowledge of the morphology, structural diversity, and taxonomic distribution of SDSGs is a fundamental step necessary for a more comprehensive understanding of their function.

The present study aimed: (1) to describe the macroscopic and microscopic structure, histochemical properties, and variation of sexually dimorphic skin glands (SDSGs) from mental and lateral regions of selected species of Cophomantini; (2) to propose hypotheses of homology for the structural variation; (3) to study the evolution of SDSGs on the basis of the most recent phylogenetic hypothesis proposed for the clade by Faivovich *et al*. (2013), and (4) to discuss their possible function considering published information on the reproductive biology of Cophomantini, and that of other species of anurans where experimental studies have demonstrated the role of these glands.

MATERIAL AND METHODS

SPECIMENS

We examined mental and lateral skin regions from adult males of 28 of the 171 species of the Neotropical tribe Cophomantini from the subfamily Hylinae (see Supporting information, Appendix S1). We have included at least two species of each of the recognized species groups in the genus *Hypsiboas*, with the exception of the *H. pellucens* and *H. punctatus* groups (only one species of each group available), three species from the *Hyloscirtus bogotensis* group, two species from the *H. larinopygion* and the *Bokermannohyla pseudopseudis* groups, one of the *B. circumdata* group, and one species from each of the three groups in the genus *Aplastodiscus* (the *A. albofrenatus*, *A. albosignatus*, and *A. perviridis* groups). In 15 of the 28 species examined, the same regions of male samples were compared against the female samples (see Supporting information, Appendix S1). To understand levels of intraspecific variation, more than one male specimen was analyzed for some species. Individuals were collected during reproductive activity and were also selected for having indirect evidence of its active reproductive condition (partially dilated vocal sac, personal communications from the collectors). Voucher specimens and locality information are listed in the Supporting information (see Supporting information, Appendix S1). Institutional codes follow Sabaj-Pérez (2013).

HISTOLOGICAL METHODS

Specimens were fixed in 4% neutral buffered formaldehyde and subsequently preserved in 70% ethanol. Small strips of skin (approximately 9–16 mm^2) were removed from mental, lateral, and dorsal regions of all specimens. Samples were dehydrated in an ascending series of ethanol, cleared in toluene, paraffin-embedded, sectioned transversely at 5 μm, and mounted onto microscope slides. Sections were stained with haematoxylin and eosin (Martoja & Martoja-Pierson, 1970), Masson–Goldner's trichrome (Martoja & Martoja-Pierson, 1970), and Masson's trichrome (Kiernan, 2003) for general histology. *Aplastodiscus leucopygius* samples from male and female specimens were also dehydrated and embedded in glycol methacrylate (Leica) and sections (2 μm) were stained with toluidine blue-basic fuchsin stain (Junqueira, 1995). Histochemical techniques included Coomassie blue R250 for detection of proteins (Kiernan, 2003), and combined Alcian blue-periodic acid Schiff (AB-PAS; Mowry & Winkler, 1956) for acid mucopolysacharides and neutral carbohydrates; for AB-PAS, all samples were counterstained with Carazzi's haematoxylin. To detect lipids, samples were washed with tap water, placed in gum sucrose solution at 4 °C for 18 h, and mounted onto a block holder (Anderson & Bancroft, 2002). Ten-micrometre thick transverse sections were cut in a cryostat and stained with Sudan black B (Sudan; Pearse, 1985). The histochemical properties of glands were classified as strongly positive (++), positive (+), and negative (–). Equivocal (±) was used eventually because of the uncertainty in the staining affinity from some samples. Although the fixation histories of the specimens may generate some confounding effects on the intensity of the histochemical reaction, this classification method allows different gland types within the same specimen to be distinguished from one another, and the same gland type between different specimens to be compared.

TERMINOLOGY

Throughout the present study, the terms 'mental gland' and 'lateral gland' in adult males are associated with the occurrence of the SDSGs in mental and lateral skin regions, respectively. We consider this as the most suitable terms to refer to these structures, even though, in most cases, the SDSGs are dispersed, and do not conform with the clusters that characterize the so-called macroglands of amphibians (Toledo & Jared, 1995). Because of the sexually dimorphic nature of SDSGs, this terminology is not applicable to the mental gland described in females of some species of Cophomantini. As demonstrated in *Hypsiboas punctatus*, the mental gland of females is macroscopically evident as a result of its distinct coloration, whereas histologically it possesses only ordinary serous glands (OSGs) similar to those homogeneously distributed throughout the body (Brunetti *et al*., 2012).

CHARACTER DEFINITION AND OPTIMIZATION

For descriptions of the external morphology, we have followed Faivovich *et al*. (2006) and Brunetti *et al*. (2012). For descriptions at microscopic levels, we have followed Romero de Perez & Ruiz Carranza (1996) and Brunetti *et al*. (2012). Also, the comparative study of mental glands in hyperoliids (Le Quang Trong, 1976) and the studies of Sever (1976, 1989) in plethodontid salamanders have provided useful information to help interpret the variation observed in the present study. The informative variation was coded as binary or multistate characters (see Appendix, Table A1; see also below). They were optimized on the phylogenetic hypothesis proposed by Faivovich *et al*. (2013), to which we added six species (i.e. *Bokermannohyla pseudopseudis*, *B. saxicola*, *Hyloscirtus caucanus*, *H. antioquia*, *H. lynchi*, and *Hypsiboas pombali*). These species were added on the basis of unpublished phylogenetic results by Faivovich, Rivera-Correa and collaborators. Two species, *Hyloscirtus alytolylax* and *Hypsiboas punctatus*, which were histologically examined in previous studies (Romero de Perez & Ruiz Carranza, 1996; Brunetti *et al*., 2012), were included in the data matrix. In addition, published external morphological observations of mental and lateral glands of 12 species included in the phylogenetic analyses of Faivovich *et al*. (2013) were added to the data matrix (Romero de Perez & Ruiz Carranza, 1996; Faivovich *et al*., 2006, 2009, 2013; Faivovich & De la Riva, 2006; Brunetti *et al*., 2012). This information only considers those reports in which the glands are macroscopically evident, excluding those in which the glands were not observable by stereomicroscope because, as demonstrated in the present study, the absence of external evidence does not necessarily imply absence of SDSGs. Character optimizations were carried out in TNT (Willi Hennig Society Edition) (Goloboff, Farris & Nixon, 2008) considering most characters as unordered, with the exception of character 3.

RESULTS

Mental glands occur in males of 16 of the 27 species for which we had mental skin samples, whereas lateral glands are present in males of 10 of the 28 species for which we had lateral skin samples (see Supporting information, Appendix S1). Results of the histological study allow the characterizion of distinct mental and lateral gland morphologies. These vary with respect to presence/absence of different SDSGs (SSGs and SMGs), morphology and appearance of their secretions, level of aggregation and disposition of different glandular types, and for mental gland, thickness of the *stratum spongiosum*. All this variation has been coded as 15 homology hypotheses (characters), which are described below. The taxonomic distribution of the character states is presented in the data matrix in Appendix (Table A1).

MACROSCOPIC STRUCTURE

The occurrence of the mental and lateral glands is independent because they do not necessarily co-occur. On the basis of this observation, the macroscopic and microscopic structures of both glands are also treated as independent characters. Our comparative data indicate the existence of three morphologies of mental and lateral glands.

In five of the 16 species having mental glands, they are discernible only through histological analysis, and the appearance of the skin of males at that region varies in colour and shape, similar to those species lacking SDSGs (Fig. 1A, B, C, D). By contrast, they are macroscopically evident in the remaining 11 species (Fig. 1E, F, G, H). Lateral glands are discernible only through histological analysis in five of the 10 species in which they occur. As in mental glands, the skin in that region varies in colour and shape independently of the presence/absence of SDSGs (Fig. 2A, B, C, D). In the remaining five species, the gland is distinguished in most cases upon careful examination (with at least ×50 magnification) and by comparison with females (Fig. 2E, F, G, H).

Character 1: Mental gland: (0) Absent. (1) Present.

Character 2: Lateral gland: (0) Absent. (1) Present.

Character 3: Macroscopic morphology of mental gland: (0) Gland not evident macroscopically, only discernible in histological sections; in some cases, the SDSGs may be inferred through dissection with a stereomicroscope (Fig. 1C, D). (1) Gland macroscopically evident by a yellowish or brownish colour in fixed specimens. Individual glands are observed under magnification (Fig. 1E, F). (2) Gland macroscopically evident by a yellowish or brownish colour in fixed specimens; individual glands are observed under magnification. The gland protrudes from surrounding skin, which becomes evident by a ridge around it (Fig. 1G, H). This character is considered ordered.

Character 4: Macroscopic morphology of lateral gland: (0) Gland not evident macroscopically (Fig. 2C, D). (1) Gland macroscopically evident by a slightly distinct yellowish or cream–coloured skin; individual glands are observed under high magnification (Fig. 2E, G). (2) Gland macroscopically evident as individual pale yellow glands distinguished without magnification (Fig. 2H).

MICROSCOPIC STRUCTURE

General observations

The skin in males and females is identical in those species lacking a mental gland and having only

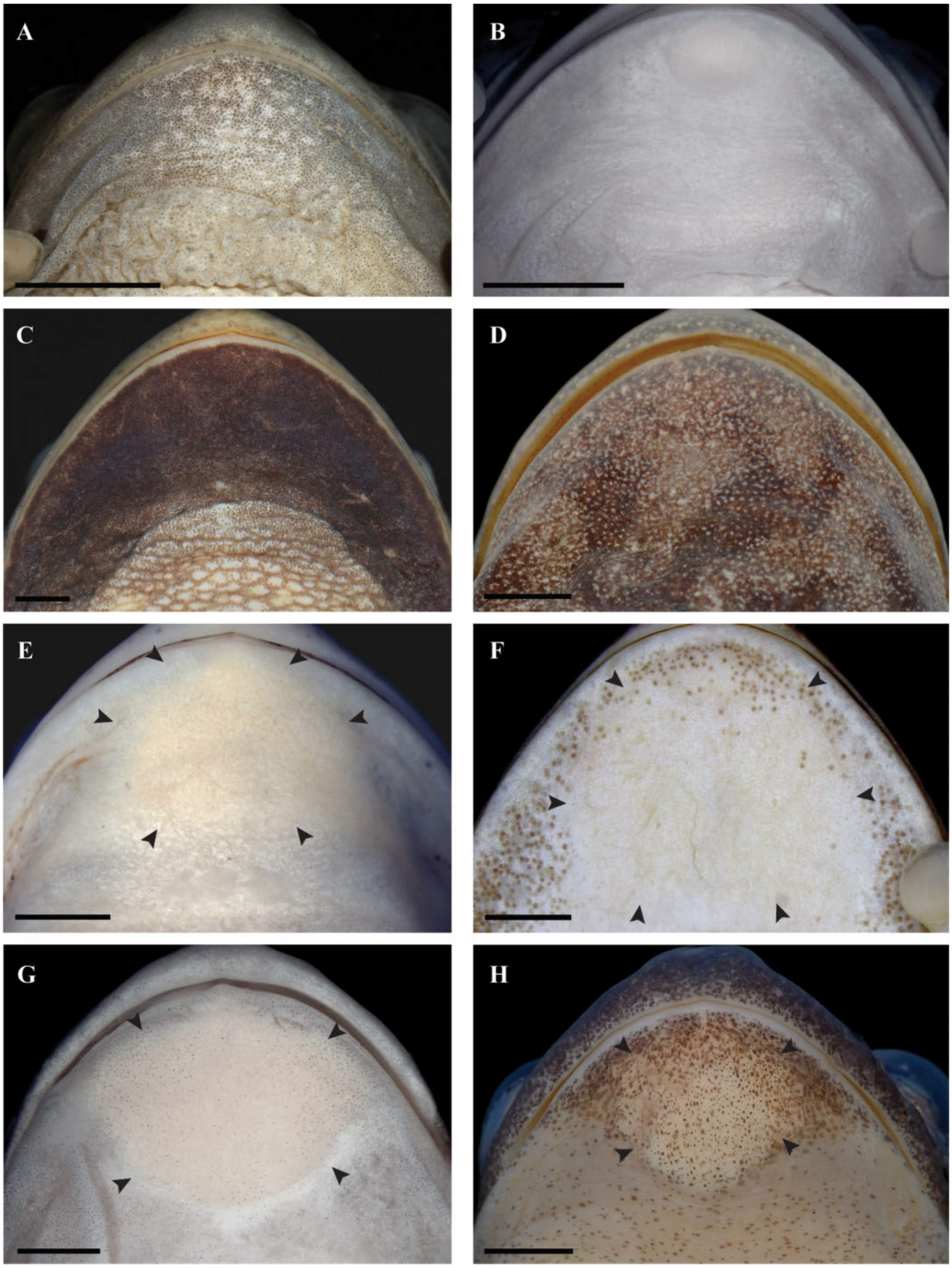

Figure 1. The mental region in preserved male specimens of some species of Cophomantini. A, *Hypsiboas pulchellus*. B, *Hypsiboas albomarginatus*. C, *Hypsiboas faber*. D, *Hyloscirtus caucanus*. E, *Aplastodiscus periviridis*. F, *Hypsiboas pombali*. G, *Hyloscirtus palmeri*. H, *Hyloscirtus colymba*. A, B, mental region in species lacking sexually dimorphic skin glands (SDSGs). C, D, mental region in species in which SDSGs are discernible only after histological analysis. Note that, in A–D, variation in the colour and structure of the skin is independent of the occurrence of SDSGs. E, F, the mental gland is distinguished by a yellowish or brownish colour, with individual glands observed under magnification. G, H, the gland protrudes from surrounding skin, which becomes evident by a ridge around it. Arrowheads indicate the limits of the glandular area, which is approximate in (C) and (D). Scale bars = 5 mm. (Colour version of figure available online.)

Figure 2. The lateral region in fixed specimens of some species of Cophomantini: A, *Hypsiboas pulchellus* (♂). B, *Hypsiboas raniceps* (♂). C, *Bokermannohyla pseudopseudis* (♂). D, *Hypsiboas albopunctatus* (♂). E, *Aplastodiscus perviridis* (♂). F, *Aplastodiscus perviridis* (♀). G, *Hypsiboas heilprini* (♂). H, *Hypsiboas punctatus* (♂; Brunetti *et al*., 2012). A, B, lateral regions in species lacking sexually dimorphic skin glands (SDSGs). C, D, lateral regions in species in which SDSGs are discernible only after histological analysis. Note that, in A–D, variation in the colour and structure of the skin is independent of the occurrence of SDSGs. E, F, the lateral gland in most cases is discernible only upon careful comparison between males (E) and females (F). E, G, H, in those cases in which it is macroscopically evident, the gland presents two different morphologies: slightly distinct skin yellowish or cream-coloured, with individual glands visible under high magnification (E, G), or pale yellow individual glands clearly distinguished without magnification (H). Note that the gland limits are often difficult to define macroscopically; broken lines indicate the putative limits of the glandular area. Scale bars = 5 mm. (Colour version of figure available online.)

ordinary glands [ordinary mucous glands (OMGs) and OSGs] in the *stratum spongiosum* of the dermis (Fig. 3A, B). There is extensive variation in diverse skin traits in different species (e.g. vascularization, pigmentation, thickness of the epidermis, characteristics of OMGs and OSGs). However, our descriptions are focused only on the arrangement and structure of SDSGs.

Mental gland structure

In those species having a mental gland, the skin of males is distinguished from that of females by the occurrence of two types of SDSGs within the *stratum spongiosum*: SMGs and SSGs (Fig. 3C, D, E, F, G, H, I, J, K). Both gland types occur independently and, in some species, they co-occur. Three characters are defined considering the arrangement of SDSGs, as well as the structure of the *stratum spongiosum*.

Character 5: Level of aggregation of SDSGs in mental gland: (0) Scattered (Fig. 3M, O). (1) Closely packed (Fig. 3C, E, G, H, J, L, N).

Character 6: SDSGs disposition in mental gland: (0) Secretory portion of all glands in a single layer (Fig. 3C, E, G, H, L, M, O). (1) Secretory portion of different glands disposed in two layers. The bottom of SMGs contacts the *stratum compactum*, and the bottom of SSGs is located close to the apex of the secretory portion of SMGs (Fig. 3J, N).

Character 7: Structure of the *stratum spongiosum* in the mental gland region: (0) *stratum spongiosum* same thickness as in the surrounding skin (Fig. 3L). (1) *stratum spongiosum* remarkably thicker than in the surrounding skin (Fig. 3G). The increase in the thickness of the *stratum spongiosum* described in state (1), occurs in conjunction with a reduction of the *stratum compactum*.

Structure of SDSGs in mental gland

Like ordinary glands (OMGs and OSGs), each SMG and SSG possesses a basic structure: an intraepidermal duct, a neck and a secretory portion surrounded by a contractile sheath of myoepithelial cells. The secretory portion of SMGs consists of radially arranged mucocytes, each of which bears a round and basal nucleus. In all species, they form a columnar epithelium but, although it is proportionally high in some species, it has medium height in others. These different heights are related to a narrow or wide lumen, respectively (for comparison, see Fig. 3C, E). The lumen of most SMGs is often (but not always) devoid of secretion. The presence of this secretion varies between different glands and does not depend on the species. Specialized mucous glands vary in their size and morphology among species; larger SMGs occur together with a thicker *stratum spongiosum* of the dermis and is related, in most cases, to a tubuloalveolar gland, whereas smaller SMGs are alveolar glands related to a thinner *stratum spongiosum* (for comparison, see Fig. 3G, L). Histochemically, the secretion of SMGs from all species stains positive for neutral mucosubstances and proteins, whereas, in a few species, they also stain positive for acidic mucosubstances (Table 1).

In all species in which they occur, SSGs share with OSGs a syncytial secretory portion, a similar size, and an oval shape in section, with their major axes lying perpendicular to the epidermis. However, these glands may be differentiated by the colloid-like appearance of the secretion of SSGs in most species, or in those species in which the secretion of both glands is granular, because SSGs have smaller granules. Further evidence to differentiate OSGs and SSGs is derived from their histochemical properties (i.e. distinct staining affinities of both glands within each species), including the remarkably strong positive reaction of OSG secretions with Sudan black B (Fig. 3O, Table 1).

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Figure 3. Light micrographs of cross sections of the mental skin region of some species of Cophomantini: A, *Hypsiboas pulchellus* (♂). B, *Hypsiboas pulchellus* (♀). C, *Hypsiboas benitezi* (♂). D, *Hypsiboas benitezi* (♀). E, *Aplastodiscus leucopygius* (♂). F, *Aplastodiscus leucopygius* (♀). G, H, *Hyloscirtus palmeri* (♂). I, *Hyloscirtus palmeri* (♀). J, *Hypsiboas faber* (♂). K, *Hypsiboas faber* (♀). L, *Aplastodiscus eugenioi* (♂). M, *Hyloscirtus caucanus* (♂). N, *Hypsiboas heilprini* (♂). O, *Bokermannohyla saxicola* (♂). A, B, C, D, E, F, G, H, I, J, K, comparative sections between males and females. A, B, There are no distinguishable differences between males and females; only ordinary mucous glands (OMGs) and ordinary serous gland (OSGs) can be recognized in both sexes. C, E, L, sexually dimorphic skin glands (SDSGs) are closely packed with their secretory portion in a single layer, and specialized mucous glands (SMGs) are of the alveolar type. Specialized serous glands (SSGs) are present in (E) and (L) but are absent in (C). Note in (L) the lack of differences in the thickness of *stratum spongiosum* (*ss*) in the mental gland region in comparison with surrounding region. Also, some SMGs are scattered out of the region of higher glandular density. G, H, the mental gland has a notorious thickening of the *ss* and a reduction of *stratum compactum*. SMGs of tubuloalveolar morphology are closely packed with their secretory portion in a single layer. Note the absence of SSGs and OSGs. J, N, mental glands are characterized by the disposition of the secretory portion of different glands in two layers, SMGs of the tubuloalveolar morphology, and the occurrence of SSGs. Note the differences in thickness of the *ss* in (N). M, O, both glands are characterized by the presence of scattered SDSG but, although they are of the mucous type in (M), they are of the serous type in (O). Histological staining: A, B, K, Masson–Goldner's trichrome; G, I, J, N, Masson's thricrome; C, D, H, L, M, Alcian blue-periodic acid Schiff; E, F, semithin sections after toluidine blue-basic fuchsin stain; (O) Sudan black B. sc, *stratum compactum*; ss, *stratum xspongiosum*. Note limits of sc and ss within arrows. Scale bars = 100 μm.

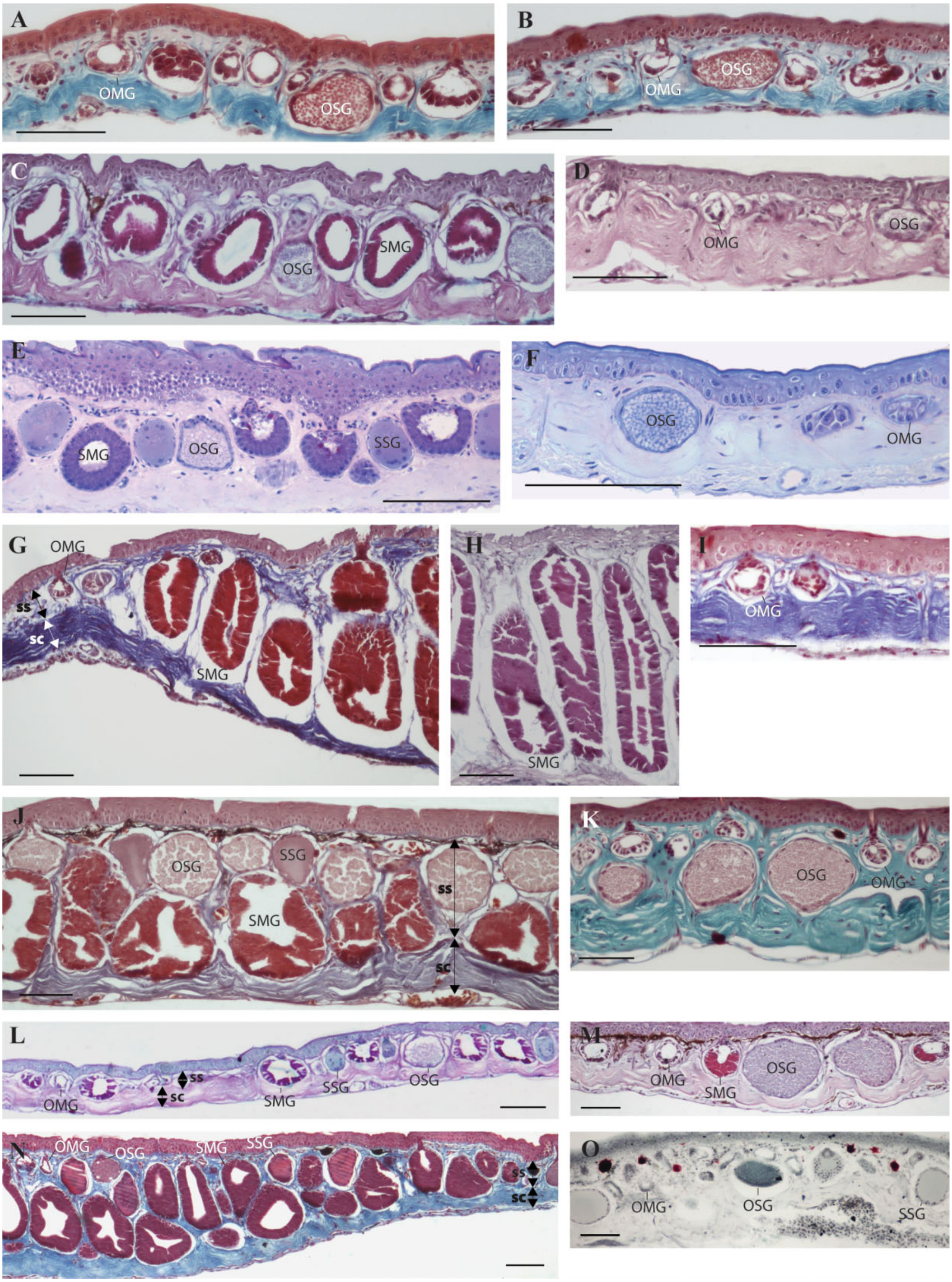
A
OMG
OSG
B
OMG
OSG
C
OSG
SMG
D
OMG
OSG
E
SMG
OSG
SSG
F
OSG
OMG
G
OMG
ss
sc
SMG
H
SMG
I
OMG
J
OSG
SSG
SMG
ss
sc
K
OSG
OMG
L
ss
sc
OMG
SMG
SSG
OSG
M
OMG
SMG
OSG
N
OMG
OSG
SMG
SSG
ss
sc
O
OMG
OSG
SSG

Table 1. Histochemical comparisons of the four gland types in different species of Cophomantini*

Species	Body region†	Gland type‡	Histochemical tests§															
			AB				PAS				CooB				Sudan			
			OMG	OSG	SMG	SSG	OMG	OSG	SMG	SSG	OMG	OSG	SMG	SSG	OMG	OSG	SMG	SSG
Aplastodiscus																		
A. eugenioi	M		–	–	+	+	+	–	++	–	–	+	++	–	–	++	–	–
	L		–	–	++	–	+	–	++	–	–	+	++	–	–	++	–	–
A. leucopygius	M		–	–	+	–	+	–	++	–	0	–	–	+	0	+	+	–
	L		–	–	+	–	+	–	++	–	0	–	–	+	0	+	+	–
A. perviridis	M		0	+	+	+	0	–	++	–	0	–	+	+	0	++	–	–
	L		0	+	+	–	0	–	++	–	0	+	++	–	0	++	–	–
Bokermannohyla																		
B. hylax	M		–	+	–	0	+	–	++	0	+	+	+	0				0
	L		+	+	–	–	+	–	++	–	+	–	++	+/–	–	++	–	–
B. pseudopseudis	M										+	+	++	++				
	L		+	+	–	–	+	–	+	–	–	+	++	++	–	++	–	–
B. saxicola	M		+	+	0	–	+	–	0	+	+	+/–	0	++	–	++	0	–
Hyloscirtus																		
H. antioquia	M		–	+	0	0	+	–	0	0	–	+	0	0			0	0
H. caucanus	M		+	+	–	0	+	–	++	0	+	–	+	0				0
H. colymba	M		–	–	+	0	+	–	++	0	+	–	++	0				0
H. larinopygion	M		++	–	0	0	++	–	0	0	+	++	0	0	–	++	0	0
H. lynchi	M		–	–	–	0	+	–	++	0	+	+	++	0	–	+	–	0
H. palmeri	M		+	–	+	0	+	–	++	0	+	–	++	0	–	+	–	0
Hypsiboas																		
H. albomarginatus	M		–	–	0	0	+	–	0	0	+	++	0	0	–	++	0	0
H. albopunctatus	L		–	–	–	–	+	–	++	–	+	+	++	+	–	++	+	–
H. benitezi	M		+	–	+	0	+	–	++	0	+/–	+	++	0	–	++	–	0
H. curupi	M		–	–	0	0	+	–	0	0	+	+	0	0	+	+	0	0
H. ericae	M		–	–	0	0	+	–	0	0	+	–	0	0			0	0
H. faber	M		+	+	+	+	+	–	++	–	+	–	++	+				
	L		+	–	+	–	+	–	++	–	+	+	++	++	–	++	–	+
H. heilprini	M		+	–	–	–	+	–	++	–	+	+	++	++				
	L		0	–	–	0	0	–	++	0	0	+	++	0				0
H. marianitae	M		+	+	0	0	+	–	0	0	+	+	0	0	–	++	0	0
H. microderma	M		+	–	+	–	+	–	++	–	–	++	+	++				
H. pellucens	M		–	+	0	0	+	–	0	0	+	–	0	0			0	0
H. picturatus	M		–	+	0	0	+	–	0	0	+	+	0	0			0	0
H. pombali	M		+	+/–	–	–	+	–	++	–	+	++			–	++	–	–
	L		++	–	–	–	–	–	++	–	+	++	++	+	–	++	–	–
H. pulchellus	M		+	+	0	0	+	–	0	0	+	+	0	0	–	++	0	0
H. raniceps	M		+	–	0	0	+	–	0	0	+	++	0	0	–	++	0	0
H. semilineatus	M		+	+	+	0	+	–	+	0								
	L		++	+	+	–	+	–	+	+	+	++	++	–	–	+	+	–
Myersiohyla																		
M. neblinaria	L		–	–	0	0	+	–	0	0	+	+	0	0			0	0

*Histochemical results are expressed as: ++, strongly positive; +, weakly positive; –, negative; +/–, equivocal. 0, gland not present; blank, data not available.

†L, lateral; M, mental. Samples from M and/or L skin regions are included considering the occurrence of sexually dimorphic skin glands (SDSGs). In those species lacking SDSGs, only M skin regions are included, except for *M. neblinaria* (L region included).

‡OMG, ordinary mucous gland; OSG, ordinary serous gland; SMG, specialized mucous gland; SSG, specialized serous gland.

§AB, Alcian Blue pH 2.5; CooB, Coomassie blue R250; PAS, periodic acid Schiff; SB, Sudan black B.

Character 8: Specialized mucous glands (SMGs) in mental gland: (0) Absent. (1) Present.

Character 9: Morphology of SMGs in mental gland: (0) Alveolar gland (Fig. 3C, E, L, M). (1) Tubuloalveolar gland (Fig. 3G, H, J, N).

Character 10: Specialized serous glands (SSGs) in mental gland: (0) Absent. (1) Present.

Character 11: Characteristics of the content of SSGs in mental gland: (0) Granular content (Brunetti *et al.*, 2012: fig. 2B). (1) Colloid-like content (Fig. 2J).

Lateral gland structure: Males from species lacking lateral glands have only ordinary glands within the *stratum spongiosum* (Fig. 4A, B). The lateral glands from all species examined have SMGs, whereas SSGs co-occur in most of the species, except *Hypsiboas heilprini*. Two characters are defined when considering the arrangement of these glands.

Character 12: Level of aggregation of SDSGs in lateral gland: (0) Scattered (Fig. 4C, D). (1) Closely packed (Fig. 4E, F, G, H, I, J, K, L).

Character 13: SDSGs disposition in lateral gland: (0) Secretory portion of all glands in a single layer (Fig. 4C, D, E, F). (1) Secretory portion of different glands disposed in two layers. The bottom of the SMGs contacts the *stratum compactum*, and the bottom of the SSGs is located close to the apex of the secretory portion of SMGs (Fig. 4G, H, I, J, K, L).

Structure of SDSGs in lateral gland: The descriptions of SDSGs from the mental gland are also applicable to those of the lateral gland, with the exception of the appearance of secretion of SSGs. In the lateral gland, the content of SSGs is granular in all species, which makes it similar to OSGs. However, as is the case in the mental gland, OSGs and SSGs are unambiguously distinguished by the strong positive reaction of OSGs secretions to Sudan black B (performed in all species having both, SSGs and OSGs), by a negative reaction in all SSGs (with the single exception of *Hypsiboas faber*), and finally by the distinct staining affinities of both glands within each species (Fig. 4I, J, K, L; Table 1). Two characters are defined considering the occurrence of SSGs and the morphology of SMGs.

Character 14: Morphology of SMGs in lateral gland: (0) Alveolar gland (Fig. 4C, D, E, F, I, J, K, L). (1) Tubuloalveolar gland (Fig. 4G).

Character 15: SSGs in lateral gland: (0) Absent (Fig. 4E). (1) Present (Fig. 4C, D, F, G, H, I, J, K, L).

CHARACTER OPTIMIZATION

The optimization of the characters on the phylogenetic hypothesis modified from Faivovich *et al.* (2013) indicates multiple instances of homoplasy, with all non-autapomorphic transformations having between one and six extra steps (Figs 5, 6; for the minimum number of steps implied by the topology for each character, see Appendix, Table A1). Further comments are provided in the Discussion.

DISCUSSION

OCCURRENCE AND MORPHOLOGY OF MENTAL AND LATERAL GLANDS

Mental glands in Cophomantini are already known in most species of *Myersiohyla* (Faivovich *et al.*, 2013), the *Hyloscirtus bogotensis* group (Duellman, 1972; Ruiz-Carranza & Ardila-Robayo, 1991), the *H. armatus* group (Faivovich & De la Riva, 2006), at least 16 species of *Bokermannohyla* (Faivovich *et al.*, 2009), the *Hypsiboas benitezi* group (Faivovich *et al.*, 2006), some species of the *H. punctatus* group (Hoogmoed, 1979; Brunetti *et al.*, 2012), and *H. heilprini* (Trueb & Tyler, 1974). The present study is the first to report the occurrence of a mental gland in males of *Aplastodiscus eugenioi*, *A. leucopygius*, *A. perviridis*, *Bokermannohyla hylax*, *Hyloscirtus caucanus*, *Hypsiboas faber*, *H. pombali*, and *H. semilineatus*.

The first report of the occurrence of lateral glands in a Cophomantini, *Hypsiboas punctatus*, is recent (Brunetti *et al.*, 2012). The present study reports, for the first time, lateral glands in males of *A. eugenioi*, *A. leucopygius*, *A. perviridis*, *B. hylax*, *B. pseudopseudis*, *H. albopunctatus*, *H. faber*, *H. heilprini*, *H. pombali*, and *H. semilineatus*.

The occurrence of mental and lateral glands is independent in Cophomantini as indicated by the presence of mental glands in several species of *Hyloscirtus*, in *B. saxicola*, and in the *Hypsiboas benitezi* group, and the presence of lateral glands in *H. albopunctatus*. Both glands occur simultaneously in *A. eugenioi*, *A. leucopygius*, *A. perviridis B. hylax*, *B. pseudopseudis*, *H. faber*, *H. heilprini*, *H. pombali*, *H. punctatus*, and *H. semilineatus* (Brunetti *et al.*, 2012; present study). A similar topographic pattern with mental and ventrolateral glands is observed in *Duellmanohyla chamulae* and *D. ignicolor* from Hylini (Duellman, 1961; Campbell & Smith, 1992). The presence of a ventrolateral gland in *D. schmidtorum* (Campbell & Smith, 1992) and several species of *Ptychohyla* (Campbell & Duellman, 2000) suggests that the occurrence of both glands may also be independent in Hylini.

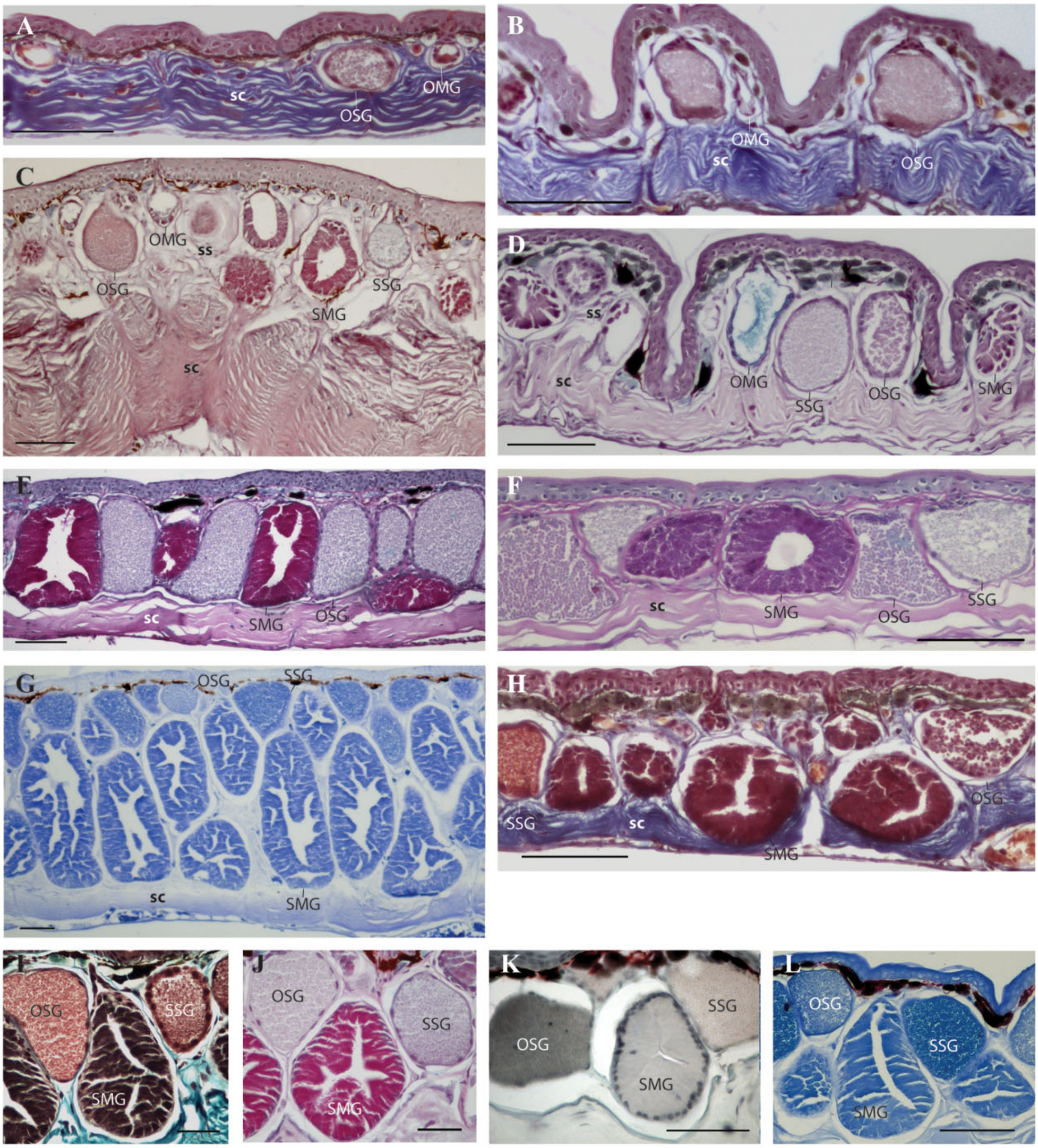

Figure 4. Light micrographs of cross-section of lateral skin regions of males of some species of Cophomantini: A, *Hypsiboas pulchellus*. B, *Hypsiboas albomarginatus*. C, *Bokermannohyla pseudopseudis*. D, *Hypsiboas semilineatus*. E, *Hypsiboas heilprini*. F, *Aplastodiscus perviridis*. G, *Hypsiboas faber*. H, *Hypsiboas albopunctatus*. I, J, K, L, *Hypsiboas pombali*. A, B, lateral glands are absent. Note the occurrence of only ordinary glands within the *stratum spongiosum*. C, D, lateral glands are characterized by the occurrence of both dimorphic skin glands, specialized mucous glands (SMGs) and specialized serous glands (SSGs), scattered in the integument. Notice differences in the size and staining properties of ordinary mucous glands (OMGs). E, F, glands are highly packed and their secretory portion disposed in a single layer. E, SMGs of the tubuloalveolar morphology. F, SMGs of the alveolar type and also SSGs. G, H, the secretory portions of glands are disposed in two layers. Notice the difference in size of the SMGs. I–L, differences in structural and histochemical properties of SMGs, ordinary serous gland (OSGs) and SSGs. Histological staining: A, B, H, Masson's trichrome; I, Masson–Goldner's trichrome; C, D, E, F, J, Alcian blue-periodic acid Schiff; G, L, Coomassie Blue R250; K, Sudan black B. sc, *stratum compactum*; SMG; ss, *stratum spongiosum*; SSG, specialized serous gland. Scale bars = 100 μm.

The optimization exercise on the topology modified from Faivovich *et al*. (2013) allows us to minimally predict that mental glands are present in all species of *Aplastodiscus* and in the *Hyloscirtus bogotensis*, *Hypsiboas benitezi* and *H. semilineatus* groups, whereas they are absent in several species of the *Hyloscirtus larinopygion* group (single known exception of *H. caucanus*; but likely present also in *H. lindae*, *H. tapichalaca*, and *H. pantostictus*), most species of the *Hypsiboas albopunctatus* group (single exception of *H. heilprini*), and all species of the *H. pellucens* and *H. pulchellus* groups. Lateral glands are most parsimoniously predicted to be present in all species of *Aplastodiscus*, as well as in the *H. albopunctatus*, *H. faber*, and *H. semilineatus* groups, and absent in all species of *Myersiohyla*, *Hyloscirtus*, and the *Hypsiboas benitezi*, *H. pellucens*, and *H. pulchellus* groups. The situation in *Bokermannohyla* deserves further study because it will be necessary to determine whether the species reported by Faivovich *et al*. (2009) that appear to lack a macroscopically visible mental gland actually have one that is visibly only microscopically, as we observed in *B. hylax*.

A few points that deserve further attention are whether mental and lateral glands co-occur with nuptial pads, other body regions where SDSGs are usually concentrated, and whether morphological transformations in the first two are related to the absence of the latter. Nuptial pads are absent in most species of *Aplastodiscus* (single exception of *A. musicus*; Lutz, 1949) and *Hypsiboas* (the exception are all species included in the *H. semilineatus* group; Faivovich *et al*., 2006; M. C. Luna & J. Faivovich, unpubl. data). They occur in all species of *Myersiohyla* (Faivovich *et al*., 2013; J. Faivovich pers. observ.), the *Hyloscirtus armatus* group (Duellman, De la Riva & Wild, 1997), and some species of the *H. larinopygion* group (Rivera-Correa & Faivovich, 2013). They are present as well in several species of *Bokermannohyla* (Leite, Pezzuti & Drummond, 2011); however, their taxonomic distribution in this genus and in the *H. bogotensis* group requires further study. Our results and the incomplete information on the occurrence of nuptial pads in Cophomantini indicate that lateral and mental glands occur both in species with (e.g. *Hypsiboas semilineatus*, *B. hylax*) and without nuptial pads (e.g. *A. leucopygius*, *Hyloscirtus palmeri*). More importantly, increased knowledge of the histological structure of the nuptial pads in Cophomantini (so far unknown) and their taxonomic distribution could prove relevant to the discussion on the origin and evolution of lateral and mental glands because SDSGs in nuptial pads have been shown to be involved in chemical communication as well (Willaert *et al*., 2013).

STRUCTURE AND HISTOCHEMICAL PROPERTIES OF SDSGs

As demonstrated by Thomas *et al*. (1993), SDSGs in anurans include two types of glands, SMGs and SSGs, which, independently of species and anatomical location, share fundamental structural and histochemical properties. Our results have also indicated the similarities within each gland type, whereas the co-occurrence of SMGs and SSGs represents a particular case within amphibians, so far known exclusively in Cophomantini (Brunetti *et al*., 2012; present study). Similar to SMGs of other anurans (Thomas *et al*., 1993; Brizzi *et al*., 2003), SMGs of Cophomantini are multicellular glands having neutral mucosubstances and a proteinaceous content (Brunetti *et al*., 2012; present study). Our comparative study additionally allows the distinction of SMGs by their shape as alveolar or tubuloalveolar glands. Although this distinction has been noted in previous studies (Thomas *et al*., 1993), we have shown that the morphology of SMGs is an informative character that may be used to provide a better description of the gland structure as a whole.

The detailed histological characterization of SSGs and OSGs in *H. punctatus* (Brunetti *et al*., 2012) allowed the identification of these glands in the species investigated in the present study using light microscopy by the appearance of their secretion and their histochemical properties. Specialized serous glands have been historically considered a rarity among SDSGs (Thomas *et al*., 1993). However, other studies in different taxonomic groups (Vences *et al*., 2007; Gonçalves & Brito-Gitirana, 2008; Brunetti *et al*., 2012, present study) demonstrate that they are more frequent than initially suspected. The reasons why the secretion of SSG appeared colloid-like in some cases and granular in others requires the investigation of their secretory cycle. A possible reason is the local fluctuation that generally causes asynchronous secretory phases within the same gland type (Toledo, Jared & Brunner, 1992). However, it is worth noting that the secretion appearance differs in the lateral and mental regions from the same specimen, and also that this phenomenon occurs in all analyzed species.

EVOLUTION OF MENTAL AND LATERAL GLANDS IN COPHOMANTINI

The taxonomic distribution and the macroscopic variation observed in Cophomantini in previous contributions (Faivovich *et al*., 2006, 2013) suggested a complex scenario with respect to the evolution of SDSGs; thus, comparative histological studies were needed to better understand these observations.

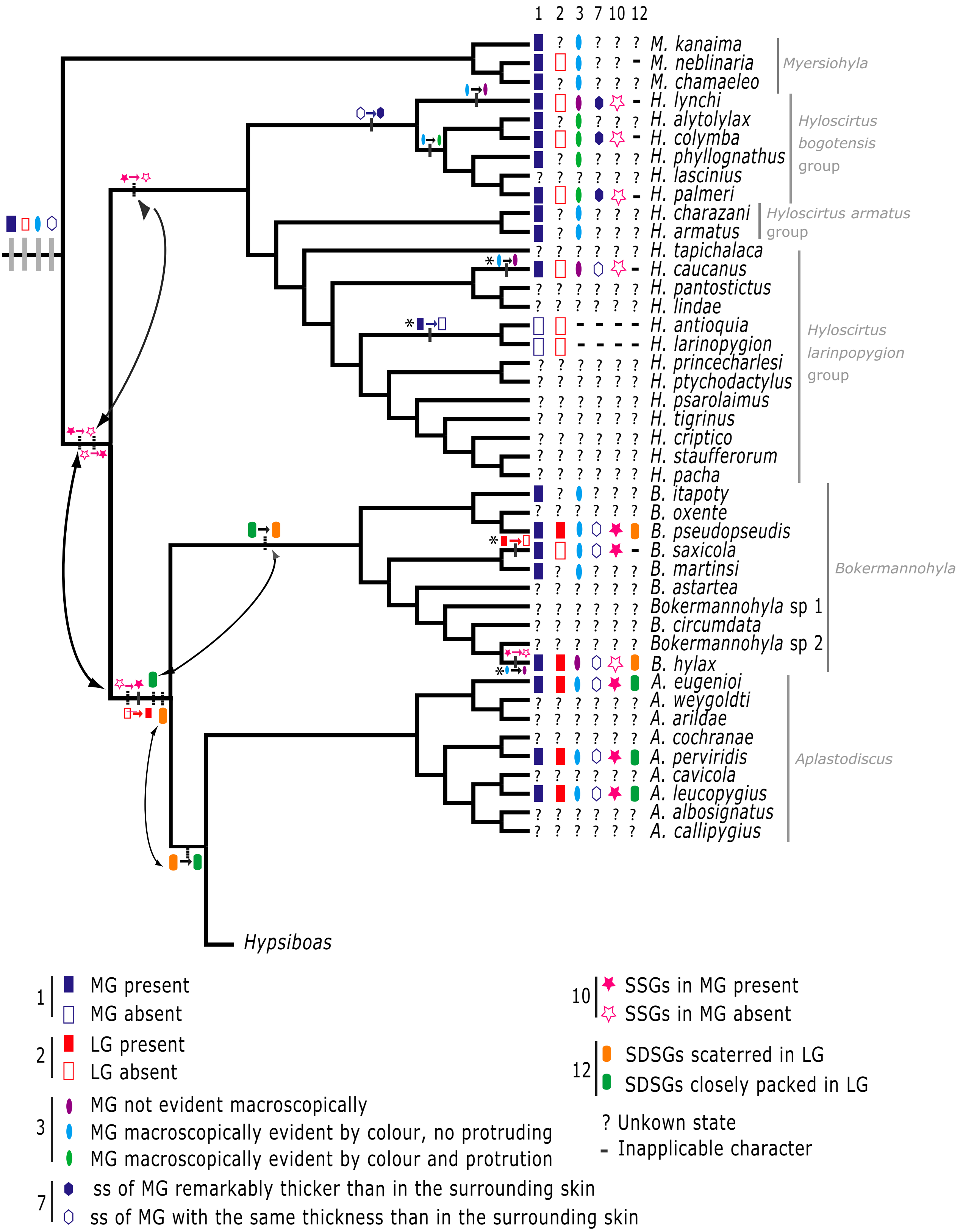
1 2 3 7 10 12
M. kanaima
M. neblinaria
M. chamaeleo
Myersiohyla
H. lynchi
H. alytolylax
H. colymba
H. phyllognathus
H. lascinius
H. palmeri
Hyloscirtus bogotensis group
H. charazani
H. armatus
Hyloscirtus armatus group
H. tapichalaca
H. caucanus
H. pantostictus
H. lindae
H. antioquia
H. larinopygion
H. princecharlesi
H. ptychodactylus
H. psarolaimus
H. tigrinus
H. criptico
H. staufferorum
H. pacha
Hyloscirtus larinpopygion group
B. itapoty
B. oxente
B. pseudopseudis
B. saxicola
B. martinsi
B. astartea
Bokermannohyla sp 1
B. circumdata
Bokermannohyla sp 2
B. hylax
Bokermannohyla
A. eugenioi
A. weygoldti
A. arildae
A. cochranae
A. perviridis
A. cavicola
A. leucopygius
A. albosignatus
A. callipygius
Aplastodiscus
Hypsiboas
1 MG present
MG absent
2 LG present
LG absent
3 MG not evident macroscopically
MG macroscopically evident by colour, no protruding
MG macroscopically evident by colour and protrution
7 ss of MG remarkably thicker than in the surrounding skin
ss of MG with the same thickness than in the surrounding skin
10 SSGs in MG present
SSGs in MG absent
12 SDSGs scaterred in LG
SDSGs closely packed in LG
? Unkown state
- Inapplicable character

Figure 5. Taxonomic distribution and optimization of six selected characters in the phylogenetic hypothesis of Cophomantini modified from Faivovich *et al*. (2013) for *Myersiohyla*, *Hyloscirtus*, *Bokermannohyla*, and *Aplastodiscus*. For *Hypsiboas*, see Fig. 6. Note that we have excluded the outgroups because SDSGs remain poorly known outside Cophomantini. Numbers refer to characters described in more detail in the text. For the data matrix, see Appendix (Table A1). The multistate character is considered ordered. Transformations that may be synapomorphies of Cophomantini or a more inclusive clade are shown with grey bars. The coloured lines indicate alternative optimizations. Asterisks (*) indicate transformations that occur in that node or in a more inclusive clade. Further studies on taxonomic distribution will help to clarify its position. Further discussion is provided in the text. LG, lateral gland; MG, mental gland; ss, *stratum spongiosum*; SSGs, specialized serous glands.

We have optimized and examined the evolution of 15 characters on the phylogenetic hypothesis of Faivovich *et al*. (2013) modified by us (Figs 5, 6). Of those, we have chosen six to show and discuss: two characters related to presence/absence of mental and lateral glands; one character associated to the macroscopic morphology of mental glands; and three characters derived from microscopic structure from both glands. Optimization of the remaining characters (excluding those that are autopomorphic) is shown in the Supporting information (Figs S1, S2).

The presence/absence of mental and lateral glands has an intricate taxonomic distribution, with different levels of homoplasy (Figs 5, 6). The intricacy is magnified by the fact that we employed only a subset of 42 out of 171 species of the tribe (28 species included in the present study, and 14 from previous studies; two of them with histological information, and 12 based only on macroscopic observations). Nonetheless, some patterns can be distinguished. The optimization of the presence/absence of mental glands, results in several alternative optimizations requiring at least five steps (Figs 5, 6). The optimization implies that the presence of the mental gland may be a synapomorphy of Cophomantini or a more inclusive clade. It presents at least three or even four reversions because there is an ambiguous optimization in the common ancestor of the *Hypsiboas pellucens* + *H. faber* + *H. pulchellus* groups, and an independent origin within a clade of the *H. faber* group that excludes *H. albomarginatus*.

The optimization of the presence/absence of lateral glands suggest the presence of these glands as a putative synapomorphy of the clade *Bokermannohyla* + *Aplastodiscus* + *Hypsiboas* with five instances of reversions in *B. saxicola*, *H. raniceps*, *H. picturatus*, the *H. benitezi* group, and in the *H. pellucens* + *H. faber* + *H. pulchellus* groups, and an independent origin in the *H. faber* group. It is remarkable that both mental and lateral glands within Cophomantini have an independent origin in the same node within the *H. faber* group.

The plesiomorphic state in Cophomantini is the macroscopically visible mental gland (Character 3, state 1). The transformation of this state to the protruded gland is a synapomorphy of most species of the *Hyloscirtus bogotensis* group, with the exception of *H. lynchi*.

The optimization of character 7 implies that a thickening of the *stratum spongiosum* in the mental gland has at least four independent origins: in the *Hyloscirtus bogotensis* group, in *Hypsiboas heilprini*, *H. faber*, and *H. punctatus* (and possibly other species in the *H. faber* and *H. punctatus* groups). This character is associated independently to the three mental gland morphologies described in character 3.

The taxonomic distribution of the occurrence of SSGs in mental glands (Character 10) does not allow determining the plesiomorphic state because it remains unknown in *Myersiohyla*. It is worth noting that SSGs are absent in *Hyloscirtus*, and are present at least in the common ancestor of *Bokermannohyla* + *Aplastodiscus* + *Hypsiboas*. In that clade, they are subsequently lost at least two times, in *B. hylax* and in *H. benitezi*. Further studies will determine whether this loss involved other closely-related species.

The taxonomic distribution of the level of aggregation of SDSGs in the lateral glands (Character 12) does not allow determination of the plesiomorphic state. Although the glands are scattered in *Bokermannohyla*, they are concentrated in the common ancestor of *Aplastodiscus* + *Hypsiboas*. However, in this clade, there is a reversion to the scattered aggregation in *H. semilineatus*.

Function of SDSGs

The variability in the occurrence of mental and lateral glands, and the extent of homoplasy of these characters in Cophomantini is striking, as is the presence of signals derived from different sensory modalities (i.e. visual, tactile, chemical, and acoustic) in several species (Table 2). Given the occurrence of SDSGs exclusively in males, and their putative role as source of sexual pheromones (Willaert *et al*., 2013), the main question underlying these results is the nature of the relationship between the pattern of

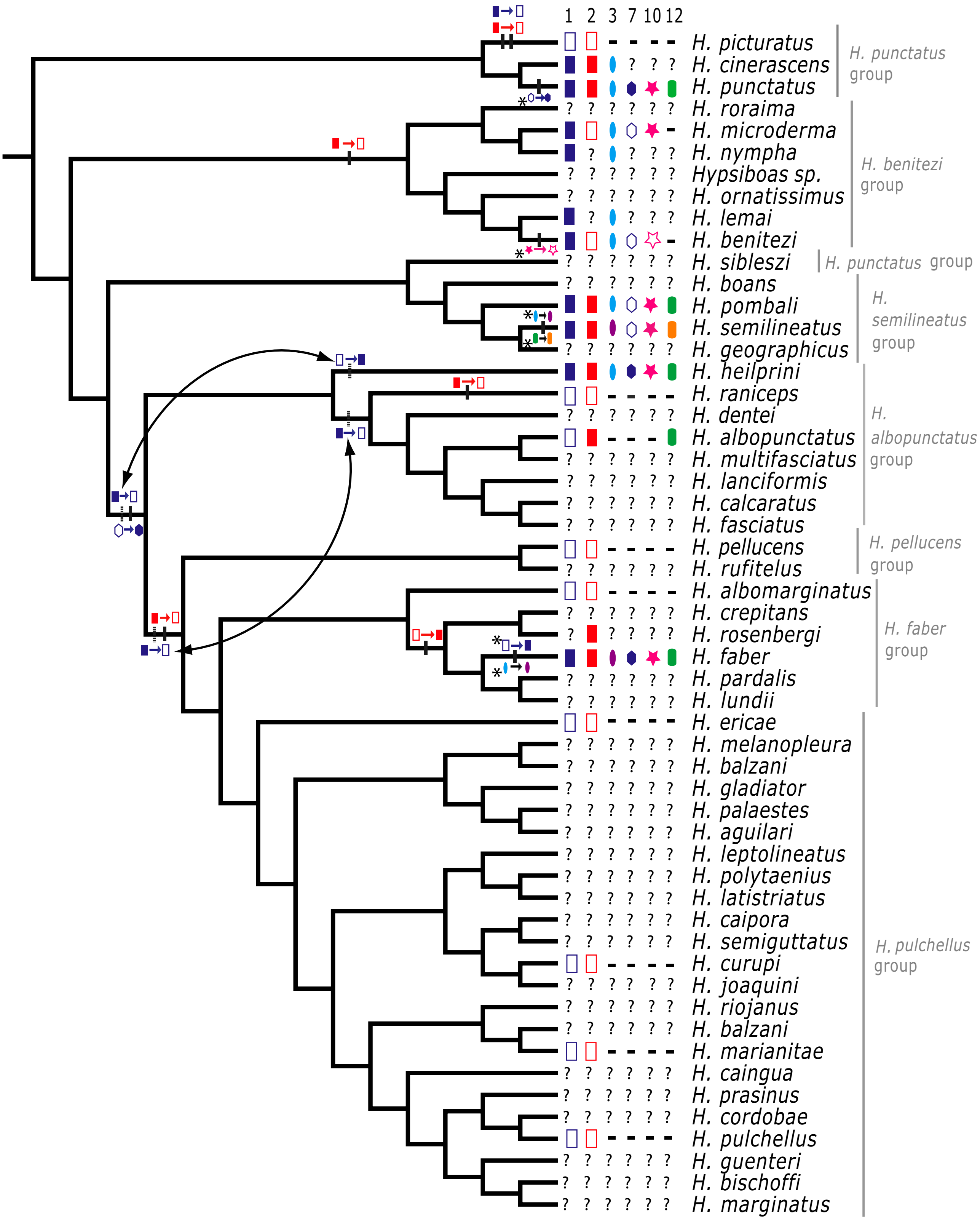

Figure 6. Taxonomic distribution and optimization of six selected characters in the phylogenetic hypothesis of Cophomantini modified from Faivovich *et al*. (2013) for *Hypsiboas*. For symbols and comments, see Fig. 5.

Table 2. Occurrence of mental and lateral glands, as well as behavioural data from the literature, in relation to courtship and amplexus in 32 species of Cophomantini[a]

	SDSGs				Sensory modality					
	Gland occurrence		Contact with glandular region[c]		Acoustic					
Species[b]	M	L	M	L	AC	CC	Visual	Tactile[d]	Amplexus[e]	References
Aplastodiscus										
A. eugenioi	+	+	+	?	+	+	+	+	?	1, 2
A. leucopygius	+	+	+	?	+	+	+	+	?	2, 3, 4
A. perviridis	+	+	+	?	+	+	?	+	?	2, 5
Bokermannohyla										
B. hylax	+	+	?	?	+	?	?	?	?	2, 6
B. ibitiguara	+	?	±[f]	?	+	?	?	+	?	7, 8, 9
B. nanuzae	+	?	±[g]	?	+	+	+	+	?	7, 10
B. pseudopseudis	+	+	?	?	+	?	?	?	?	2, 11
B. saxicola	+	–	?	–	+	?	?	?	?	2, 11
Hyloscirtus										
H. alytolylax	+	?	?	?	?	?	?	?	?	12
H. antioquia	–	–	–	–	+	+	?	?	–	2, 13, 14
H. caucanus	+	–	?	–	?	?	?	?	?	2
H. colymba	+	–	?	–	+	?	?	?	?	2, 15, 16
H. larinopygion	–	–	?	–	+	?	?	?	–	2, 13, 17
H. lynchi	+	–	?	–	+	?	?	?	?	2, 18
H. palmeri	+	–	?	–	+	?	?	?	?	2, 16
Hypsiboas										
H. albomarginatus	–	–	–	–	+	?	+	–	–	2, 19, 20
H. albopunctatus	–	+	–	±[h]	+	?	+	+	–	2, 21, 22, 23
H. benitezi	+	–	?	–	+	?	?	?	?	2, 24
H. curupi	–	–	–	–	+	?	+	?	–	2, 23, 24
H. ericae	–	–	–	–	+	?	?	?	–	2, 27
H. faber	+	+	?	?	+	+	?	+	+	2, 28, 29, 30
H. rosenbergi	?	+	±[i]	+	+	+	–	+	+	31, 32
H. heilprini	+	+	?	?	+	?	+	+	?	2, 33, 34
H. marianitae	–	–	–	–	+	?	?	?	–	2, 35
H. microderma	+	–	?	–	+	?	?	?	?	2, 36
H. pellucens	–	–	–	–	?	?	?	?	–	2
H. picturatus	–	–	–	–	?	?	?	?	–	2
H. pombali	+	+	?	?	+	?	?	?	?	2, 37
H. pulchellus	–	–	–	–	+	?	?	?	–	2, 38
H. punctatus	+	+	+	+	+	+	?	–	+	2, 39, 40, 41
H. raniceps	–	–	–	–	+	?	?	?	–	2, 42
H. semilineatus	+	+	?	?	+	?	?	?	?	2, 16
Myersiohyla										
M. neblinaria	+	–	?	–	+	?	?	?	?	2, 43

[a] Results are expressed as: +, presence; –, absence; +/–, equivocal; ?, unknown. Information from behavioural data was obtained considering different signals occurring in three sensory modalities (acoustic, visual, tactile), within the context of reproductive activity. Given the importance of sexually dimorphic skin glands (SDSGs) in the context of the present study, physical contact with areas having these glands is treated separately from tactile signals. Experimental evidence is still needed in Cophomantini to test the hypothesis that chemical signals are released during contact with SDSGs. Only those signals associated to courtship behaviour or female attraction are shown in the table.

[b] Besides those species examined in the present study, two species are included from which the histology of their SDSGs has been reported previously (*H. alytolylax* [2] and *H. punctatus* [3]). Additionally, three species (*B. ibitiguara*, *B. nanuzae*, and *H. rosenbergi*) are included in which behavioural observations report physical contact of females with the mental and/or lateral regions of males, even though there are not references to SDSGs.

[c] This refers to contact of mental and/or lateral glands of males with any part of the body of the female. This behaviour is coded as absent in those species lacking SDSGs.

[d] Behavioural descriptions in which the couple interacts by tactile stimuli excluding contacts to mental and lateral glands.

[e] Behavioural descriptions in which the male has been observed to press the mental region on the female nostrils during amplexus. This behaviour is coded as absent in those species lacking SDSGs.

[f] It was not explicitly noted by Nali & Prado (2012), although it can be inferred from their figures.

[g] Lima, Gontijo & Eterovick (2014) noted that female contacts different parts from the body of male but did not specify whether those contacts also include mental gland.

[h] Muniz *et al.* (2008) only noted that female contacts the male but did not specify which part of the body.

[i] Kluge (1981) noted that female contact the mental region of male but, although predicted by our optimization, the presence of mental gland in this species needs to be corroborated.

1, Hartmann *et al.*, 2004; 2, Present study; 3, Haddad & Sawaya, 2000; 4, Zina & Haddad, 2007; 5, Haddad *et al.*, 2005; 6, De Carvalho *et al.*, 2012; 7, Faivovich *et al.*, 2009; 8, Nali & Prado, 2012; 9, Cardoso, 1983; 10, Lima *et al.*, 2014; 11, Eterovick & Brandão, 2001; 12, Romero de Perez & Ruiz Carranza, 1996; 13, Rivera-Correa *et al.* (unpub. data); 14, Esteban Alzáte (unpub. data); 15, Duellman, 1970; 16, Ibáñez *et al.*, 1999; 17, Bernal *et al.*, 2004; 18, Ruiz-Carranza (unpub. data); 19, Giasson & Haddad, 2006; 20, Giasson & Haddad, 2007; 21, Muniz *et al.*, 2008; 22, Toledo *et al.*, 2007; 23, Dos Santos, 2008; 24, Donnelly & Myers, 1991; 25, Garcia *et al.*, 2007; 26, Lipinski *et al.*, 2012; 27, Garcia & Haddad, 2008; 28, Lutz, 1960; 29, Martins & Haddad, 1988; 30, Martins, 1993; 31, Kluge, 1981; 32, Noble, 1931; 33, Trueb & Tyler, 1974; 34, Landestoy, 2013; 35, De la Riva *et al.*, 2002; 36, Pyburn, 1977; 37, Caramaschi *et al.*, 2004; 38, Barrio, 1962; 39, Brunetti *et al.*, 2012; 40, Brunetti *et al.*, in press; 41, Brunetti, Taboada & Faivovich, 2014; 42, Guimarães & Bastos, 2003; 43, Faivovich *et al.*, 2013.

AC, advertisement call; CC, courtship call; M, mental gland; L, lateral gland.

character distribution and the reproductive biology of these species. More specifically, do the patterns reflect differences in sexual selection mechanisms? Are these patterns indicative of changing preponderant roles of chemical or acoustic signals during courtship? Do different signals in Cophomantini interact as multiple sensory modalities (multimodal communication)? These questions will remain unanswered because, as shown in Table 2, our knowledge of reproductive biology of most species is very scarce, and our knowledge of the molecules produced by these glands is completely unknown. However, there are several points that require further discussion.

Our findings and published records related to the reproductive biology of these species (Table 2), allow inferring that some of the physical contacts performed by females on males involve skin regions having SDSGs. It would be expected to observe courtship behaviours involving contact with the mental gland of males in some species of *Myersiohyla*, the *Hyloscirtus bogotensis* group, in *H. caucanus*, and in the *Hypsiboas benitezi* group. These contacts might also involve lateral glands in species of *Bokermannohyla*, and the *H. semilineatus* group. These contacts would be absent in the *H. pellucens* and *H. pulchellus* groups. With the same reasoning, although from a different perspective, the descriptions of the courtship behaviour in both *H. faber* and *Hypsiboas rosenbergi*, and the presence of mental and lateral glands in *H. faber*, suggest that *H. rosenbergi* might have a mental gland. Whether the role of the mental gland is also associated with contact during amplexus, as suggested in *H. faber* (Lutz, 1960), and in *H. punctatus* (Brunetti, Taboada & Faivovich, in press), requires further behavioural studies.

Although still speculative, it is feasible that, through these contacts, females may be sensing chemical signals secreted by SDSGs of males. This inference is supported by the behavioural and chemical studies in anurans involving SDSGs that revealed the female-attracting role of their secretion (Pearl *et al*., 2000; Poth *et al*., 2012) and, in those compounds of proteinaceous origin, the similar structure to pheromones of plethodontid salamanders (Willaert *et al*., 2013). These findings and our results suggest that there is a relationship between the presence of SDSGs and physical contact in the form of chemical communication during courtship in Cophomantini. Moreover, the occurrence of different signals during courtship (visual, tactile, acoustic, and possibly chemical) in several species (Table 2) suggests that they may interact with each other, and thus comprise complex signalling (or multimodal signalling; Hebets & Papaj, 2005).

In anurans, studies of multimodal signals have been focused on the interaction among acoustic and visual components (Taylor *et al*., 2011; Grafe *et al*., 2012; Preininger *et al*., 2013), whereas only a few studies have inferred the existence of a system that includes acoustic and chemical signals (Pearl *et al*., 2000; Starnberger *et al*., 2013; Starnberger, Preininger & Hödl, 2014). In Cophomantini, behavioural descriptions in *Hypsiboas rosenbergi* (Kluge, 1981), and *H. punctatus* (Brunetti *et al*., in press) suggest that females select or reject males based on different signals that interact in a multimodal signalling. These signals would involve acoustic (advertisement and courtship calls) and chemical signals, based on contacts to mental and lateral glands. Although these descriptions and other behavioural observations in Cophomantini (Table 2) do not constitute direct evidence, it is noticeable that all species of anurans in which chemical attractants were experimentally demonstrated also communicate acoustically (Wabnitz *et al*., 1999; Pearl *et al*., 2000; Poth *et al*., 2012).

FINAL REMARKS

According to our histological results, SDSGs may be unambiguously considered as specialized glands, *sensu* Brizzi *et al*. (2003). Although this is based on topographical and histological data, it remains unknown whether SDSGs are specialized for social communication and reproduction in most species of anurans. We consider that at least four important issues have contributed to this lack of information. First, knowledge of the reproductive biology of most species is scarce and fragmentary. Particularly in Cophomantini, with more than 170 species described, there are good descriptive observations for only some species of the genus *Aplastodiscus*, two species of *Bokermannohyla*, and four species of *Hypsiboas* (Table 2). Second, being the most conspicuous form of communication, studies on mating behaviour of anurans were traditionally focused on acoustic signals, whereas studies published in the last decade have revealed that many species may also use other signals during social interactions (Stephenson & Verrell, 2003; Giasson & Haddad, 2006; Grafe *et al*., 2012; Poth *et al*., 2012; Preininger *et al*., 2013; Starnberger *et al*., 2013, 2014). Third, as suggested by the present study, SDSGs appear to be present in many more species than previously assumed. Fourth, there are few experimental studies involving SDSGs: so far only two studies have demonstrated its role as source of chemical signals during mating (Pearl *et al*., 2000; Poth *et al*., 2012).

Finally, the occurrence of different signal modalities (i.e. acoustical, tactile, and visual) in several species suggests that they may be used as multimodal components in courtship signals (Table 2). The study of different signals and their interactions, as well as the levels in which they occur during female choice, represents an exciting framework. Our findings should be useful in guiding future research in the field of behavioural and chemical studies in frogs.

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APPENDIX

Table A1. Data matrix and number of minimum steps for each character implied by the phylogenetic hypothesis modified from Faivovich *et al*. (2013)

	Characters															
Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	Reference
Aplastodiscus																
A. eugenioi	1	1	1	1	1	0	0	1	0	1	1	1	0	0	1	a
A. leucopygius	1	1	1	1	1	0	0	1	0	1	1	1	0	0	1	a
A. perviridis	1	1	1	1	1	0	0	1	0	1	1	1	0	0	1	a
Bokermannohyla																
B. hylax	1	1	0	0	0	0	0	1	0	0	–	0	0	0	1	a
B. itapoty	1	?	1	?	?	?	?	?	?	?	?	?	?	?	?	b
B. martinsi	1	?	1	?	?	?	?	?	?	?	?	?	?	?	?	b
B. pseudopseudis	1	1	1	0	1	1	0	1	1	1	1	0	0	0	1	a, b
B. saxicola	1	0	1	–	0	0	0	0	–	1	1	–	–	–	–	a, b
Hyloscirtus																
H. alytolylax	1	?	2	?	?	?	?	?	?	?	?	?	?	?	?	c
H. antioquia	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	a
H. armatus	1	?	1	?	?	?	?	?	?	?	?	?	?	?	?	d
H. caucanus	1	0	0	–	0	0	0	1	0	0	–	–	–	–	–	a
H. charazani	1	?	1	?	?	?	?	?	?	?	?	?	?	?	?	d
H. colymba	1	0	2	–	1	0	1	1	1	0	–	–	–	–	–	a, c
H. larinopygion	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	a
H. lynchi	1	0	0	–	1	0	1	1	1	0	–	–	–	–	–	a, e
H. palmeri	1	0	2	–	1	0	1	1	1	0	–	–	–	–	–	a, f
H. phyllognathus	1	?	2	?	?	?	?	?	?	?	?	?	?	?	?	c
Hypsiboas																
H. albomarginatus	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	a
H. albopunctatus	0	1	–	0	–	–	–	–	–	–	–	1	1	1	1	a
H. benitezi	1	0	1	–	1	0	0	1	0	0	–	–	–	–	–	a, f
H. cinerascens	1	1	1	2	?	?	?	?	?	?	?	?	?	?	?	g, h
H. curupi	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	a
H. ericae	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	a
H. faber	1	1	0	0	1	1	1	1	1	1	0/1	1	1	1	1	a
H. heilprini	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	a, i
H. lemai	1	?	1	?	?	?	?	?	?	?	?	?	?	?	?	a, f
H. marianitae	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	a
H. microderma	1	0	1	–	1	0	0	1	0	1	1	–	–	–	–	a, f
H. nympha	1	?	1	–	–	–	–	–	–	–	–	–	–	–	–	f
H. pombali	1	1	1	0	1	0	0	1	0	1	1	1	1	0	1	a
H. pellucens	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	a
H. picturatus	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	a
H. pulchellus	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	a
H. punctatus	1	1	1	2	1	1	1	1	1	1	0	1	1	1	1	g, j
H. raniceps	0	0	–	–	–	–	–	–	–	–	–	–	–	–	–	a
H. rosenbergi	?	1	?	–	–	–	–	–	–	–	–	–	–	–	–	k
H. semilineatus	1	1	0	0	0	0	0	1	0	1	–	0	0	0	1	a
Myersiohyla																
M. chamaleo	1	?	1	?	?	?	?	?	?	?	?	?	?	?	?	l
M. neblinaria	1	0	1	–	?	?	?	?	?	?	?	–	–	–	–	a, l
M. kanaima	1	?	1	?	?	?	?	?	?	?	?	?	?	?	?	l
Min. steps	5	7	6	3	3	3	3	1	4	3	1	2	2	3	1	

[a]Present study; [b]Faivovich *et al*., 2009; [c]Duellman, 1972; [d]Faivovich & De la Riva, 2006; [e]Ruiz-Carranza & Ardila-Robayo, 1991; [f]Faivovich *et al*., 2006; [g]Hoogmoed, 1979; [h]Photographs in Telles, Vaz & Menin, 2013; [i]Trueb & Tyler, 1974; [j]Brunetti *et al*., 2012; [k]Noble, 1931; [l]Faivovich *et al*., 2013.
?, unknown state; –, non-applicable.

SUPPORTING INFORMATION

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

Figure S1. Taxonomic distribution and optimization of characters four, five, six, nine, 13, and 14 in the phylogenetic hypothesis of Cophomantini modified from Faivovich *et al*. (2013) for *Myersiohyla*, *Hyloscirtus*, *Bokermannohyla*, and *Aplastodiscus*. For *Hypsiboas*, see Fig. S2. Note that we have excluded the outgroups because SDSGs remain poorly known outside Cophomantini. For the data matrix, see Appendix (Table A1). Multistate characters are treated as non-additive. Transformations that may be synapomorphies of Cophomantini or a more inclusive clade are shown with grey bars. Asterisks (*) indicate transformations that occur in that node or in a more inclusive clade. Further studies on taxonomic distribution will help to clarify its position. Further discussion is provided in the text. LG, lateral gland; MG, mental gland; SDSGs, sexually dimorphic skin glands; SMGs, specialized mucous glands.

Figure S2. Taxonomic distribution and optimization of characters four, five, six, nine, 13, and 14 in the phylogenetic hypothesis of Cophomantini modified from Faivovich *et al*. (2013) for *Hypsiboas*. For symbols and comments, see Fig. S1.

Appendix S1. Specimens examined. Superscripts after ccollection numbers indicate the figure in which the specimen appears.