Supplementary Information for:

Diversity and evolution of squamate hemipenes: an overview with particular reference to the origin and early history of snakes

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Supplementary Appendix 16.S1

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1. Extended Acknowledgements

We thank V. Deepak, Pedro M. S. Nunes and Ana L. C. Prudente for constructive reviews of the submitted manuscript. DJG thanks Thomas Ziegler for answering some questions about lizard hemipenes. We are deeply grateful to the following colleagues for allowing and facilitating examination and preparation of hemipenes from specimens under their care: Patrick Campbell (NHM, London: thanks also to M. Wilkinson for help with GGM's visit); Ana L. C. Prudente (MPEG, Belém); Greg Schneider (UMMZ, Ann Arbor); Alain Dubois, Annemarie Ohler, Ivan Ineich (MNHN, Paris), Darrel Frost, Charles W. Myers, Christopher Raxworthy, Frank Burbrink (AMNH); Georges Zug, Roy McDiarmid, Kevin de Queiroz (USNM, Washington); Giuseppe Puorto, Francisco L. Franco, Hebert Ferrarezzi, Felipe G. Grazziotin (IBH, São Paulo); Ronald Nussbaum (UMMZ, Ann Arbor); Harold Voris, Alan Resetar (FMNH, Chicago); Ulisses Caramaschi, Paulo Passos, Ronaldo Fernandes, José Pombal Jr. (MNRJ, Rio de Janeiro); William Duellman, Linda Trueb (KU, Lawrence). We are indebted to the following colleagues for generously sharing photographs of hemipenes for this chapter: Ana Lucia Prudente (*Anilius scytale*), Felipe Curcio (*Tropidophis paucisquamis*, *Trachyboa boulengeri*), Paulo Passos (*Epicrates cenchria*, *Trilepida macrolepis*), Pedro Nunes (*Amphisbaena brasiliana*, *Teius teyou*).

2. Hemipenial Specimens Figured in this Article

Fig. 16.2
Delma inornata (BMNH 98.10.19.6);
Pygomeles braconnieri (BMNH 1930.7.2.5);
Mabuya sp. (UIS-R 3908);
Platysaurus sp. (MZUSP 98894);
Gallotia stehlini (BMNH 1977.1253);
Amphisbaena brasiliana (TM 180)

Fig. 16.3
Heterodactylus imbricatus (BMNH 1913.9.30.3);
Teius teyou (AMNH 65221);
Chamaeleo sp. (BMNH 1903.1.28.6);
Uromastyx aegyptia (BMNH 1975.958);
Agama paragama (BMNH 1982.14.19);
Anguis fragilis (BMNH 94.5.7.23)

Fig. 16.4
Pseudopus apodus (BMNH 1972.1115);
Varanus prasinus (BMNH 1897.12.10.18-19);
Amerotyphlops minuisquamus (MZUSP 21447);
Amerotyphlops paucisquamus (AGARDA 2786);
Amerotyphlops brongersmianus (MZUSP 14678);
Amerotyphlops reticulatus (MZUSP 23526)

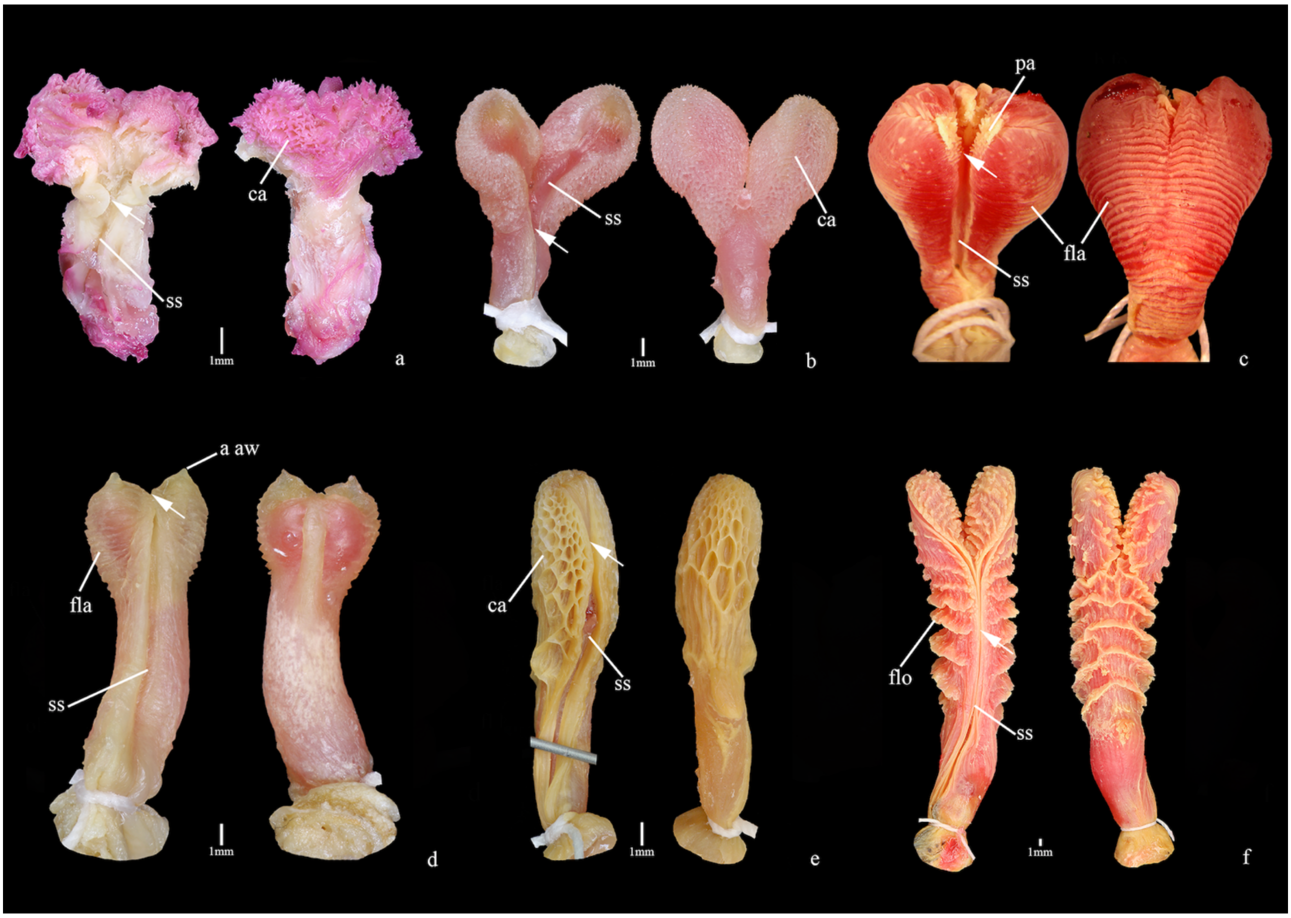
Fig. 16.5
Afrotyphlops lineolatus (BMNH 1975.569);
Trilepida macrolepis (ICN 7677);
Cylindrophis melanotus (BMNH 1871.7.20.214);
Trachyboa boulengeri (BMNH 1913.11.12.38);
Tropidophis paucisquamis (IBSP 77872);
Anilius scytale (MPEG 15007)

Fig. 16.6
Morelia spilota (BMNH 84.9.13.20);
Xenopeltis unicolor (BMNH uncatalogued);
Epicrates cenchria (MNRJ 2723);
Charina bottae (BMNH 94.3.24.5);
Candoia bibroni (BMNH 1875.26.46);
Calabaria reinhardtii (BMNH 1897.72.2)

Collection abbreviations

AGARDA, Adrian Garda field series; BMNH, Natural History Museum, London, UK; IBSP, Instituto Butantan, São Paulo, Brazil; ICN, Instituto de Ciencias Naturales de Colombia, Bogotá, Colombia; MNRJ, Museu Nacional, Rio de Janeiro, Brazil; MPEG, Museu Paraense Emílio Goeldi, Belém, Brazil; MZUSP, Museu de Zoologia da Universidade de São Paulo, Brazil; TM, Tami Mott field series.

3. Supplementary Figure 16.S1



Sulcate (left) and asulcate (right) views of hemipenes of (a) *Pygopus lepidonotus* (Pygopodidae: BMNH 1966.387); (b) *Draco maximus* (Agamidae: BMNH 1974.3815); (c) *Kentropyx calcarata* (Teiidae: MZUSP 67665; scale not available: courtesy P. Nunes); (d) *Lanthanotus borneensis* (Lanthanotidae: BMNH 1906.271) (e) *Morelia viridis* (Pythonidae: BMNH 1876.18.10); (f) *Chilabothrus subflavus* (Boidae: BMNH 50.5.2.8.6). Abbreviation: **a aw,** apical awn; **ca,** calyces; **fla,** ridge- or lamina-like flange; **flo,** flounce; **pa,** papillae; **ss,** sulcus spermaticus; arrow indicates point at which ss expands or bifurcates.

4. Character optimization

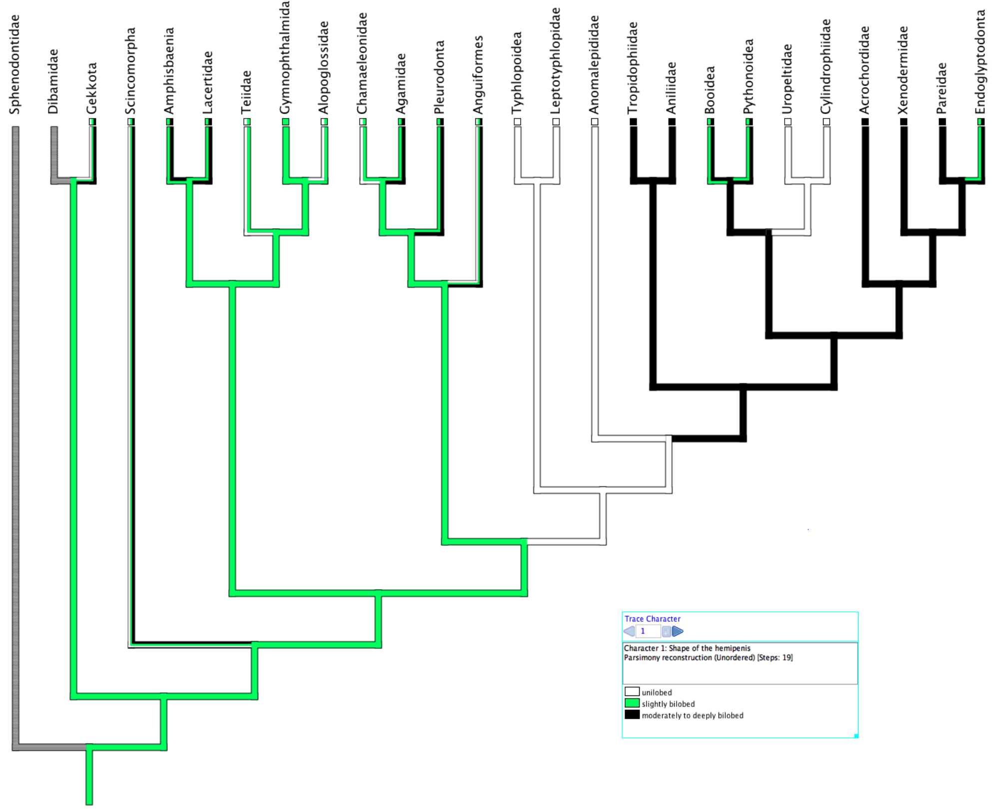
We used parsimony as implemented in Mesquite 3.7 to optimize character states for the ten characters summarized in Fig. 16.1. For this, we deleted values in Fig. 16.1 presented in parentheses—states that are rare and probably not plesiomorphic for particular lineages. Beyond the exclusion of these rare character states, we included polymorphic traits and did not score major lineages for possible or probable plesiomorphic states, such that optimizations reported here should be viewed as indicative and preliminary.

Matrix:

Sphenodontidae	??????????
Dibamidae	????0??0??
Gekkota	(0 1 2)0(0 1)00(0 2)(0 1)0(0 1)0
Scincomorpha	(0 1 2)(0 1)(0 1)0000(1 3)(0 1)0
Amphisbaenia	(1 2)111000(0 1)0(0 1)
Lacertidae	(1 2)0100(0 2)0100
Teiidae	(0 1)(0 1)10000120
Gymnophthalmidae	1(0 1)(0 1)(0 1)100120
Alopoglossidae	(0 1)11(0 1)000120
Chamaeleonidae	(0 1)0000012(1 2)0
Agamidae	(1 2)0100(0 2)1000
Pleurodonta	(1 2)0100(0 2)1200
Anguiformes	(0 1 2)0000002(0 3)(0 1)
Leptotyphlopidae	00010(0 2)0(0 2)0(0 1)
Typhlopoidea	0001000(0 2)0(0 1)
Anomalepididae	0001110?11
Tropidophiidae	2111000020
Aniliidae	2111000000
Pythonoidea	(1 2)11100(0 1)2(0 1)0
Booidea	(1 2)11100(0 1)2(0 1)0
Uropeltidae	0101110000
Cylindrophiidae	00010000??
Acrochordidae	2111010001
Xenodermidae	21110(0 1)(0 1)001
Pareidae	2111001001
Endoglyptodonta	(1 2)1111(0 1 2)(0 1)000

Tree:

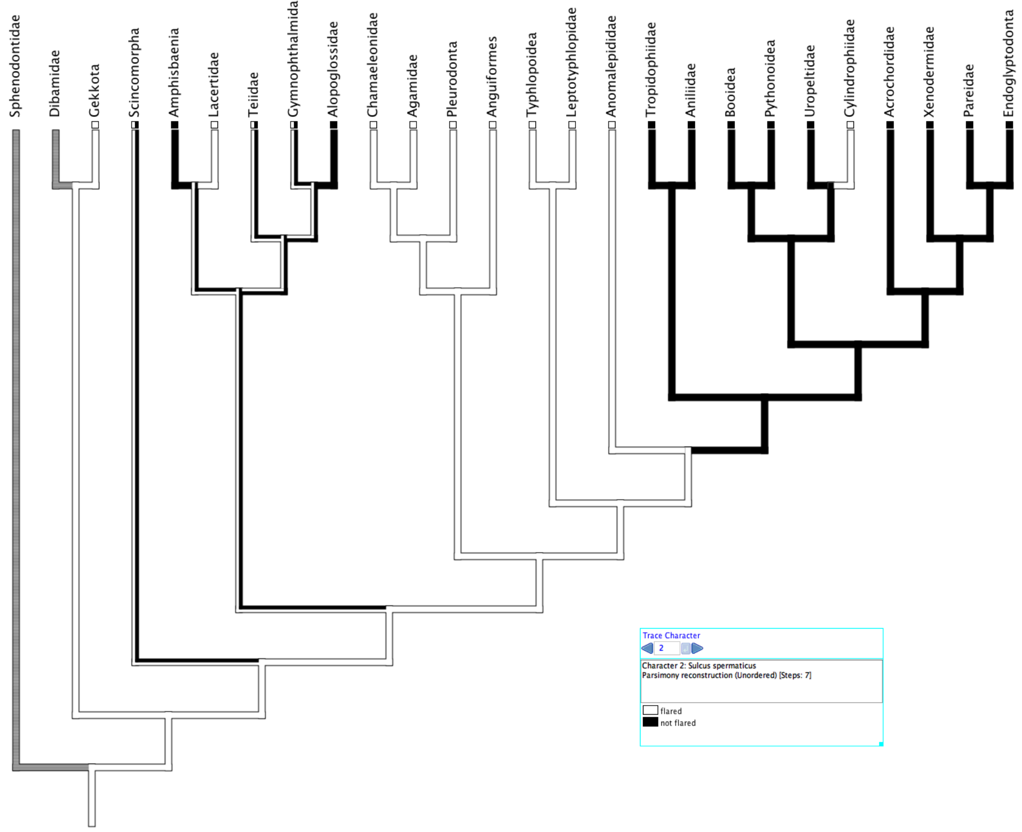
(Sphenodontidae,((Dibamidae,Gekkota),(Scincomorpha,(((Amphisbaenia,Lacertidae),(Teiidae,(Gymnophthalmidae,Alopoglossidae))),((((Chamaeleonidae,Agamidae),Pleurodonta),Anguiformes),((Typhlopoidea,Leptotyphlopidae),(Anomalepididae,((Tropidophiidae,Aniliidae),(((Booidea,Pythonoidea),(Uropeltidae,Cylindrophiidae)),(Acrochordidae,(Xenodermidae,(Pareidae,Endoglyptodonta)))))))))))));



Character 1: Shape of the hemipenis
Parsimony reconstruction (Unordered) [Steps: 19]

☐ unilobed
🟩 slightly bilobed
■ moderately to deeply bilobed

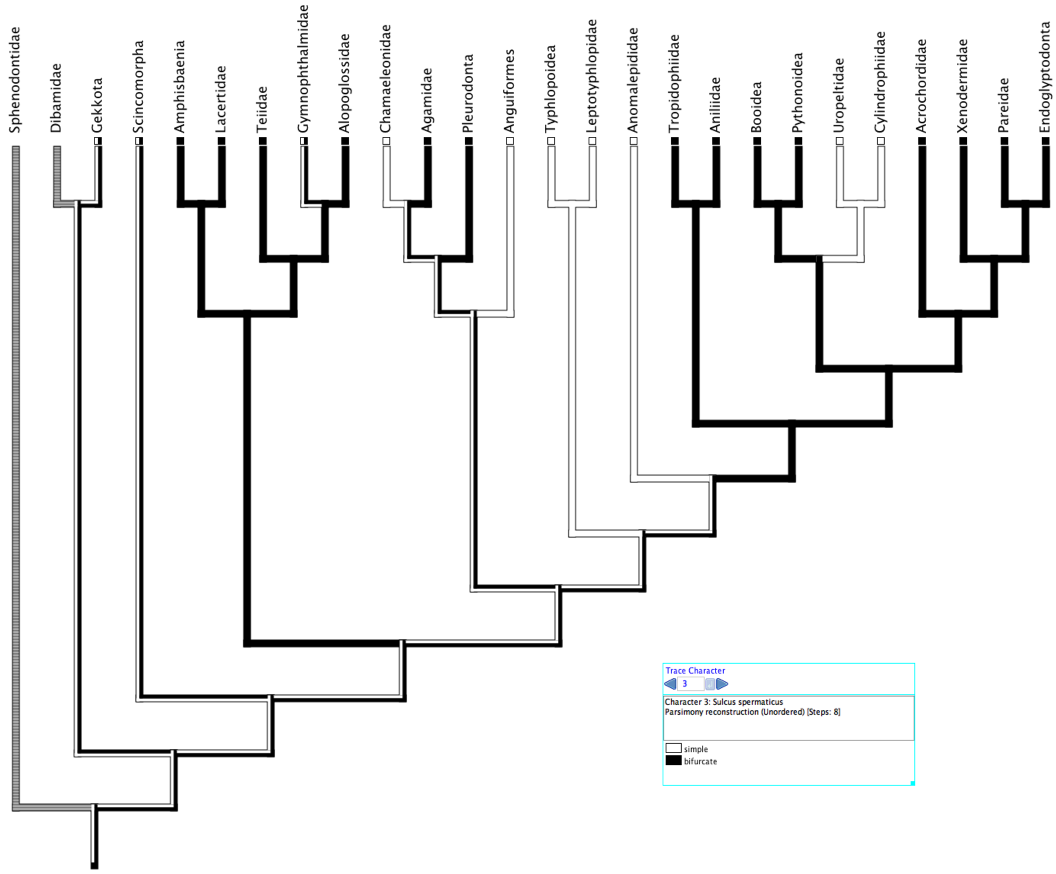
Character 1



Character 2: Sulcus spermaticus
Parsimony reconstruction (Unordered) [Steps: 7]

☐ flared
■ not flared

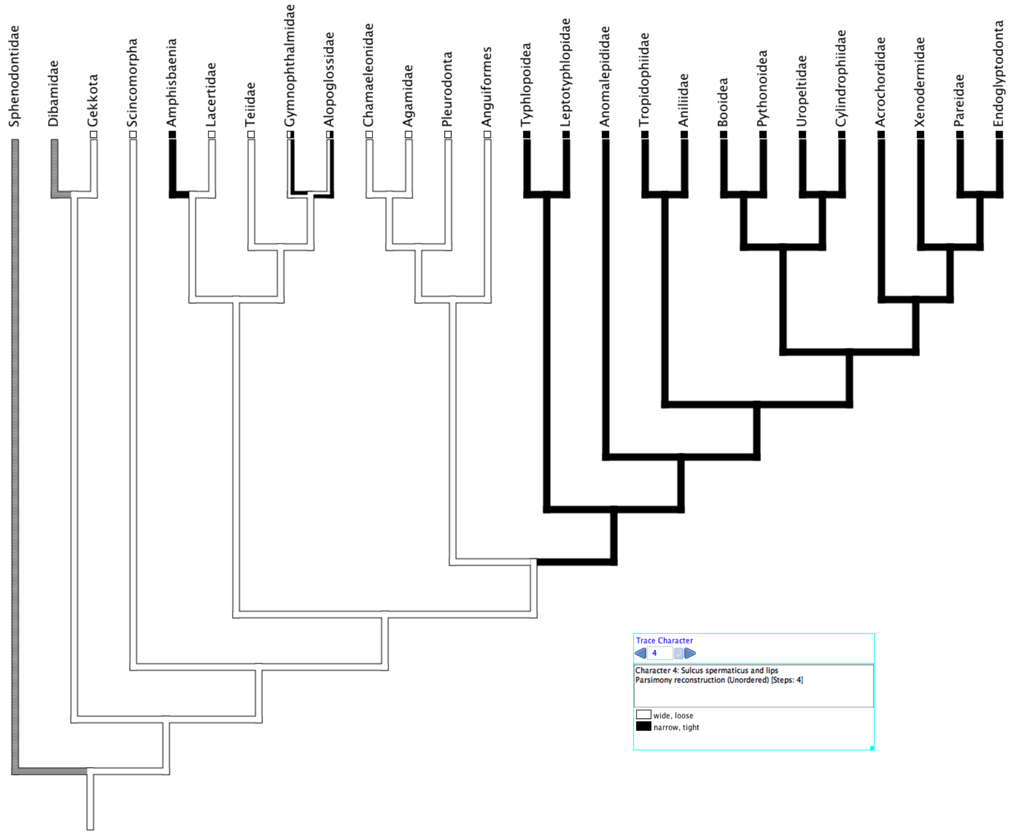
Character 2



Character 3: Sulcus spermaticus
Parsimony reconstruction (Unordered) [Steps: 8]

☐ simple
■ bifurcate

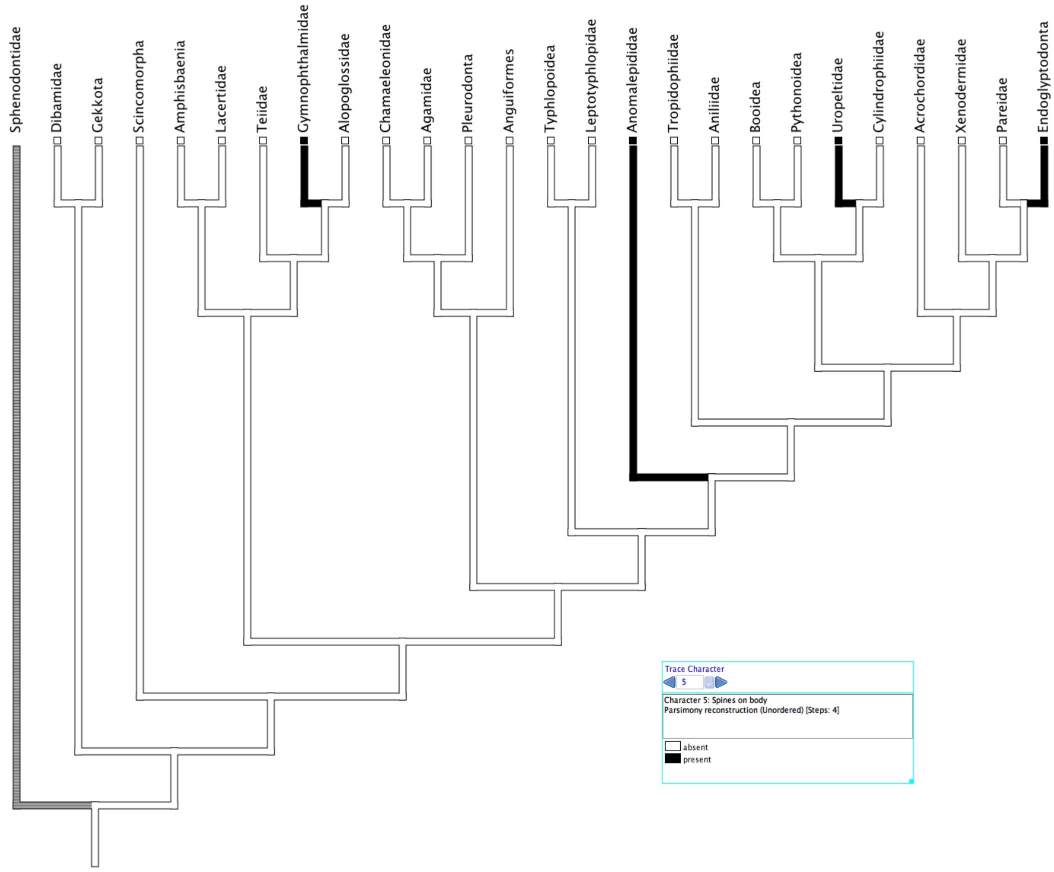
Character 3



Character 4: Sulcus spermaticus and lips
Parsimony reconstruction (Unordered) [Steps: 4]

☐ wide, loose
■ narrow, tight

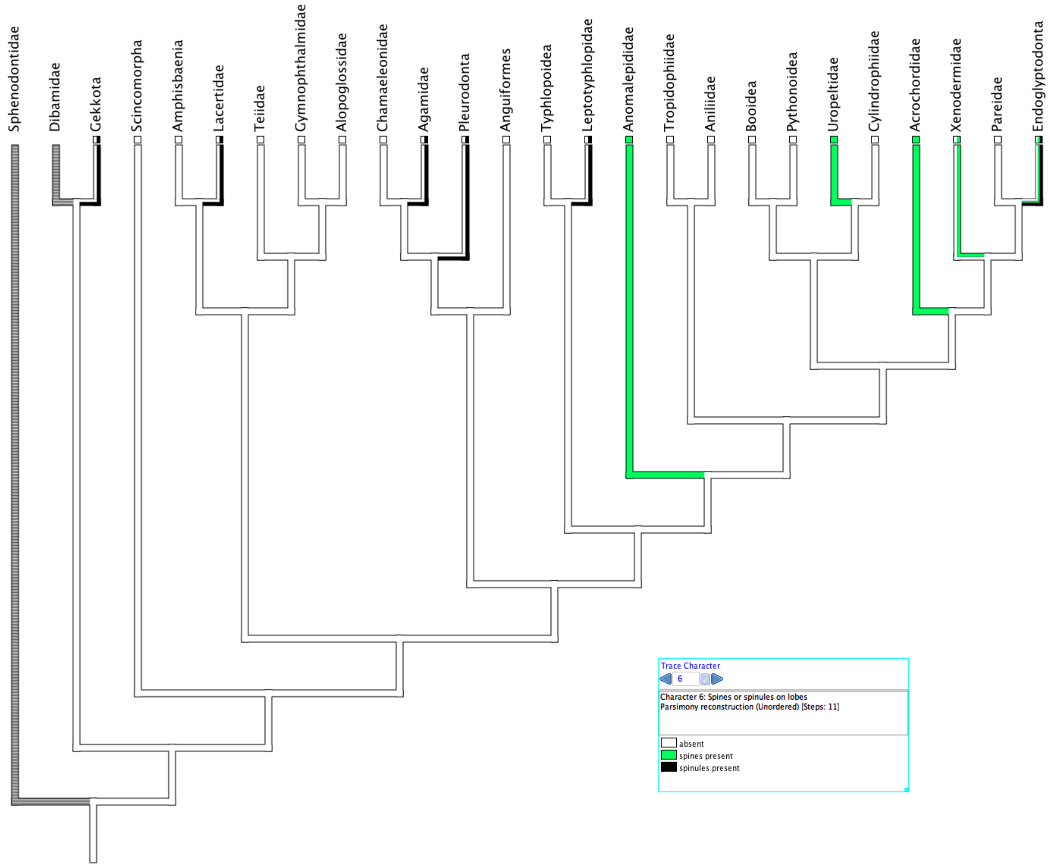
Character 4



Character 5: Spines on body
Parsimony reconstruction (Unordered) [Steps: 4]

☐ absent
■ present

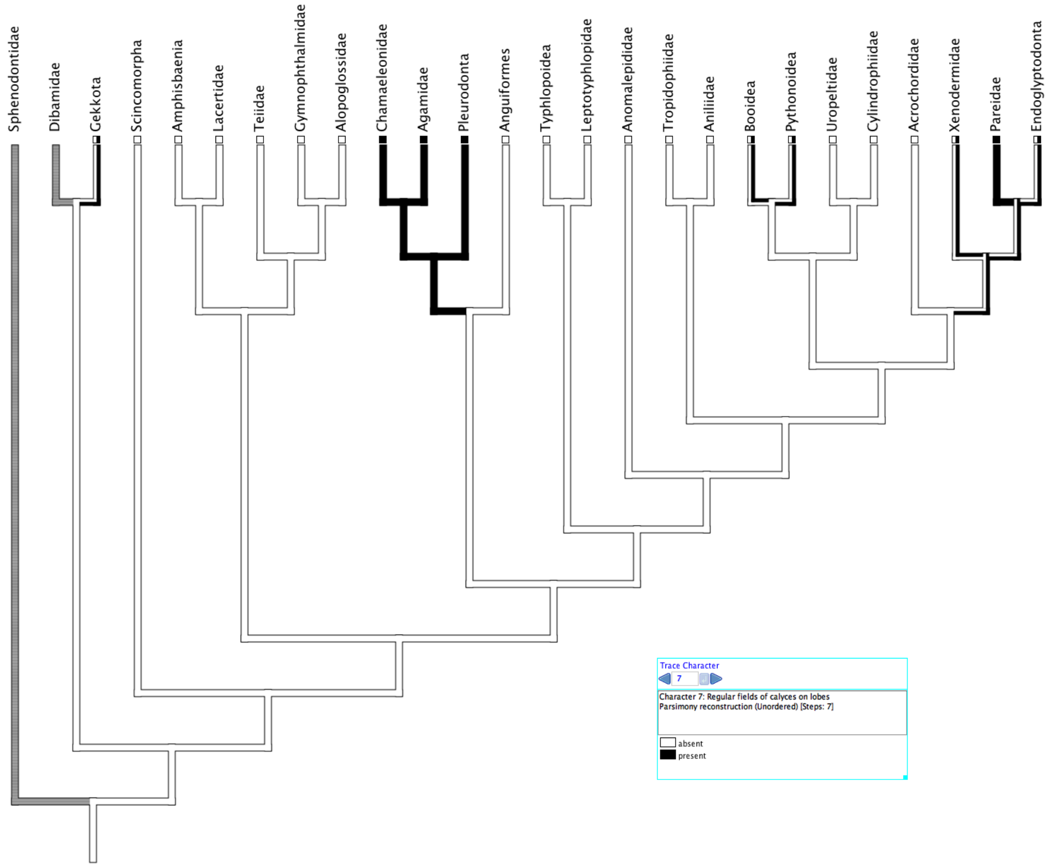
Character 5



Character 6: Spines or spinules on lobes
Parsimony reconstruction (Unordered) [Steps: 11]

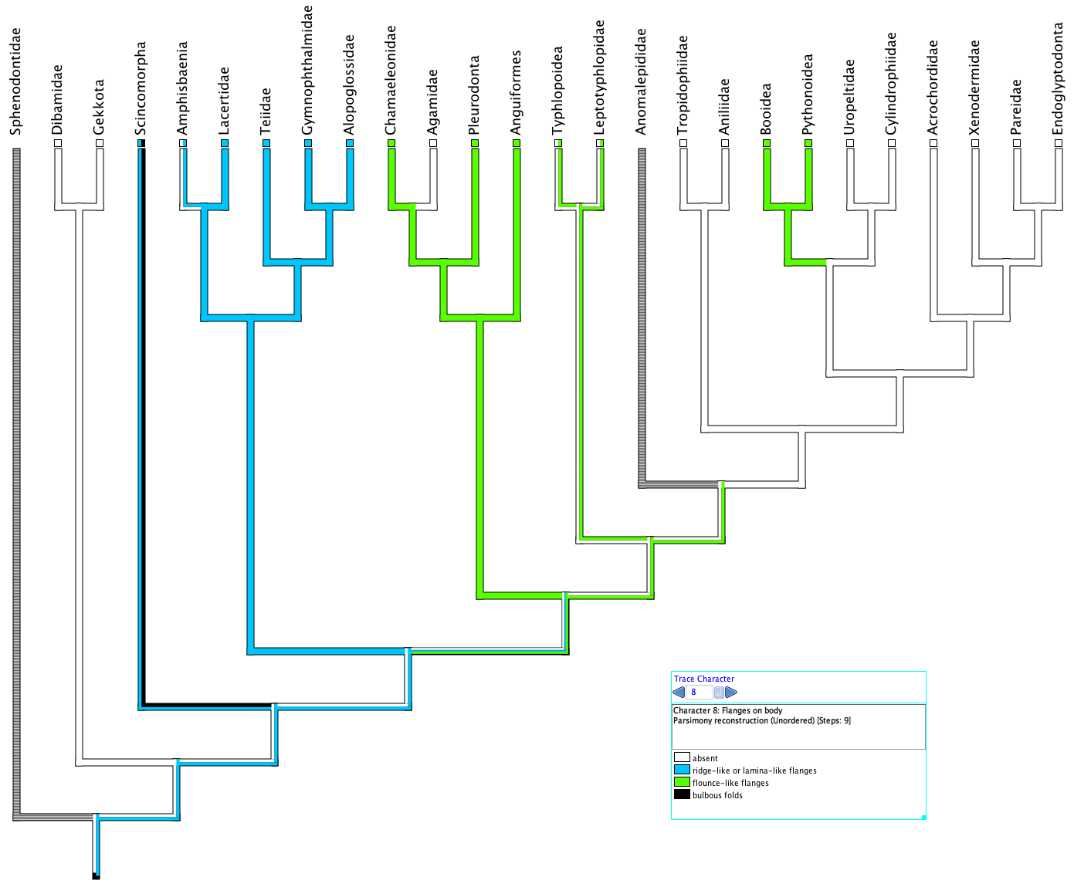
☐ absent
🟩 spines present
■ spinules present

Character 6



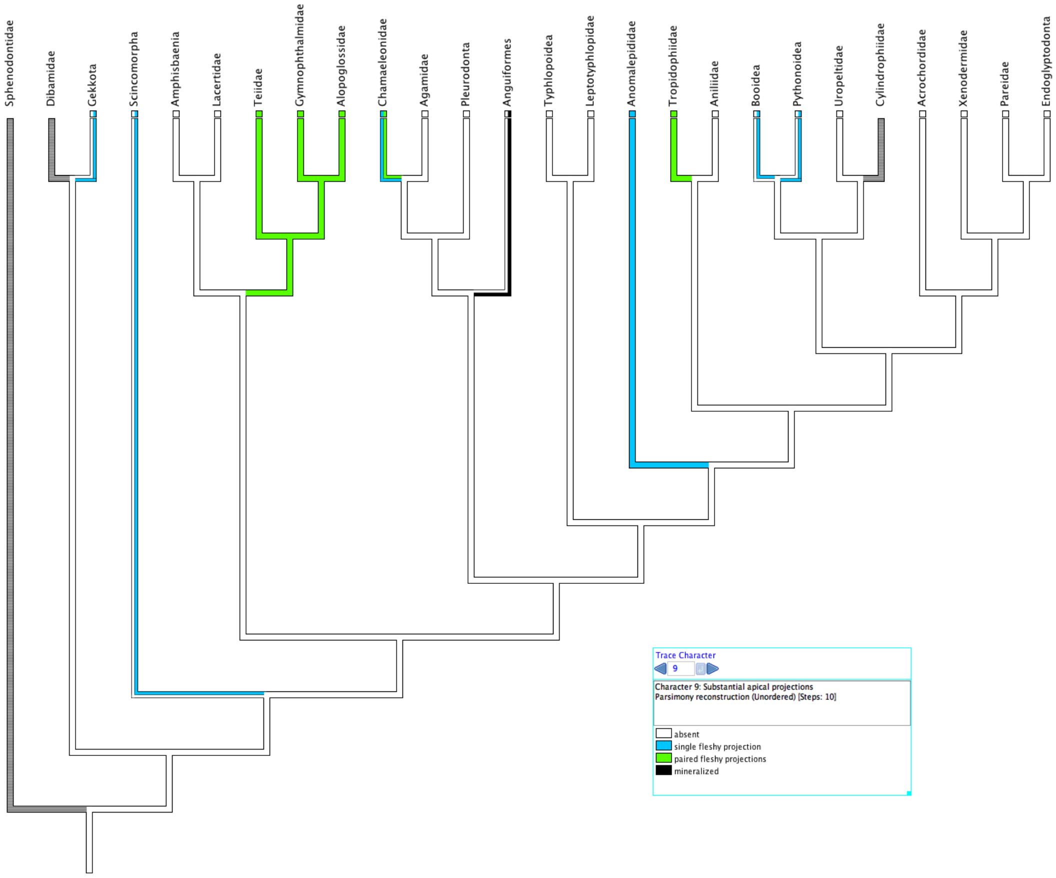
Character 7: Regular fields of calyces on lobes
Parsimony reconstruction (Unordered) [Steps: 7]

Character 7



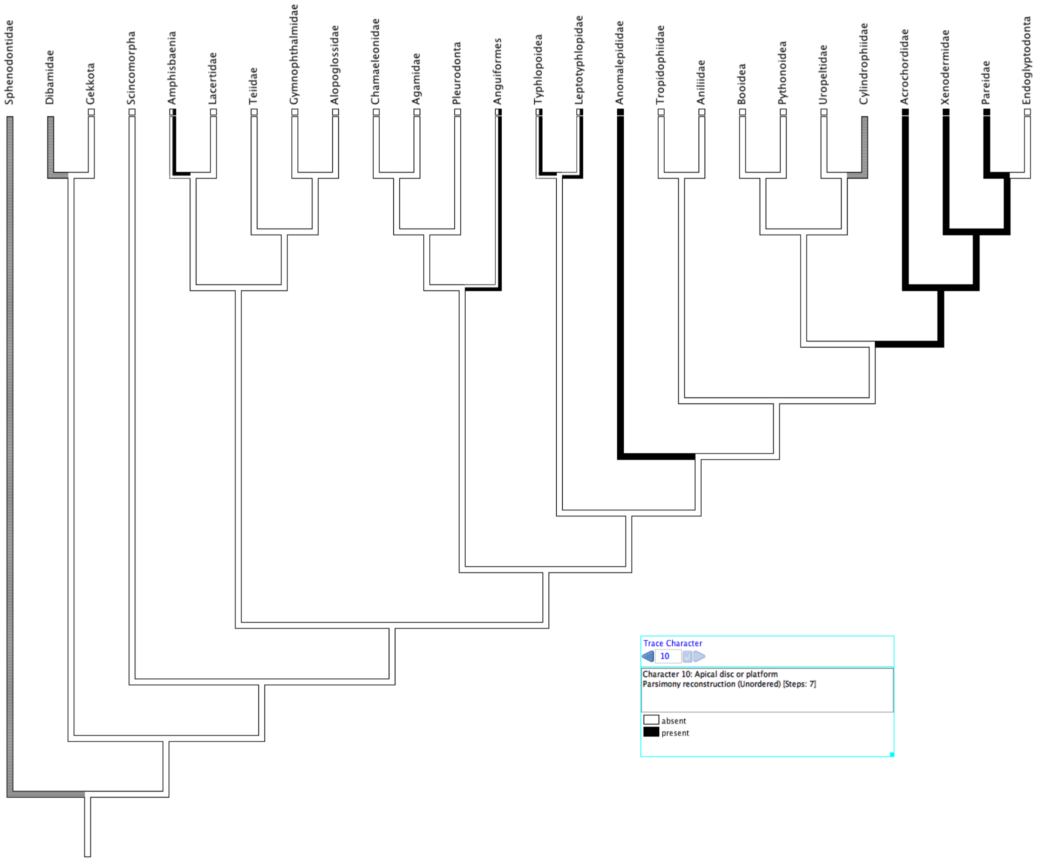
Character 8: Flanges on body
Parsimony reconstruction (Unordered) [Steps: 9]

Character 8



Character 9: Substantial apical projections
Parsimony reconstruction (Unordered) [Steps: 10]

Character 9



Character 10: Apical disc or platform
Parsimony reconstruction (Unordered) [Steps: 7]

Character 10

5. Form and function

Several previous authors (Presch 1978; Böhme 1988; Arnold 1986a; Böhme & Ziegler 2008; King et al 2009; Nunes et al. 2014; Brennan 2016; Andonov et al. 2017; Gillman et al 2018) have identified, proposed or discussed potentially causative correlations between hemipenial morphology and copulatory behaviour, reproductive ecology, female reproductive tract form and function, body morphology, and life habits. A correlation between hemipenial morphology and couple-anchoring reproductive strategies to improve mating success have been postulated for several groups, including the hypothesis that larger and/or more numerous spines in limbless animals will enhance copulatory time and improve sperm transfer (see e.g., Nunes et al. 2014). However, many limbless lizards (amphisbaenians, some pygopodids, many scincoids) have spineless organs, as do almost non-caenophidian lineages of extant snakes (with a few exceptions among "scolecophidians" and uropeltids). Most of these taxa are constrictors so that longer duration copulation aided by coiling (e.g., Rivas et al. 2007) might be achievable in the absence of hemipenial spines, and many of these taxa also possess pelvic spurs used during copulation (e.g., Gillingham & Chambers 1982) to likely improve mating success.

It is often assumed that tail and hemipenis size are correlated, but both longer and shorter tails co-occur with hemipenes and retractor muscles of many different sizes and forms. Phylogenetically restricted correlations might occur. For example, surface-dwelling or arboreal snakes with long tails generally have long and voluminous hemipenes, such as in the colubrids *Chironius, Drymarchon, Palusophis*, *Ptyas* and *Spilotes* (Montingelli et al. 2019; Zaher et al. 2019), and many short-tailed fossorial snakes, such as uropeltids, have short tails and hemipenes. However, a long and slender hemipenis, sometimes notably longer than the tail, is found in some small terrestrial, zoocryptic or fossorial snakes, including asiatyphlopines and *Prosymna* (Myers and McDowell 2014), as well as in some larger snakes with shorter tails such as *Pseudaspis cana* (Myers and McDowell 2014). To fit the hemipenis and associated retractor muscles inside short tails demands the elaboration of strategies such as coiling of hemipenes, insertion of the retractor muscle close to the tip of the tail, folding the retractor muscle, or having protrusible awns on the apex of the hemipenis that can be projected with full eversion of the organ inside of female cloaca (as in e.g., *Typhlops, Pseudaspis,* and *Prosymna*; McDowell 1974; Myers and McDowell 2014).

In general, there appears to be notable phylogenetic signal in the taxonomic distribution of major hemipenial features, and less clear ecological signal. However, thorough quantitative comparative analyses across Squamata have not been carried out, and available data on hemipenis morphology and (especially reproductive) ecology are patchy. The functional significance of finer-scale features has occasionally been considered (e.g., apical microstructure as discussed by Rosenberg et al. 1991) but largely remains speculative. As has been pointed out elsewhere (Brennan 2016; de-Lima et al. 2019), a detailed understanding of hemipenial diversity and evolution will also require a better understanding of the even less-well studied anatomy of the female squamate cloaca, and of the female hemiclitoris (e.g., Böhme 1995; Ziegler & Böhme 1997).

Besides their main reproductive function, some snakes can partially evert hemipenes as defensive strategies to indicate fright, possibly mimicking defensive structures such as stingers or claws (see Greene, 1988). During copulation only one hemipenis is used at a time (Cope, 1894), even when explosive mating aggregations occur (Doan and Arriaga, 1999). Alternative use of each hemipenis in successive copulations events over a short period of time (e.g., Tokarz 1988; Zweifel, 1997) may occur generally among squamates (but see Shine et al., 2000), as a mechanism to maximize sperm delivery given that each hemipenis is independently connected to each testis (Tokarz, 1988). Females have been rarely observed mating with two males simultaneously (Marinho et al., 2020).

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7. Colour version of Figures 16.2, 167.3, 16.5, and 16.6

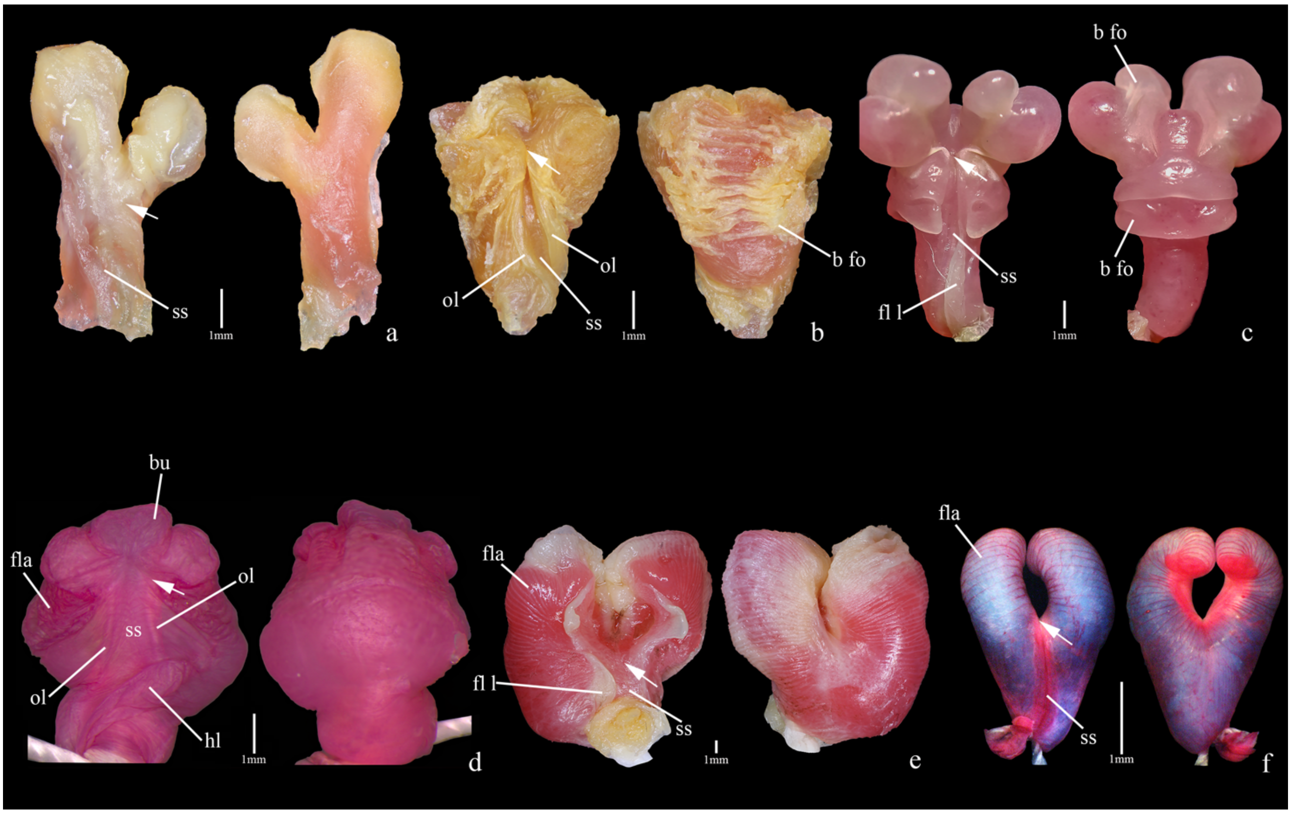

Figure 16.2 Hemipenes of (a) *Delma inornata* (Pygopodidae); (b) *Pygomeles braconnieri* (Scincidae; possibly incompletely everted); (c) *Mabuya* sp. (Scincidae; reproduced with permission [36]); (d) *Platysaurus* sp. (Cordylidae); (e) *Gallotia stehlini* (Lacertidae; incompletely inflated); (f) *Amphisbaena brasiliana* (Amphisbaenidae; reproduced with permission [14]). Sulcate (left) and asulcate (right) views. See Box 16.1 for abbreviations.

Figure 16.3 Hemipenes of (a) *Heterodactylus imbricatus* (Gymnophthalmidae); (b) *Teius teyou* (Teiidae; courtesy P. Nunes); (c) *Chamaeleo* sp. (Chamaeleonidae); (d) *Uromastyx aegyptia* (Agamidae; incompletely everted); (e) *Agama paragama* (Agamidae); (f) *Anguis fragilis* (Anguidae). Sulcate (left) and asulcate (right) views. See Box 16.1 for abbreviations.

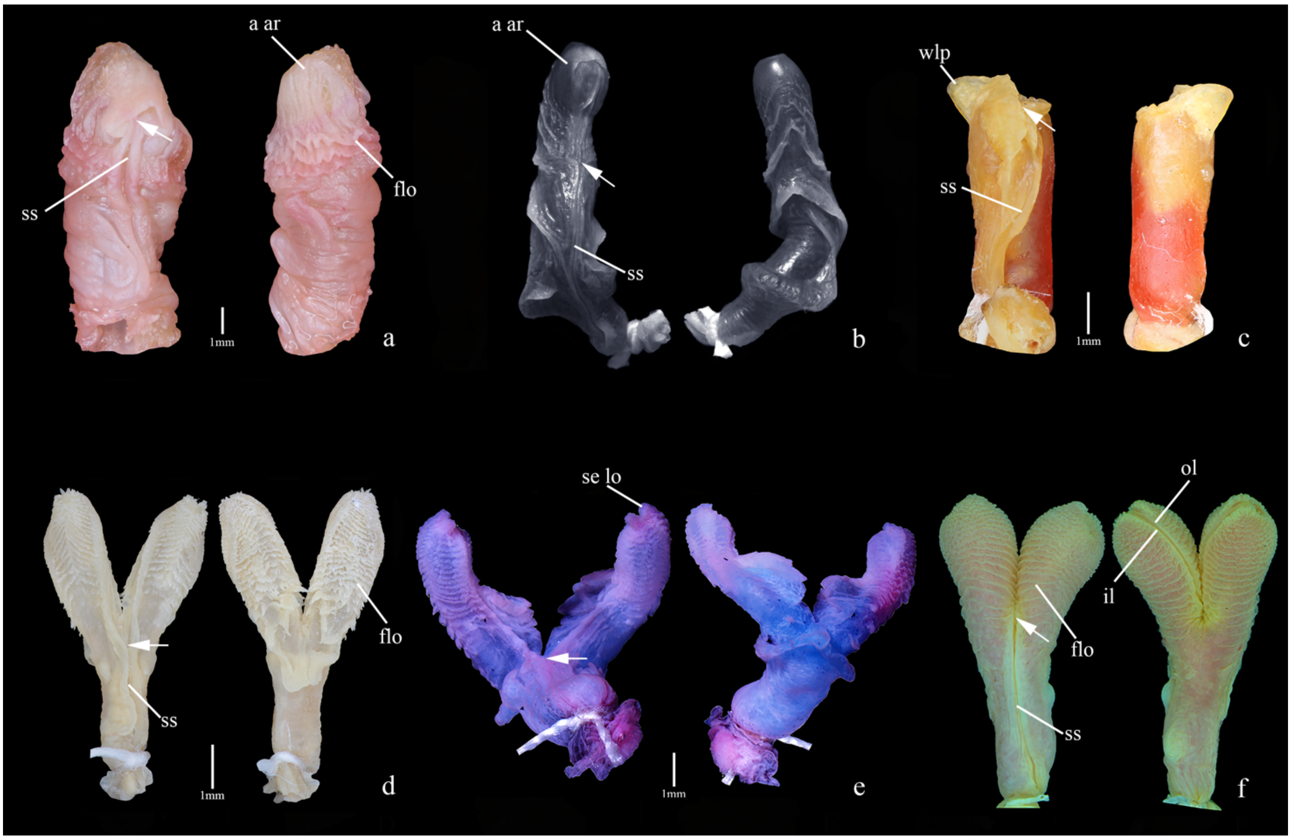

Figure 16.5 Hemipenes of (a) *Afrotyphlops lineolatus* (Typhlopidae); (b) *Trilepida macrolepis* (Leptotyphlopidae); (c) *Cylindrophis melanotus* (Cylindrophiidae; possibly incompletely everted); (d) *Trachyboa boulengeri* (Tropidophiidae); (e) *Tropidophis paucisquamis* (Tropidophiidae); (f) *Anilius scytale* (Aniliidae; courtesy A. Prudente). Sulcate (left) and asulcate (right) views; (b) and (e) reproduced with permission [109, 120]. See Box 16.1 for abbreviations.

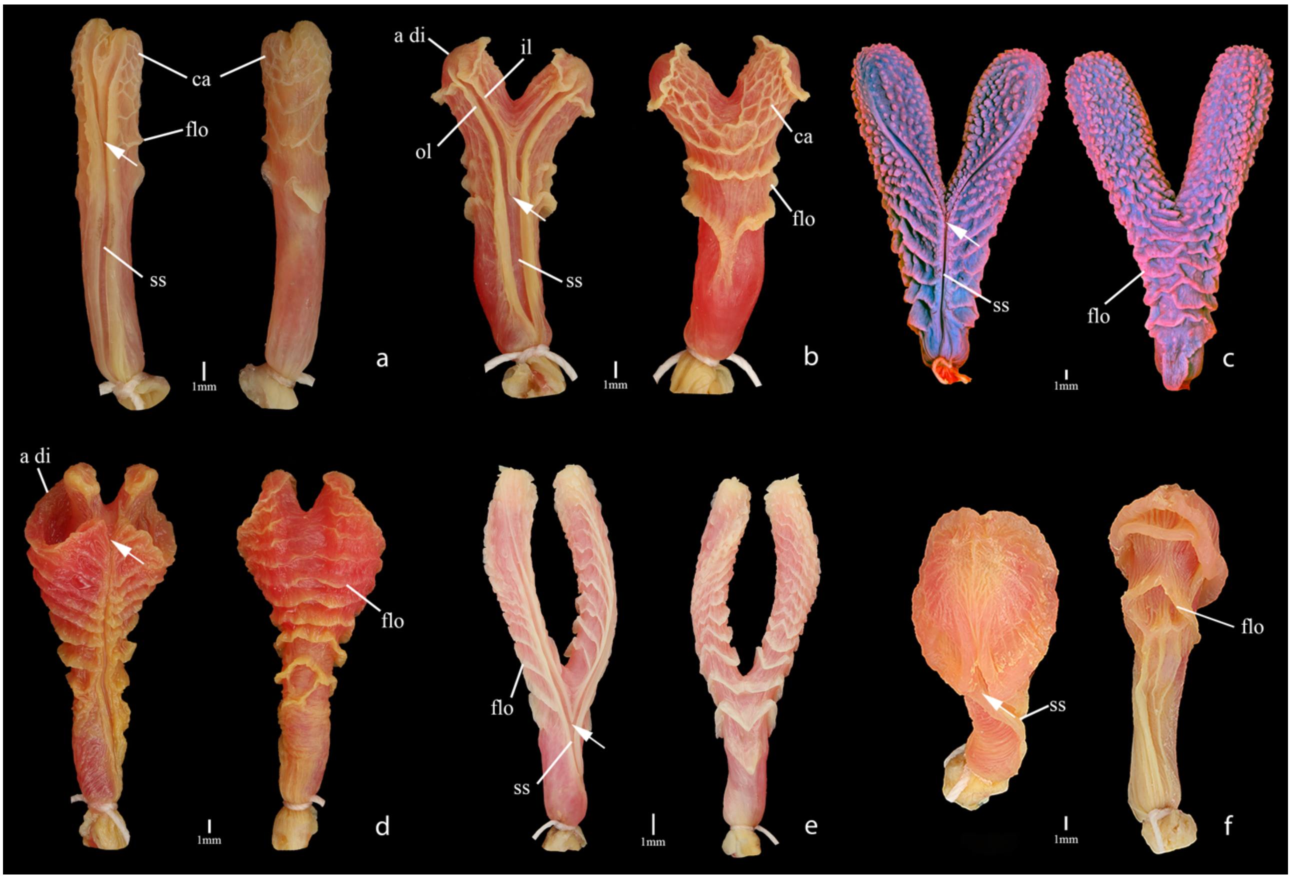

Figure 16.6 Hemipenes of (a) *Morelia spilota* (Pythonidae); (b) *Xenopeltis unicolor* (Xenopeltidae); (c) *Epicrates cenchria* (Boidae; courtesy P. Passos); (d) *Charina bottae* (Charinidae); (e) *Candoia bibroni* (Candoiidae); (f) *Calabaria reinhardtii* (Calabariidae). Sulcate (left) and asulcate (right) views, except left of (f), an apical view of the sulcate surface. See Box 16.1 for abbreviations.