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Comparative anatomy of the middle ear in lizards with comments on the evolutionary changes of its structures

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The middle ear in Squamates is composed of three elements: columella, extracolumella, and tympanic membrane, and its main function is transforming sound pressures into vibrations and transmitting these to the inner ear. Most middle ear studies mainly focus on its functional aspects, while few describe the anatomy in detail. In lizards, the morphology of the columella is highly conservative, while the extracolumella shows the variation in its presence/absence, size, and the number of processes present on the structure. There are three typical middle ear organization patterns observed in lizards: the gekkonid, iguanid, and scincid types, which are defined by their morphology and describe most of the variation found across lizards. However, some forms do not correspond to any of these types and are called “divergent” or “degenerate” forms. These occur less frequently among lizards, and most of them result from extreme modifications of the iguanid type. In this paper, we used cleared and double-stained specimens to study the middle ear in 38 species belong to 24 genera, in a comparative framework and described substantial variation in the shape of the pars superior and anterior process of the extracolumella. Geckos showed a more complex morphology in the shape of the extracolumella, including an expansion of this structure. The data collected were combined with data from previous descriptions, and these characters were used for ancestral state reconstruction using parsimony and Bayesian approaches. One of the characters studied shows high levels of homoplasy, while two of them would serve to diagnose some clades. We identified synapomorphies for the clades Gekkota, Gymnophthalmidae, and Pleurodonta, in addition to some extracolumellar features that complement the morphological definition of the three standard middle ear types described for lizards.

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Abstract

The middle ear in Squamates is composed of three elements: columella, extracolumella, and tympanic membrane, and its main function is transforming sound ~~pressures~~ into vibrations and transmitting these to the inner ear. Most middle ear studies mainly focus on its functional aspects, while few describe the anatomy in detail. In lizards, the morphology of the columella is highly conservative, while the extracolumella shows the variation in its presence/absence, size, and the number of processes present on the structure. There are three typical middle ear organization patterns observed in lizards: the gekkonid, iguanid, and scincid types, which are defined by their morphology and describe most of the variation found across lizards. However, some forms do not correspond to any of these types and are called "divergent" or "degenerate" forms. These occur less frequently among lizards, and most of them result from extreme modifications of the iguanid type. In this paper, we used cleared and double-stained specimens to study the middle ear in 38 species belong to 24 genera, in a comparative framework and described substantial variation in the shape of the pars superior and anterior process of the extracolumella. Geckos showed a more complex morphology in the shape of the extracolumella, including an expansion of this structure. The data collected were combined with data from previous descriptions, and these characters were used for ancestral state reconstruction using parsimony and Bayesian approaches. One of the characters studied shows high levels of homoplasy, while two of them would serve to diagnose some clades. We identified synapomorphies for the clades Gekkota, Gymnophthalmidae, and Pleurodonta, in addition to some extracolumellar features that complement the morphological definition of the three standard middle ear types described for lizards.

Introduction

The ear is a morphologically complex system that performs a dual function – equilibrium and hearing. The ear has been described in three divisions: the outer, middle, and inner ear (Baird, 1970). The outer ear includes the meatal cavity, closure muscles, and modifications of skin that detect sound waves and conduct them to the middle ear. In the middle ear (composed by the tympanic membrane, extracolumella, and columella) the sound pressures are transformed into vibrations, which are transmitted to the inner ear. Finally, the inner ear is constituted by the membranous or endolymphatic labyrinth where the sense organs are located, and the perilymphatic labyrinth that is an area of fluid-filled cavities in which the movements continue as fluid oscillations, impacting the cochlea (Baird, 1960, 1970; Wever, 1978). Most of the studies around the lizard ear are focused on the study of processes of conductivity of sound, and the electrophysiological aspects of the inner ear (e.g., Shute & Bellairs, 1953; Baird, 1960; Wever et al., 1963; Schmidt, 1964; Wever et al., 1965; Baird, 1967; Suga & Campbell, 1967; Wever, 1967, 1970; Baird & Marovitz, 1971; Wever, 1971; Manley, 1972a; Wever & Gans, 1972; Miller, 1974; Werner, 1976; Manley, 2000; Werner & Igić, 2002; Wibowo, Brockhausen & Köppl, 2009; Manley, 2011). The standard approach of studies on the middle ear has been mainly focused on investigating the functional aspects of the transformation of sound waves into vibrations, with some work describing a few morphological features (e.g., Wever & Peterson, 1963; Wever & Wener, 1970; Manley, 1972b; Werner & Wever, 1972; Wever, 1973; Manley, 2011; Han & Young, 2016). Other studies, although less common, have concentrated specifically on the anatomy of the middle and outer ear (e.g., Versluys, 1898; Earle, 1961a; Earle, 1961b; Earle, 1961c; Posner & Chiasson, 1966; Iordansky, 1968; Wever, 1978). The studies that could be considered the most relevant contributions to knowledge of the middle ear in lizards are those by Versluys (1898) and Wever (1978). Versluys (1898) described essential information about the morphology of the structures and associated muscles, whilst Wever (1978) contributed to the knowledge of the function of the inner ear, describing details of the structures of the middle and outer ear and its taxonomic distribution, information that has been used in cladistics studies (e.g., Kluge, 1987).

In lizards, the general pattern of the middle ear (Fig. 1) is a simple structure composed of the columella and extracolumella that are suspended in the tympanic cavity, and the tympanic membrane (Versluys, 1898; Baird, 1970; Wever, 1978; Saunders et al., 2000). The columella (Fig. 1A) is a slender rod whose main part is osseous, and its distal end is cartilaginous. The proximal end is formed by a footplate, which inserts into the oval window, and connects with the cochlea; the distal end is connected to the extracolumella. The extracolumella (Fig. 1A–B) is a cartilaginous structure formed by a shaft that might have a variable number of processes (two to four), namely: superior and inferior ~~pars~~, and the anterior and posterior processes. These processes meet the internal surface of the tympanic membrane in a cruciform arrangement. The principal extracolumellar processes are the pars superior and pars inferior, which form a vertical shaft whose function is to transmit the vibrations and tense the tympanic membrane. In most of the species, the pars superior and inferior are associated with the extracolumellar and ~~the~~ intratympanic ligaments, respectively. Also, in most of the gekkotans, the pars superior is associated with the extracolumellar muscle that probably exercises tension on the membrane and the other structures of the middle ear (Wever & Werner, 1970; Wever, 1978). The anterior and posterior processes arise from the pars superior and pars inferior and are smaller than the structures from where they originate, sometimes being poorly defined or absent in some species (Wever, 1978). These four processes (Fig. 1B) help to tense and to stabilize the extracolumella (Baird, 1970; Wever, 1978; Saunders et al., 2000). The extracolumella is the element of the middle ear in lizards that displays the most morphological variation. This variation tends to occur in the shape and number of the extracolumella processes, in the form of the connection between the columella and the extracolumella, and in the shape of the internal process, which is an additional cartilaginous element connected to the quadrate that can be either present or absent (Wever, 1978).

Based on the overall morphology, Wever & Werner (1970) defined three main patterns of middle ears in lizards, namely the gekkonid, iguanid, and scincid types. Additionally, different forms that do not correspond to the previous patterns were considered as “divergent” types, which mostly were morphologies that departed the iguanid type (Wever, 1978). These three standard types exhibit the same primary structure described above but differ in some details associated with both presence and form of certain structures. In the iguanid type (Wever, 1978, Fig. 6-10), the most generalized type in lizards, there is an additional cartilaginous shaft termed the ‘internal process’ by Versluys (1898), which arises from the extracolumellar shaft and expands dorsally and anteriorly to attach to the quadrate bone. In the gekkonid type (Wever, 1978, Fig. 6-30), there is no internal process, but there is a tympanic muscle called the ‘extracolumellar muscle’ (Wever & Werner, 1970), that runs from the distal edge of the pars superior to the ceratohyal process. The scincid type (Wever, 1978, Fig. 6-42) lacks both the internal process and the tympanic muscle; and the divergent types show features that do not match with any of the aforementioned types (Wever, 1978).

The middle ear has evolved independently several times in vertebrates (Lombard & Bolt, 1979; Clack, 1997; Clack, 2002; Manley, 2010). This structure is absent in the stem reptiles, but changes in the whole-body structure of these early reptiles during the transition to the different orders of living reptiles resulted in unique middle ear morphologies developing in each one of the subclasses, Anapsida, Diapsida and Synapsida (Saunders et al., 2000). In lizards, the studies presented by Versluys (1898), Olson (1966), and Baird (1970) made anatomical comparisons of the outer and middle ear among taxa making some evolutionary assumptions. According to Olson (1966), the middle ear is associated with the masticatory apparatus and is therefore highly susceptible to adaptive modifications and, although some morphological types are conservative, others are rather diverse. Thus, the middle ear structures could prove to be useful in providing phylogenetic information within major morphological types, but not when relationships between these types are considered (Olson, 1966). Baird (1970) suggests that in most terrestrial and arboreal lizards, the middle ear corresponds to the iguanid pattern, but it is common to find related taxa that show morphological variations correlated to other features of the ear, or variations that may relate more directly to habits or habitats. However, this kind of affirmation is preliminary because the diversity of morphologies of the external and middle ear across lizards is barely understood and requires further investigation (Wever, 1968; Baird, 1970). This study aims to describe morphological features of the middle ear of lizards, comparing some of the component structures in a phylogenetic context in order to draw a preliminary scenario of middle ear evolution during lizard diversification.

Materials & Methods

Comparative Anatomy

We examined the middle ear of cleared and double-stained specimens of 38 species of lizards, belonging to 24 genera and 12 families (Table 1). The specimens examined belong to the Herpetological Collection of the Museo Javeriano de Historia Natural Lorezo Uribe, S.J. (MUJ) in Pontificia Universidad Javeriana, (Bogotá, Colombia), the Herpetological Collection of the

Instituto de Ciencias Naturales (ICN) in Universidad Nacional de Colombia (Bogotá, Colombia), the Collection of Reptiles of the Museo de Herpetología (MHUA) in Universidad de Antioquia (Medellín, Colombia), and the Museu de Zoologia da Universidade de São Paulo (MZUSP) (São Paulo, Brazil). Voucher specimen information is provided in Table S1. The middle ears of the species studied were described following the nomenclature proposed by Wever (1978) and analyzed in a comparative framework with the data available in the literature. The summary of the variation described is presented in Tables 2 and 3.

As a note on taxonomy within this paper, we have considered the genus *Mabuya* in the broad sense. The genus *Mabuya* was extensively rearranged in 2012, and here we examined species from the clade referred to as "American Mabuyas," which now encompasses eight genera (Hedges & Conn, 2012). In this study, we used specimens from two of these American genera – *Copeoglossum nigropunctatum* and *Marisora falconensis* – together with other undescribed species, but for simplicity, we have referred all of them to the genus *Mabuya* s.l.

Ancestral Reconstruction

Character states were coded from direct observations of the material described and from published data. The sources of the information published for each species included in the analysis are given in Table 4. In order to reconstruct the evolutionary changes, the morphological characters defined were optimized on the phylogenetic hypothesis based on molecular data proposed by Zheng & Wiens (2016), using maximum parsimony (MP) and Bayesian approaches. The parsimony analysis used equal weighting, the characters were considered as unordered and the analysis was performed using MESQUITE 3.5 (Maddison & Maddison, 2018). The Bayesian analysis used the "ARD" (backward & forward rates between states) and "ER" (single-rate) models, and was conducted using R 4.0.2 (R Core Team, 2020) and the phytools package (Revell, 2012). To perform the parsimony analysis, we pruned the tree to include only the species studied here, and in some cases, we substituted certain tips following two rules: 1) if several species from a single genus had the same character state, these were collapsed into a single terminal with the genus name (the list of species collapsed and their corresponding terminal taxon are provided in Table S2); 2) if one or more examined taxa were not included in the molecular phylogenetic analysis, these taxa were included as terminals in a polytomy, assuming that the genera are monophyletic. Features with unknown character states were treated as missing "?", and inapplicable characters as dash "-". To conduct the Bayesian analysis, we pruned the topology by collapsing the genera without data to a single terminal for family.

Results

Middle ear

Lizards occupy a wide diversity of habitats (e.g. terrestrial, arboreal, fossorial, aquatic), and for this reason it is expected that they exhibit significant variation in their middle ear structure according to the way, and medium through which, they perceived sounds. As anticipated, according to the literature, the columella bone retains relatively the same shape but changes in proportions, displaying minor differences among lizards' clades. Nevertheless, the extracolumella shows more significant variation in the number and shape of its processes (Fig. 1).

Columella. The main body of the columella is an elongated osseous rod (Fig. 1A). Its proximal end is formed by an expanded footplate, which inserts into the oval window (the opening that leads to the inner ear); while at its distal end, the columella connects to the extracolumella. The variation found among the specimens examined was mainly in the presence of the stapedial foramen, the presence of a cartilaginous stalk on the distal end, differences in the length of the columella in relation to the extracolumellar vertical axis, and a slight expansion of the distal end. The variation of the columella observed in the examined specimens is summarized in Table 2.

The stapedial foramen (Fig. 2A) pierces the columella near the proximal end, and this opening allows the passage of the stapedial artery (Greer, 1976). In the present study, this character was observed in the gekkotans *Gonatodes albogularis*, *G. concinnatus*, *Hemidactylus brasilianus*, *Phelsuma madagascariensis*, and *Tarentola mauritanica* (Fig. 2A). This foramen is absent (Fig. 2B) in the remaining species studied, although it has been reported in lizards of the family Dibamidae (Greer, 1976; Estes, de Queiroz & Gauthier, 1988; Gauthier, Estes & de Queiroz, 1988) and embryonic stages of amphisbaenians (Kearney, 2003).

There are some differences in the relationship between the length of the columella and extracolumella. The length of the columella (measured from the footplate to the joint with the extracolumella; Fig. 1A), can be longer (Fig. 2C), subequal (Fig. 3A), or shorter (Fig. 3B), than the length of the extracolumellar vertical axis (taken from the upper edge of the pars superior to the lower edge of the pars inferior; Fig. 1B). In the specimens studied, the length of the columella was longer in *Acanthocercus atricollis* (Agamidae); *Mabuya nigropunctata* (Scincidae); *Tarentola mauritanica* (Phyllodactylidae); and *Tretioscincus bifasciatus* (Gymnophthalmidae; Fig. 2C). The columella length is similar to the extracolumella vertical axis in *Acanthodactylus* cf. *schmidti* (Lacertidae); *Anolis* spp., (Dactyloidae); *Hemidactylus brasilianus* and *Phelsuma madagascariensis* (Gekkonidae); *Mabuya* spp. (except in *M. nigropunctata*; Scincidae); *Riama striata* (Gymnophthalmidae); *Stenocercus trachycephalus* (Tropiduridae; Fig. 3A); and *Thecadactylus rapicauda* (Phyllodactylidae). The columella was shorter in *Anadia bogotensis, Gelanesaurus cochranae, Loxopholis rugiceps, Neusticurus medemi, Pholidobolus montium,* and *P. vertebralis* (Gymnophthalmidae); *Gonatodes albogularis, G. concinnatus* (Sphaerodactylidae; Fig. 2A); *Hoplocercus spinosus, Morunasaurus groi* (Hoplocercidae); *Lialis jicari* (Pygopodidae; Fig. 3B); and *Tropidurus pinima* (Tropiduridae).

A slight expansion of the osseous distal end of the columella was observed in *Acanthocercus atricollis* (Agamidae); *Acanthodactylus* cf. *schmidti* (Lacertidae); *Anolis* spp. (Dactyloidae; Fig. 3C); *Mabuya* spp. (Scincidae); *Morunasaurus groi* (Hoplocercidae); *Lialis jicari* (Pygopodidae; Fig. 3B); *Loxopholis rugiceps, Pholidobolus vertebralis, Tretioscincus bifasciatus* (Gymnophthalmidae; Fig. 2C); *Phelsuma madagascariensis* (Gekkonidae); *Stenocercus trachycephalus* (Fig. 3A); and *Tropidurus pinima* (Tropiduridae). The remaining species do not show this expansion. Two conditions of the distal end of the columella – expanded end or constant size along the columellar shaft – were observed in different specimens of *Anadia bogotensis* (Gymnophthalmidae), specimen ICN 2987 (slight expansion) and ICN 2178 (constant width).

We detected a slight difference in the cartilaginous rim of the footplate. The rim can form a complete ring around the footplate of the columella, as observed in *Gonatodes albogularis* MUJ-665, or be a discontinuous and very thin ring, as observed in *Anolis auratus* MUJ 590. In some specimens this ring is absent altogether (e.g. *Pholidobolus vertebralis* ICN 5719). We do not discount that differences in the development of the cartilaginous ring of the footplate could be an artifact of the staining used in the preparations, and may not represent true morphological variation.

Columella–extracolumella joint. This joint varies in the presence/absence of connective tissue and the form of the joint. Connective tissue was observed in *Acanthocercus atricollis* (Agamidae); *Acanthodactylus* cf. *schmidti* (Lacertidae); *Anolis* spp., except *A. auratus* (Dactyloidae; Fig. 3C); *Hoplocercus spinosus* (Hoplocercidae; Fig. 2B); *Mabuya nigropunctata, Mabuya* sp. 2 (Scincidae); *Riama striata, Tretioscincus bifasciatus* (Gymnophthalmidae; Fig. 2C); *Stenocercus trachycephalus* (Tropiduridae; Fig. 3A); and *Tarentola mauritanica* (Phyllodactylidae). When the two elements are joined by connective tissue, the lateral end of the columella is cartilaginous. This condition was observed in *Anolis antonii*, *A. chrysolepis*, *A. fuscoauratus, A. maculiventris, A. trachyderma* (Dactyloidae); *Hoplocercus spinosus* (Hoplocercidae; Fig. 2B); *Mabuya nigropunctata* and *Mabuya* sp. 2 (Scincidae). When the connective tissue is surrounding the columella–extracolumella joint, the cartilaginous shaft of the columella is hidden. This formation of joint and connective tissue was observed in *Acanthocercus atricollis* (Agamidae); *Acanthodactylus* cf. *schmidti* (Lacertidae); *Anolis tolimensis* (Dactyloidae); *Riama striata, Tretioscincus bifasciatus* (Gymnophthalmidae; Fig. 2C); *Stenocercus trachycephalus* (Tropiduridae); and *Tarentola mauritanica* (Phyllodactylidae). The remaining specimens do not show connective tissue (Fig. 2A, 3B). The specimens of *Anolis mariarum* and *A. ventrimaculatus* exhibit variation in the presence of the connective tissue. In specimens ICN 5808 and MHUA 10014 of *A. mariarum* the connective tissue is seen between the joint, while specimen MHUA 10013 does not have connective tissue; and in *A. ventrimaculatus,* the specimens MHUA 10671 and MHUA 10672 display the connective tissue between the joint, while in specimen PUJ 338 connective tissue is absent.

Extracolumella. Usually, this element is cartilaginous. The extracolumella is composed of a small shaft and two to four processes attached to the tympanic membrane (Fig. 1B). This structure is present in all the specimens examined, and represents the most variable element from the middle ear of lizards. The variation in this element involves the presence/absence of the anterior and/or posterior process, the shape of the four processes, and the presence/absence of the internal process. The extracolumella variation observed in the examined specimens is summarized in Table 3.

The general pattern of the extracolumella exhibits four processes – superior and inferior pars, and the anterior and posterior processes – all attached to the tympanic membrane (Fig. 1B). The pars superior and the pars inferior form the vertical axis of the extracolumella, and from this axis, the anterior and posterior processes arise laterally. The variation observed in this pattern is the lack of the anterior process in some species, or the lack of both processes (anterior and posterior) in others. The general pattern (the presence of four processes of the extracolumella; Fig. 1B), was observed in the specimens of *Acanthocercus atricollis, Leiolepis belliana* (Agamidae); *Acanthodactylus* cf. *schmidti* (Lacertidae); *Anolis* spp. (Dactyloidae); *Hemidactylus brasilianus, Phelsuma madagascariensis* (Gekkonidae; Fig. 4A); *Tarentola mauritanica, Thecadactylus rapicauda* (Phyllodactylidae; Fig. 4B); *Lialis jicari* (Pygopodidae; Fig. 4C); *Gonatodes albogularis, G. concinnatus* (Sphaerodactylidae; Fig. 5A); *Hoplocercus spinosus, Morunasaurus groi* (Hoplocercidae; Fig. 5B); *Stenocercus erythrogaster, S. trachycephalus,* and *Tropidurus pinima* (Tropiduridae; Fig. 5C). The anterior process is absent in *Anadia bogotensis, Gelanesaurus cochranae* (Fig. 6A)*, Loxopholis rugiceps, Neusticurus medemi, Pholidobolus montium, P. vertebralis, Riama striata, Tretioscincus bifasciatus* (Gymnophthalmidae); *Stellagama stellio* (Agamidae; Fig. 6B); *Cnemidophorus lemniscatus* (Teiidae); and *Mabuya* spp.

(Scincidae; Fig. 6C). The posterior process is absent in *Cnemidophorus lemniscatus* (Teiidae); *Loxopholis rugiceps, Pholidobolus vertebralis* (Gymnophthalmidae); *Mabuya* spp. (Scincidae; Fig. 6C); and *Stellagama stellio* (Agamidae; Fig. 6B).

All four extracolumellar processes display some morphological variation in their shape. The pars superior shows two principal variations, determined by the presence of an extension of the upper edge, which varies in the orientation of the extension (anterior or posterior). The upper edge of the pars superior has one posterior extension in the gekkotans *Gonatodes albogularis, G. concinnatus* (Fig. 5A), *Hemidactylus brasilianus, Lialis jicari* (Fig. 4C)*, Phelsuma madagascariensis* (Fig. 4A)*, Tarentola mauritanica,* and *Thecadactylus rapicauda* (Fig. 4B); while in *Tropidurus pinima* (Tropiduridae), the extension is anterior (Fig. 5C). In all of these species, the distal end of the posterior extension of the pars superior is curved downward, except in *Lialis jicari* (Fig. 4C) in which this distal end is slightly straight, like the anterior extension in *Tropidurus pinima* (Fig. 5C). The remaining species lack any of these extensions. Additionally, the upper edge of the pars superior displays three kinds of surfaces: a slightly plane edge (Fig. 4A–C; 5A, C; 6A), a rounded edge (Fig. 5B; 6B), and an edge with small peaks (Fig. 6C). The upper edge is slightly plane in *Acanthocercus atricollis,, Leiolepis belliana* (Agamidae); *Acanthodactylus* cf. *schmidti* (Lacertidae); *Anadia bogotensis, Gelanesaurus cochranae* (Fig. 6A), *Loxopholis rugiceps, Neusticurus medemi, Pholidobolus montium, P. vertebralis, Riama striata, Tretioscincus bifasciatus* (Gymnophthalmidae); *Anolis* spp. (Dactyloidae); *Cnemidophorus lemniscatus* (Teiidae); *Gonatodes albogularis*, *G. concinnatus* (Sphaerodactylidae; Fig. 5A); *Hemidactylus brasilianus, Phelsuma madagascariensis* (Gekkonidae; Fig. 4A); *Lialis jicari* (Pygopodidae; Fig. 4C); *Stenocercus erythrogaster, S. trachycephalus, Tropidurus pinima* (Tropiduridae; Fig. 5C); *Tarentola mauritanica* and

Thecadactylus rapicauda (Phyllodactylidae; Fig. 4B); while the edge is rounded in *Stellagama stellio* (Agamidae; Fig. 6B); *Hoplocercus spinosus* and *Morunasaurus groi* (Hoplocercidae; Fig. 5B). Finally, an edge with three small peaks is observed in the specimens of *Mabuya* spp. (Scincidae; Fig. 6C).

The pars inferior is the extracolumellar process with the most conservative morphology. This process displays an inverted triang~~le~~ shape, with the thicker portion contacting the pars superior (Fig. 1B), and the thinner portion at the distal end. The only variation observed is in the distal end which can appear sharp or thick. The sharp distal end (Fig. 1B) is present in all the specimens studied except in *Gonatodes albogularis*, *G. concinnatus* (Sphaerodactylidae; Fig. 5A); *Hemidactylus brasilianus* (Gekkonidae); and *Thecadactylus rapicauda* (Phyllodactylidae; Fig. 4b); which show a thick distal end with small projections on the pars inferior.

Both processes, anterior and posterior, arise from the superior half of the vertical axis of the extracolumella, which is formed by the pars superior and inferior (Fig. 1B). Usually, the processes are thin and extended laterally, but in some species, these are thick and/or turned downward (see below).

The anterior process appears in three main shapes: short (Fig. 3C), long and pointed (Fig. 4C, 5B–C), or long with some small and sharp projections (Fig. 4A–B). The first type, a short and pointed anterior process, is the simplest morphology for this process, and was observed in the studied specimens of *Anolis* spp. (Fig. 3C), except *A. ventrimaculatus* (Dactyloidae) which shows a short process, but its distal end has two small pointed prolongations (see below). The second type, a long and pointed process, was observed in *Acanthocercus atricollis, Leiolepis belliana* (Agamidae); *Acanthodactylus* cf. *schmidti* (Lacertidae); *Hoplocercus spinosus, Morunasaurus groi* (Hoplocercidae; Fig. 5B); *Lialis jicari* (Pygopodidae; Fig. 4C); *Stenocercus* *erythrogaster, S. trachycephalus,* and *Tropidurus pinima* (Tropiduridae; Fig. 5C). In *Lialis jicari* (Fig. 4C), the anterior process is oriented downward, while in the other species this process is straight. The third type, a long thick extension with some small and sharp prolongations (Fig. 4A–B), was observed in *Hemidactylus brasilianus* and *Phelsuma madagascariensis* (Gekkonidae; Fig. 4A); *Tarentola mauritanica* and *Thecadactylus rapicauda* (Phyllodactylidae; Fig. 4B). Unlike the previous species, *Gonatodes albogularis* and *G. concinnatus* (Sphaerodactylidae; Fig. 5A) present short anterior processes with the distal ends turning downward, simulating a hook that is rounded in *G. albogularis*, while it forms a right angle in *G. concinnatus* (Fig. 5A). There is no anterior process in the specimens of *Anadia bogotensis, Gelanesaurus cochranae* (Fig. 6A)*, Loxopholis rugiceps, Nesticurus medemi, Riama striata, Tretioscincus bifasciatus* (Gymnophthalmidae); *Cnemidophorus lemniscatus* (Teiidae); all specimens of *Mabuya* spp. (Scincidae; Fig. 6C); or *Stellagama stellio* (Agamidae; Fig. 6B).

The posterior process shows a slight variation in both the length and thickness of its extension. Among the specimens studied, most of them show an extended and thin, or a short and acute process, except for *Lialis jicari* (Pygopodidae) which shows a short thick posterior process turned upward, simulating a hook (Fig. 4C). The extended thin posterior process was observed in *Acanthocercus atricollis* (Agamidae); *Anolis ventrimaculatus* (Dactyloidae); *Hoplocercus spinosus* (Hoplocercidae); *Nesticurus medemi* (Gymnophthalmidae); *Phelsuma madagascariensis* (Gekkonidae; Fig. 4A); *Stenocercus erythrogaster, S. trachycephalus, Tropidurus pinima* (Tropiduridae; Fig. 5C); *Tarentola mauritanica* and *Thecadactylus rapicauda* (Phyllodactylidae; Fig. 4B); while the short and acute posterior process was observed in *Acanthodactylus* cf. *schmidti* (Lacertidae); *Anadia bogotensis, Gelenasaurus cochranae* (Fig. 6A)*, Pholidobolus montium, Riama striata, Tretioscincus bifasciatus* (Gymnophthalmidae);

Anolis spp., except *A. ventrimaculatus* (Dactyloidae); *Gonatodes albogularis*, *G. concinnatus* (Sphaerodactylidae; Fig. 5A); *Hemidactylus brasilianus* (Gekkonidae); *Leiolepis belliana* (Agamidae); and *Morunasaurus groi* (Hoplocercidae; Fig. 5B). The specimens of *Cnemidophorus lemniscatus* (Teiidae); *Loxopholis rugiceps* (Gymnophthalmidae); *Mabuya* spp. (Scincidae; Fig. 6C); and *Stellagama stellio* (Agamidae; Fig. 6B) do not show the posterior process.

The internal process originates from the extracolumellar shaft, and extends until it establishes contact with the tympanic conch of the quadrate bone. Its origin is thin, but as it extends, its edge widens. There was no obvious morphological variation observed in this feature. This process was only found in *Acanthocercus atricollis, Leiolepis belliana, Stellagama stellio* (Agamidae); *Acanthodactylus* cf. *schmidti* (Lacertidae); *Anolis* spp. (Dactyloidae; Fig. 3C); *Cnemidophorus lemniscatus* (Teiidae); *Hoplocercus spinosus*, *Morunasaurus groi* (Hoplocercidae; Fig. 5B); and *Stenocercus erythrogaster, S. trachycephalus,* and *Tropidurus pinima* (Tropiduridae; Fig. 5C). This process is absent in the remaining studied species.

In some specimens, the extracolumella, usually cartilaginous, exhibits a red-stained region of different sizes and in different degrees of staining, in the central axis, and the lateral processes, indicating the presence of osseous tissue. This feature was observed in *Acanthocercus atricollis, Leiolepis belliana, Stellagama stellio* (Agamidae; Fig. 6B); *Anolis* spp. (Dactyloidae; Fig. 3C); *Hemidactylus brasilianus* (Gekkonidae); *Morunasaurus groi* (Hoplocercidae; Fig. 5B); *Stenocercus trachycephalus* (Tropiduridae; Fig. 3A); and *Thecadactylus rapicauda* (Phyllodactylidae; Fig. 4B). This feature is particularly noticeable in some specimens of the *Anolis* species in which the red-stained area appears bigger and more intense than in the other species.

Ancestral Reconstruction

Definition of characters: Based on the morphological descriptions presented above, the following middle ear characters were defined to analyze them in a phylogenetic framework. Despite the limited sampling, the results of this survey provide a baseline to understand overall variation and outline a general scenario about the evolutionary changes of selected features of the middle ear in lizards.

- Character 1. Length of the columella relative to the extracolumella central axis length. [0] equal length (Fig. 2C); [1] longer (Fig. 3A); and [2] shorter (Fig. 3B).
- Character 2. Extracolumella. [0] reduced (Fig. 4A); [1] expanded; [2] absent; [3] extensive. To test if there is a general pattern in the reduction of the extracolumella processes, we summarized the available information on this structure into four states, including the absence of the extracolumella. A reduced extracolumella refers to a lack of one or more processes. An expanded extracolumella indicates the presence of four developed processes – the superior and inferior pars, and the anterior and posterior processes. Finally, an extensive extracolumella refers to a case where this structure runs anteriorly along, and contacts, the skin.
- Character 3. Nature of the Internal Process. [0] Absent (Fig. 2A); [1] present (Fig. 2B).

Character mapping: Characters were optimized using parsimony with unordered states and equally weights, and Bayesian analyses with the all rates different (ARD) and the equal rates (ER) models. The summaries of the optimization of characters with parsimony are presented in Figures 7 and 8, and the values of the posterior probabilities of the Bayesian reconstructions in Table 5 (see the complete mapping with parsimony in Fig. S1, and Bayesian reconstructions in Fig. S2-S4, and the posterior probability values in Table S3).

Character 1. Length of the columella relative to the extracolumella central axis length. The parsimony approach (Fig. 7; Fig. S1) shows the ancestral condition of the columella's length relative to the extracolumella central axis length for Squamata [node 2] as ambiguous between the states shorter and longer. Also, there is ambiguity between the three states of the character for the ancestor of Teiioidea [27], and between the states longer and equal length in Lacertoidea [26], Lacertidae [39], (Amphisbaenidae + Lacertidae) [33]. The shorter columella state was the reconstructed state for the ancestral node of Gekkota [4] and Pygopodidae [7]; and the longer columella state for the nodes of (Xantusiidae (Gerrhosauridae + Cordylidae)) [19], Scincoidea [18], Anguimorpha [43], Agamidae [55], Acrodonta [51], Phrynosomatidae [73], Pleurodonta [60], Iguania [50] (Anguimorpha + Iguania) [42], (Lacertoidea (Serpentes (Anguimorpha + Iguania))) [25], (Scincoidea (Lacertoidea (Serpentes (Anguimorpha + Iguania)))) [17]. There is no available information for the clades Amphisbaenia [34], (Amphisbaenidae + Trogonophidae) [38], in (Bipedidae ((Cadeidae + Blanidae) (Amphisbaenidae + Trogonophidae))) [35].

The Bayesian analysis (Table 5; Table S2; Fig. S2) with both models shows ambiguity for the ancestral node of Squamata [2] with equal probabilities for all states (Table 5). The ARD reconstruction found ambiguity for all other clades with similar values for each state. However, the higher support values for these clades are for the longer columella. Similarly, the ER reconstruction found ambiguity for all clades with equal values of probability for each character state for all these clades (Table 5).

Character 2. Extracolumella. The parsimony approach (Fig. 8A, Fig. S1) defines the reduced extracolumella as the ancestral condition for Squamata [node 2]. This state was also reconstructed for the nodes of the clades Scincoidea [18], Teiioidea [27], Lacertidae [39], Amphisbaenia [34], (Amphisbaenidae + Lacertidae) [33], Lacertoidea [26], Anguimorpha [43], Agamidae [55], Acrodonta [51], Iguania [50], (Anguimorpha + Iguania) [42], and (Lacertoidea (Serpentes (Anguimorpha + Iguania))) [25], (Scincoidea (Lacertoidea (Serpentes (Anguimorpha + Iguania)))) [17]. The expanded extracolumella was the estimated ancestral state in Gekkota [4], Pygopodidae [7], and Phrynosomatidae [73]; the extensive extracolumella in (Amphisbaenidae + Trogonophidae) [38]; and the absence of extracolumella in (Bipedidae ((Cadeidae + Blanidae) (Amphisbaenidae + Trogonophidae))) [35]. This reconstruction showed an ambiguous state result for the ancestral nodes of the clades (Xantusiidae (Gerrhosauridae + Cordylidae)) [19], and Pleurodonta [60].

There was no conflict between the parsimony method and both models of the Bayesian approach (Table, 5; Table S2; Fig. S3) used to reconstruct the ancestral state of Squamata [2] since the Bayesian analyses show a greater certainty for the reduced extracolumella as the ancestral state (Table 5) although also show a minimum probability for the expanded state. The ARD model reconstruction mostly agrees with the parsimony results except for the following exceptions. At the nodes for Gekkota [4], Pleurodonta [60], Pygopodidae [7], and Phrynosomatidae [73], the higher probability for the ancestral state is for the expanded extracolumella, and for the first three clades (Gekkota [4], Pleurodonta [60], and Pygopodidae [7]) the lower probability is for the absence of it. The ancestral node of Phrynosomatidae [73] shows lower and similar probabilities for the reduced columella and its absence. The ancestral node for the family Lacertidae shows a higher probability for the reduced extracolumella and a lower probability for the expanded one. At the ancestral nodes of (Amphisbaenidae + Trogonophidae) [38], and (Bipedidae ((Cadeidae + Blanidae) (Amphisbaenidae + Trogonophidae))) [35] there is great certainty for the extensive extracolumella state, as the probabilities are very low values for other states. The clade (Xantusiidae (Gerrhosauridae +

Cordylidae)) [19] shows a high probability for the reduced state, and a lower probability for the expanded state.

The ER model reconstruction mostly agrees with the parsimony results but shows the following differences (Table 5). In the ancestral node for Phrynosomatidae [73] there is a high probability for the expanded columella state and a lower one for a reduced columella; the ancestral node of (Amphisbaenidae + Trogonophidae) [38] has a major probability for the extensive state compared to lower likelihood for the absent condition, but at the node for (Bipedidae ((Cadeidae + Blanidae)(Amphisbaenidae + Trogonophidae))) [35] the higher probability is the absence of extracolumella with lower values for the extensive and reduced state. For the ancestral node of Pleurodonta, there is a greater certainty for the expanded extracolumella; and for (Xantusiidae (Gerrhosauridae + Cordylidae)) [19] the higher value is for the reduced state and the lower for the expanded one. With the reconstruction of the ARD model, the ancestral node estimate for the family Lacertidae shows a higher probability for the reduced extracolumella and a lower probability for the expanded one.

Character 3. Nature of the Internal Process. The parsimony reconstructions (Fig. 8B; Fig. S1) estimated the ancestral condition for Squamata [2] is the absence of internal process, which was also the reconstructed state for Gekkota [4] and Gymnophthalmidae [30]; while the evolutionary novelty, the presence of the process, was reconstructed in the ancestral nodes for Teiioidea [27], Lacertoidea [26], Anguimorpha [43], (Anguimorpha + Iguania) [42], Iguania [50], (Lacertoidea (Serpentes (Anguimorpha + Iguania))) [25], Anguidae [47], Acrodonta [51], Pleurodonta [60], and Phrynosomatidae [73]. This reconstruction shows as ambiguous states the ancestral nodes of the clades Scincoidea [18], (Xantusiidae (Gerrhosauridae + Cordylidae)) [19], Xantusiidae [20], (Scincoidea (Lacertoidea (Serpentes (Anguimorpha + Iguania)))) [17], and (Alopoglossidae + Gymnophthalmidae) [29]. The character is not applicable for amphisbaenians.

Contrary to the parsimony results, the reconstructions obtained for this character using the ARD (Table 5; Table S2; Fig. S4); model defined the presence of the internal process as the ancestral state of Squamata [2] with great certainty, while for the ER model (Table 5; Table S2; Fig. S4) it remains ambiguous, showing similar probabilities for both states (Table 5). The ARD model reconstruction mostly agrees with the parsimony results but shows the following exceptions. The presence of an internal process has a high probability in the reconstruction of the nodes of Scincoidea [18], (Xantusiidae (Gerrhosauridae + Cordylidae)) [19], Xantusiidae [20]; (Scincoidea (Lacertoidea (Serpentes (Anguimorpha + Iguania)))) [17]. This reconstruction results in ambiguous state estimations for the ancestral node of Gymnophthalmidae [30] with a higher probability for the absence than the presence of the internal process, while in (Alopoglossidae + Gymnophthalmidae) [29], the higher probability is for the presence. In the amphisbaenian clade [34] the highest likelihood is for the presence of the process and a lower probability for the inapplicability of the character, while the clades (Amphisbaenidae + Trogonophidae) [38], and ((Bipedidae ((Cadeidae + Blanidae) (Amphisbaenidae + Trogonophidae)) [35] show the contrary.

There are a few differences between the reconstructions obtained with the ER model (Table 5) and the parsimony analysis (Fig. 8B; Fig. S1). The ED model found a higher probability for the presence of the process in the ancestral node of the clades Teiioidea [27] and Gymnophthalmidae [30]. For the nodes of the clades where the character is not applicable, the ED model found a higher probability for the presence of the process in the ancestor of amphisbaenians [34], contrary to the values found for the ancestral node of (Amphisbaenidae + Trogonophidae) [38] and (Bipedidae ((Cadeidae + Blanidae) (Amphisbaenidae + Trogonophidae))) [35]. The ancestral nodes of the clades (Amphisbaenidae + Lacertidae) [33], Lacertoidea [26], and Pygopodidae [7] show lower probabilities for the inapplicability of the character, with a higher probability for the presence of the process in the two first clades and the absence in the last one. The ED model analysis found the higher probability for the presence of the process in the ancestral nodes of the clades Xantusiidae [20], (Xantusiidae (Gerrhosauridae + Cordylidae) [19], Scincoidea [18], (Alopoglossidae + Gymnophthalmidae) [29], (Scincoidea (Lacertoidea (Serpentes (Anguimorpha + Iguania)))) [17], that were defined as ambiguous by the parsimony approach.

Discussion

Although there is a lot of information available about the skull of lizards, most of these publications provide incomplete information about the middle ear, being limited to only a few details of the columella and even less about the extracolumella. The main studies regarding the middle ear as an anatomical complex, were realized by Versluys (1898) and Wever (1973, 1978). These authors described morphological details of each structure for many species within a comparative framework that has allowed the establishment of morphological patterns of the middle ear of lizards. Our study adds some substantial details that show more significant morphological variation of the structures of the middle ear across Lacertilia.

The most common pattern of the middle ear in lizards is constituted by columella and extracolumella, structures that display morphological variation (Wever, 1978). A few groups of species show extreme modifications or reductions of some structures of the middle ear (e.g., *Blanus* and *Bipes*, Wever & Gans, 1973; Wever, 1978; *Chamaeleo*, Wever, 1968; and

Rhampholeon, Toerien, 1963), or even the total absence of the middle ear (e.g., *Aprasia* spp., Baird, 1970; Wever, 1978; Daza & Bauer, 2015).

Columella. The typical pattern of the middle ear in lizards shows a quite conservative columella (Wever, 1978). However, in some cases, it is complicated to compare the scarce variation that it presents, due to the terminology used to describe this structure in the published descriptions.

The presence of the stapedial foramen (Fig. 2A) is accepted as a primitive condition in reptiles (Goodrich, 1958; Underwood, 1957; Greer, 1976; Estes, de Queiroz & Gauthier, 1988; Gauthier, Estes & de Queiroz, 1988). The only living lepidosaurs that exhibit this foramen are *Anelytropsis*, *Dibamus*, and some gekkotans (Kamal, 1961; Greer, 1976; Rieppel, 1984; Estes, de Queiroz & Gauthier, 1988; Gauthier, Estes & de Queiroz, 1988; Bauer, 1990). Although this foramen may be present in embryos of amphisbaenians, it is always absent in the adults (Versluys, 1898; Gans, 1978; Kearney, 2003). In gekkotans the foramen has been recorded in all genera of Sphaerodactylidae (Bauer et al., 2018), and some representatives of Eublepharidae (Posner & Chiason, 1966), Gekkonidae (Kluge & Eckardt, 1969; Bauer, 1990; Daza, Aurich & Bauer, 2012; Villa et al., 2018), and Phyllodactylidae (Daza et al., 2017; Villa et al., 2018). As expected, we recorded the presence of the stapedial foramen in all the gekkotans examined (Table 2), confirming its presence in *Gonatodes* (Sphaerodactylidae), *Hemidactylus* and *Phelsuma* (Gekkonidae), and *Tarentola mauritanica* (Phyllodactylidae), as previously registered by Villa et al. (2018) in this last species. We also confirmed the absence of the stapedial foramen in *Lialis* (Pygopodidae) and *Thecadactylus* (Phyllodactylidae), as was previously recorded by Kluge & Nussbaum (1995) and Wever (1974) for these genera. The absence of the stapedial foramen has also been recorded in several genera of Gekkonidae, such as *Christinus* (Bauer, Good & Branch, 1997), *Ebenavia*, *Gehyra*, *Gekko*, and *Paroedura* (Kluge & Nussbaum, 1995); and both states have been described in the genus *Homonota* (Phyllodactylidae) – the absence by Kluge & Nussbaum (1995), and the presence by Daza et al. (2017).

There are some relative differences in the size of the rod and footplate of the columella in lizards. According to Wever (1978), the rod is usually slender and flexible, although in a few species it is thick and sturdy; and the footplate is mostly broadly flared, while a rounded knob footplate, a little larger than the rod itself, is present in just a few instances (Wever, 1978). Evans (2008) describes the sizes of the rod and footplate and its variation using the more common morphological pattern (referred to as the "normal" pattern) as a point of comparison: a slender rod with a small footplate, typical pattern present in iguanians. Thus, according to Evans (2008), the columellar rod is: "normal" in iguanians, gekkotans, and scincids; shorter and usually with an expanded footplate, as in *Anguis*, *Saurodactylus*, *Xenosaurus* (Rieppel, 1980, Fig. 21), Agamidae, and Dibamidae; or longer, as in *Shinisaurus*. It can also vary from long to short within the same genus, as in *Ceratophora* (Pethiyagoda & Manamendra-Arachchi, 1998), or show tendencies towards the reduction of the rod and enlargement of the footplate, as observed in gymnophthalmids (Evans, 2008). In some of the previously published morphological descriptions, there are a few specific remarks made regarding the size of the columellar rod, such as noting the extremely short length in amphisbaenians (Wever & Gans, 1973), and the agamid *Ceratophora* (Pethiya & Manamendra-Arachchi, 1998). While substantial differences in the increased size of the footplate are also frequently described, for example, the relatively immense stapedial footplate of amphisbaenians and anniellids (Baird, 1970; Wever & Gans, 1973), the noticeable asymmetrical footplate of *Draco volans* (Wever, 1978), and the large footplates of *Anniella pulchra*, *Cophosaurus texanus* (Wever, 1973), *Ceratophora stoddartii* (Wever, 1978), and *Rhineura floridana* (Baird, 1970; Olson, 1966). Most of the specimens examined in this study exhibit a slender columellar rod with a proportionally small footplate, except in the case of *Lialis jicari* (Fig. 3B) which shows an evident short, but not stout, rod with a small footplate. This description differs from that of *L. burtonis* by Wever (1974), who described a short and sturdy columella with a relatively large footplate. In this case, according to the figure of the middle ear of *L. burtonis* (Wever, 1974, Fig. 4), it is possible to assume that there are no significant differences between the columella of *L. jicari* and *L. burtonis*, except in the references used to describe their sizes. It is difficult to compare the morphology of the columella between species due to the different parameters and criteria used by each author to estimate the size of the structures. For this reason, we chose to define a ratio between the size of the columella and one of its associated structures. Thus, given the functional role of the complex formed by the columella and extracolumella pointed out by Wever (1978), we used the ratio between the relative length of the columellar rod and the length of the central axis of the extracolumella (Fig. 1, 2C, 3A–B), previously defined as ANC – "total anchorage length" by Werner & Igic (2002). Using our observations and some illustrations available in the literature (see Table 4), we were able to estimate the different conditions of this feature in some species. We are aware that gathering information on this feature without precise measures, as well as estimating the measures from published illustrations is not the most accurate method; however, this provides some assessment regarding the existing variation in this ratio and affords a preliminary estimation of the evolutionary history of variation in this feature. Based on the current information available, there is no phylogenetic signal to the variation of the columella-extracolumella ratio we observed in the major groups of lizards, since the parsimony ancestral states reconstruction shows multiple independent appearances of all three states of this character in less inclusive groups, and the Bayesian approach found similar probabilities for each state at all ancestral nodes (Fig. 7; Table 5).

The expanded distal end of the osseous columella (Fig. 3) is not explicitly mentioned in the available descriptions of the lizard columella; however, Wever (1978) described and illustrated a thin, delicate and rather flexible mid-portion in the columella of *Trachylepis brevicollis* (= *Mabuya brevicollis*) that was also illustrated in other species, such as *Crotaphytus collaris, Callisaurus draconoides, Holbrookia maculata*, and *Sceloporus magister* (Wever, 1978). These records make evident the observation of a widening of the distal end of the columella in these species, a feature that we also registered in some species (see Table 2). Werner & Igic (2002) measured different elements of the middle ear to establish the effects of the dimensions of these structures on the auditory sensitivity of gekkonid lizards. Their results suggest that part of the sensitivity in these lizards would depend on the sizes of the structures of the middle ear. The columella measures used in that study were: the length of the columella and its diameter in the midpoint, and the diameter of the footplate (Werner & Igic, 2002, Fig. 1). Thus, the presence (Fig. 3) or absence of a widening in the distal end of the columella could also be related to auditory sensitivity. However, our observations show the existence of both states of this feature (presence and absence of the widening) in *Anadia bogotensis,* implying this trait displays individual variation, and hence we flag the necessity of evaluating this feature across a larger sample of individuals.

According to Wever (1978), in some species the cartilaginous joint between columella and extracolumella shows a discontinuity comprised of dense connective tissue that gives rigidity to this point, and that can surround the joint, or occur between both structures. Apparently however, the only specific record of this feature was made by Wever (1978) mentioning the absence of this kind of joint in *Trachylepis brevicollis* (= *Mabuya brevicollis*). In our study, both the presence and absence of the connective tissue in this joint were observed in different groups and families (Table 2), and even in the same species, *Anolis marianum*, which suggests this feature possibly displays intraspecific variation. With the current data we cannot address the amount of variation, thus it is necessary examine more specimens of *Anolis marianum* to establish if it could be due to ontogenetic variation or a polymorphism that could support the presence of cryptic species. We also suggest making an in-depth exam using more detailed sampling methods, such as histological techniques, to confirm the kind of tissue involved and determine its definite association with both the columella and the extracolumella.

Extracolumella. Several descriptions and illustrations of the extracolumella exist, which present accurate and detailed information and show significant morphological variation of this structure (e.g., Versluys, 1898; Peterson, 1966; Posner & Chiason, 1966; Wever, 1968; & Wever & Werner, 1970, 1972; Wever, 1973, 1978; Werner et al., 2005). Some variations of the extracolumella are relatively rare, such as the extreme reduction observed in *Varanus bengalensis* (Varanidae, McDowell, 1967); a distinct rough oval form in *Lanthanotus borneensis* (Lanthanotidae, McDowell, 1967); a short structure with a dense mass of ligament fibers split into two branches, one extending along the lower jaw, and the other along the upper jaw in *Rhineura floridana* (Rhineuridae, Wever, 1978); and an elongated structure that extends along the quadrate and laterally connects with the labial skin in Amphisbaenidae and Trogophidae, (Versluys, 1898; Wever & Gans, 1973; Kearney, 2003; Kearney, Maisano & Rowe, 2005). The absence of the extracolumella in lizards has only been registered in the species of *Aprasia* (Pygopodidae, Wever, 1978), *Bipes* (Bipedidae, Wever & Gans, 1973), and *Blanus* (Blanidae, Wever & Gans, 1973). On the other hand, the more common morphological pattern found in lizards is an extracolumella with four principal processes. Some of the variation described for this element refers to the size or lack of one or more of these processes. In most species, all these processes are easily distinguished, but in a few cases, as in *Ceratophora stoddartii* (Agamidae) and *Chamaeleo* (Chamaeleonidae), there is some uncertainty about a processes' presence and correspondence (Wever, 1973, 1978).

The four extracolumellar processes have been either described or illustrated in *Callisaurus* (Phrynosomatidae); *Coleonyx variegatus* and *Eublepharis macularius* (Eublepharidae); *Chondrodactylus bibronii* (=*Pachydactylus bibronii*) and *Gekko gecko* (=*Gekko verticillatus*) (Gekkonidae); *Crotaphytus collaris* (Crotaphytidae); *Iguana iguana* (= *Iguana tuberculata*) (Iguanidae); and *Lialis burtonis* (Pygopodidae) (Versluys, 1898; Iordansky, 1968; Posner & Chiason, 1966; Werner & Wever, 1972; Wever, 1974, 1978; Werner et al., 2005). In this study, we found these four processes to be present in Agamidae, Dactyloidae, Hoplocercidae, Lacertidae, Phyllodactylidae, Sphaerodactylidae, and Tropiduridae, and in two additional species of Gekkonidae and one of Pygopodidae (Table 3). In all these cases, the pars superior and inferior, and the anterior and posterior processes are evident and easily recognized. The presence of the four processes registered here in the species of Gekkota agrees with the literature records for this group, and we also add information on these features to the morphology previously described in Agamidae and Lacertidae (see below).

The absence or extreme reduction of the pars superior only has been registered in *Draco volans* and *Phrynocephalus maculatus* (Agamidae), and *Cophosaurus texanus* (Phrynosomatidae) (Wever, 1973, 1978), and there are no records indicating the absence of the pars inferior in any of the lizard groups. In contrast, the lack of the anterior, posterior or both processes are more frequent within some families and genera. In Gymnophthalmidae, the genera

– *Anadia*, *Gelanesaurus*, *Neusticurus*, *Riama*, and *Tretioscincus* the anterior process is absent; while *Loxopholis* lacks both processes (Table 3). In Teiidae, the genera – *Pholidoscelis lineolatus* (= *Ameiva lineolata*), and *Tupinambis teguixin* (= *T. nigropunctatus*) do not have the anterior process (Versluys, 1898; Wever, 1978), while *Cnemidophorus lemniscatus* lacks both processes. In Lacertidae, there is no anterior process present in *Timon lepidus* (= *Lacerta ocellata*) (Versluys, 1898), but we recorded the presence of a very short and thin anterior process in *Acanthodactylus* cf. *schmidti.* The agamids *Draco volans* and *Phrynocephalus maculatus* do not have any of these processes (Wever, 1973, 1978), and this feature corresponds to our observations in *Stellagama stellio*, but differs from those in *Acanthocercus atricollis* and *Leiolepis belliana*, species that exhibit all four extracolumellar processes. The variation in this structure has also been described within some genera. According to Earle (1961a; 1961b), the genera *Callisaurus* and *Holbrookia* (Phrynosomatidae) have four extracolumellar processes, while Wever (1973, 1978) points out that *C. draconoides* and *H. maculata* do not have either the anterior nor the posterior processes. Furthermore, *H. maculata* also shows an extreme reduction of the pars superior and inferior. Similarly, according to Wever (1973), and Han & Young (2016), *Phrynosoma coronatum* (Phrynosomatidae) and *Varanus salvator* (Varanidae) do not present the anterior process; while Versluys (1898), McDowell (1967), and Wever (1973) stated that *P. platyrhinos*, *V. bengalensis,* and *V. niloticus* do not exhibit either process. We observed interspecific variation in *Pholidobolus* (Gymnophthalmidae), since *P. montium* does not have the anterior process and *P. vertebralis* does not have either of them.

The absence of both processes, anterior and posterior, has been recorded in *Anguis fragilis* and *Anniella pulchra* (Anguidae), and *Trachylepis brevicollis* (= *Mabuya brevicollis*) (Scincidae) (Versluys, 1898; Wever, 1973, 1978). We found this condition in *Cnemidophorus lemniscatus*

(Teiidae) and the species of *Mabuya* (Scincidae). The absence of the posterior process, when the anterior process is present, has only been reported in *Heloderma suspectum* (Helodermatidae) and *Xenosaurus grandis* (Xenosauridae) (Versluys, 1898; Wever, 1973, 1978).

The available information about the shapes of the extracolumellar processes describes them as pointed and long or short cartilaginous structures, without any further descriptive detail. There are no specific descriptions of the shape of each extracolumellar process, except for a few mentions and illustrations of the anterior process in some species of Gekkota (Versluys, 1898; Posner & Chiason, 1966; Werner & Wever, 1972; Wever, 1978; Werner et al., 2005, 2008). In the specimens available for this study, we found some differences in the shapes of the extracolumellar processes, which illustrates wide variation in these structures. Although our sample is not representative of all groups of lizards, it was enough to display such variation, mainly in the pars superior and the anterior process. Thus with the available information, the pars superior, which shows noticeable variation in its shape (Table 3), characterizes the species of Gekkota with a posterior prolongation of its upper edge (Fig. 4A–C, 5A); while Hoplocercidae (Fig. 5B, 6B) can be differentiated by a rounded upper edge; Scincidae (Fig. 6C) by a tridentate upper edge; and *Tropidurus pinima* (Tropiduridae) by a anteriorly prolonged and shorter upper edge (Fig. 5C).

Among the species studied which show an anterior process, the more frequently observed shape is a pointed cartilaginous extension that can be short (Fig. 3C), or long (Fig. 4C, 5B–C), which corresponds with the shape most commonly described in the literature. However, we found that in specimens of Gekkonidae and Phyllodactylidae examined (Table 3), the anterior process is a long and thick extension with some small and sharp prolongations (Fig. 4A–B). This shape has also been described or illustrated in Eublepharidae (*Coleonyx variegatus, Eublepharis* *macularius*), and Gekkonidae (*Chondrodactylus bibronii* and *Gekko gecko*) (Versluys, 1898; Posner & Chiason, 1966; Werner & Wever, 1972; Wever, 1978; Werner et al., 2005). The remaining species of Gekkota examined (Table 3) did not show these sharp prolongations in the anterior process. One example is *Lialis jicari* (Pygopodidae, Fig. 4C), which shows a long and pointed process that is not oriented anteriorly, but downward; as well the distal end of the anterior process that turns downward in *Gonatodes* (Sphaerodactylidae, Fig. 5A).

The pars inferior and the posterior process are more morphologically conserved. The pars inferior shows a sharp distal end in most of the species with available information, but a thicker distal end in Gekkonidae, Phyllodactylidae, and Sphaerodactylidae (Table 3). In the posterior process the only variation observed was the overall size, except in *Lialis jicari* that shows both a short and thick posterior process that turns upward resembling a hook (Fig. 4C). These features – the shapes of the pars superior, the anterior process, and the shape of the distal end of the pars inferior – should be evaluated in greater detail and in a larger sample, to confirm if the variation observed has any taxonomic relevance within Gekkota.

The middle ear types in lizards. The three types of middle ear described by Wever & Werner (1970) represent the more common morphologies observed in lizards and show an important morphological variation within each one. Despite the morphological differences between the types, all of these are highly effective in sound reception and transmission (Wever, 1973). According to Wever (1978), the most common type in lizards is the iguanid type that is present in Iguanidae, Agamidae, Cordylidae, Gerrhosauridae, Helodermatidae, Lacertidae, Teiidae, Varanidae, and Xantusiidae (see Wever, 1978, Table 5-III, p. 132). The species that Wever (1978) originally included in Iguanidae now belong to the families Corytophanidae, Crotaphytidae, Dactyloidae, Tropiduridae, Opluridae, Phrynosomatidae, and Iguanidae (see Wever, 1978, p.215-216). In addition, in our work we found this pattern in species from some of these families and from Hoplocercidae (Table 3) that we add to the list. According to Wever & Werner (1970), the iguanid type is characterized by the presence of the internal process. To this, we add that this type is further characterized by the presence of at least three well-defined extracolumellar processes, since all species that exhibit the internal process also have these additional processes. Given the variation observed in the shape and number of the extracolumellar processes within the iguanid type, we suggest greater evaluation of these characters within the families that possess them, in order to determine whether the variation in the morphology of these processes provides further systematic information at a finer taxonomic scale.

The gekkonid middle ear type is only present in the families of Gekkota (Werner & Wever, 1972; Wever, 1978). Although we did not have available material to check the presence of the extracolumellar muscle in any specimen within our sample, we recorded that none of the species of Gekkota studied showed internal processes. Additionally, all the specimens from these families exhibited: i) four extracolumellar processes, ii) a posterior extension in the pars superior, and iii) an anterior process with some small and sharp projections. Thus, we add these three features to the definition of the gekkonid type described by Wever & Werner (1970). The posterior extension of the pars superior and the shape of the anterior process and its projections, could be diagnostic characters for Gekkota, and the variation present within these features may even be further diagnostic within the group as well. For this reason, we recommend more detailed analysis in a systematic context.

The simplest type of the middle ear is that of the scincids, which was described in Scincidae, Anguidae, and Xantusiidae (see Wever, 1978; Table 5-III). Interestingly however, the family Xantusiidae actually shows two different middle ear types: the scincid type is seen in *Lepidophyma flavimaculatum* and *L. smithi*, that do not possess both the internal process and the extracolumellar muscle; and the iguanid type is observed in *Xantusia henshawi*, which does have the internal process (Wever, 1978). The absence of the extracolumellar muscle was not evaluated in the latter species, but the absence of the internal process was corroborated here in the genus *Mabuya* (Scincidae).

The "divergent" or "degenerate" middle ears are those with a morphology that does not match with any of the three previously mentioned types (Wever & Werner, 1970; Wever, 1973, 1978). However, all genera described by Wever (1973) as divergent forms, except those in the genus *Anguis,* exhibit an internal process, which is small and, in some cases, extremely reduced (Wever, 1973). According to Wever (1978), divergent middle ears are present in Chamaeleonidae, and Xenosauridae, as well as in some species of Agamidae and Scincidae, and less frequently in some species of the families Anguidae, Pygopodidae, Teiidae, and in several families of Iguania (Wever, 1978; Table 5-III). The genus *Feylinia* and the families Dibamidae and Lanthanotidae also show this type of middle ear (McDowell, 1967; Baird, 1970; Wever, 1978). The genera *Anguis*, *Anniella*, *Callisaurus*, *Ceratophora*, *Cophosaurus*, *Draco*, *Holbrookia*, *Phrynocephalus*, *Phrynosoma*, and *Xenosaurus* show a divergent pattern (Wever, 1973). All of them lack the tympanic membrane and exhibit an extreme reduction in the extracolumella.

Ancestral state reconstructions. Ancestral state reconstructions of the available information indicated that at least some extracolumella features can be a useful source of systematic information within Squamata. The great uncertainty shown by the analyses for the ancestral state of the length of the columella relative to the extracolumella central axis length (character 1, Fig. 7) suggests that there is no phylogenetic signal associated with this feature. The variation observed in this ratio could be related to the auditory sensitivity associated with the inner ear, as well as morphological or morphometrical features of the skull and the outer ear, or even ecological conditions.

Although the different variations of the extracolumellar morphology, as well as the specific shapes of its processes, should be evaluated in more detail and within less inclusive groups. The summarization of the available information into only four states: expanded, reduced, absent, and extensive (character 2, Fig. 8A) provides an idea of the general evolutionary history of the extracolumella in lizards. The ancestral state in Squamata reconstructs to the reduced form of the extracolumella that is the predominant condition within the group. The expanded columella appears to have arisen via convergence in Gekkota, Pleurodonta, and Xantusiidae, and could be a diagnostic character (along with other features) for members of these groups. The families Agamidae, Lacertidae, and Phrynosomatidae are polymorphic in that different members of these clades exhibit reduced and expanded extracolumellas (Fig. 8A). Although there are four extracolumellar processes exhibited in Xantusiidae (Wever, 1978), Agamidae and Lacertidae (this study), the anterior process in the first family, and the anterior and posterior processes in the latter two, are extremely small and thin structures, giving the expanded extracolumella a similar appearance to the reduced extracolumella, emphasizing the necessity for detailed observation in species that apparently lack any processes. The genus *Aprasia*, the families *Bipedidae* and

Blanidae, and the clade of serpentes do not have extracolumellas, indicating at least three independent losses of the extracolumella in Squamata. The genus *Aprasia* (Pygopodidae) does not have a tympanic membrane, a columellar apparatus, or a tympanic cavity (Baird, 1970; Wever, 1978), although some species might have a small tympanic membrane and a very rudimentary columella. The morphology of the inner ear and some anatomical modifications in the pterygoid and quadrate of *Aprasia repens* denote normal auditory function, where the quadrate plays a role in sound transmission (Daza & Bauer, 2015). These observations suggest a limited ability to hear airborne sounds, but also potential capacity to hear "underground sound" (Greer, 1989; Daza & Bauer, 2015). The ear modifications are one distinctive feature of the extremely divergent morphological condition of the fossorial adaptation that this genus shows (Baird, 1970). The loss of the extracolumella also occurred in the ancestor of the clade (Bipedidae + (Blanidae + Cadeidae) (Amphisbaenidae + Trogonophidae)), but it appears again as an expanded structure in Amphisbaenidae and Trogonophidae. In this clade, we could expect that Cadeidae, a family with no current information, does not have an extracolumella (see below), similar to *Bipes* (Bipedidae) and *Blanus* (Blanidae) that lack the external ear and only have a columella that ends in a disk of fibrous tissue beneath skin, resulting in a very aberrant sound receiving system, but with a high level of sensitivity stimulated by aerial sounds (Wever & Gans, 1972, 1973). Finally, the clade of serpentes is the third group that does not exhibit extracolumellas. This group have long and narrow columellas with a cartilaginous end that connects with the quadrate through an articulatory process, and in some groups, intermediate cartilages may also be observed between both structures (Wever, 1978). The adaptations in the peripheral and cochlear processes ensure the performance of the ear in most species of snakes is similar to that observed in the majority of lizards within the restricted range of low frequencies

(Wever, 1978). The extensive extracolumella is extremely different morphologically and is present only in Amphisbaenidae and Trogonophidae. It is a cartilaginous structure that runs anteriorly along the quadrate and is attached to the skin which functions as a sound-receptive surface (Wever & Gans, 1973; Wever, 1978). The origin of the amphisbaenian extracolumella has been a controversial topic since Fürbringer (1919, 1922) proposed that it originated from the epihyal portion of the hyoid apparatus, while Camp (1923) stated that these structures are not related. Later, based on their personal observations, Wever & Gans (1972, 1973) supported Fürbringer's proposal, suggesting that the amphisbaenian extracolumella is not homologous with that of lizards, but instead a modification of a dorsal portion of the hyoid (see Wever & Gans, 1973). However, according to Kearney (2003), this hypothesis has not been tested since there are no studies about the development of amphisbaenians that have found any relation between the extracolumella and the hyoid. Considering the statement of Kearney (2003), we consider the extracolumella of Amphisbaenidae and Trogonophidae as a structure homologous with the lizard extracolumella. Whenever it is present, the extracolumella always connects with the dermal layer of the skin in members of the amphisbaenian clade. Aside from this however, members of this group exhibit wide variation in extracolumellar morphology. This variation is present in the family Rhineuridae that, despite the presence of a reduced extracolumella, also exhibits an unusual morphology in that it has two branches of ligament fibers – one connected with the lower jaw and the other with the upper jaw (Wever, 1978). These modifications are part of a suite of features that are advantageous for a fossorial lifestyle (Baird, 1970; Wever & Gans, 1972, 1973).

The comparison of results between the ARD and ED Bayesian approaches show some differences in the probability values for the ancestral state estimates for the clades Gekkota, Pleurodonta, and Xantusiidae. However, both analyses show the highest support for the expanded extracolumella at the ancestral node of the three clades, consistent with the parsimony results. A second difference between the two Bayesian analyses was in the probability values of the nodes within the amphisbaenian clade. In this case, both analyses still estimated the highest probability for the extensive extracolumella at the ancestral node of (Amphisbaenidae + Trogonophidae), agreeing with the parsimony results. Contrary to this, the ARD model shows the highest probability values for the extensive extracolumella in the ancestral nodes of (Bipedidae + (Blanidae + Cadeidae) (Amphisbaenidae + Trogonophidae)), ((Blanidae + Cadeidae) (Amphisbaenidae + Trogonophidae)), and (Blanidae + Cadeidae), suggesting an extensive extracolumella in Cadeidae. In contrast, the ER model, concordant with the parsimony results, shows the highest support for the absent extracolumella at the ancestral nodes for these clades, proposing the absence of an extracolumella in Cadeidae.

The internal process (character 3; Fig. 8B) is an additional extracolumellar structure that arises close to the joint with the columella, running anteriorly to attach to the quadrate. The proposed function of this process is mainly to protect the middle ear complex (Wever, 1978). The differences between analyses do not permit establishing the ancestral state (presence or absence) for this character for Squamata, along with some of the other more ancestral nodes within this group (Fig. 8B). The absence of this process is likely a result of convergence occurring between the groups of Gekkota, Gymnophthalmidae and Scincidae (Fig. 8B); while the presence of this process is the more common state within Squamata. Based on the available information, the families Anguidae and Xantusiidae are the only ones which are polymorphic for this character state.

The columella and extracolumella morphology have not been associated functionally with lizards' vocalizing capabilities. However, given the high morphological complexity of the extracolumella described in the geckos' clade, probably it could be correlated with the vocalizations that they produce, which are complex and exhibit variation in amplitude and frequency. On the other hand, Wever (1978) considered a correlation between the vocalization and the meatal closure muscle of the outer ear in these lizards. According to Wever (1978), the function of the meatal closure muscle is to protect the ear; although it is no clear if this protection is only against mechanical damage or also against particularly loud sounds. This muscle could be related to the fact that these lizards produce vocalizations, and hence the muscle plays a role in protecting the individual's ears against its own vocal sounds, which can be extremely loud in some species. However, in some individuals of the family Sphaerodactylidae and the gekkonid genus *Phelsuma*, which are considered to be mute species, or with tenuous vocalization, don't have this muscle; other species (e.g., *Gehyra variegata*, *Oedura monilis* (= *Oedura ocellata*), and *Strophurus elderi* (= *Diplodactylus elderi*)) that also do not produce vocalizations, do have the meatal closure muscle in their outer ears (Wever, 1978). Thus, while the production of loud vocalization might be related to the presence of the meatal closure muscle, it is clear that other conditions may also produce the development of this muscle (Wever, 1978). Alternatively, it can be assumed that the presence of the meatal closure muscle and vocalization are the ancestral condition for gekkotans, and in some groups the muscles have been lost along with vocalization, whilst in others the muscles haven't been lost yet. We cannot also ~~discard~~ that this muscle has an alternative unknown function. The combined analysis of morphological and functional information is necessary to establish the possible relation between the outer and middle ear with geckos' vocalizations.

Despite the general morphology of the lizard middle ear being quite well known, and there being no particularly notable variation in the lizard columella, the morphological variation of the extracolumella structure is evidently more significant than previously described. We have presented evidence of that extensive variation here and demonstrated that some features of the extracolumella could potentially provide a source of phylogenetic information for some groups. However, in some clades, other ear modifications may be more closely related to adaptations for navigating and functioning within particular habits. It is necessary to perform a more detailed and comprehensive study around each of the specific morphologies of the extracolumella, here defined as expanded, reduced, and extensive, to understand better the variation present within each particular clade. This kind of detailed information will possibly let us know about more morphological features that may be useful to the systematic and understanding of the functioning of the middle ear in certain groups of lizards.

Conclusions

The middle ear in lizards shows a considerable morphological variation, especially in the structures that conform to the extracolumella. This study provides an overall overview of the variation of these structures across lizards. We also provided some details of the morphological descriptions of the middle ear, presenting new information about the features of the extracolumellar processes. The analysis of this morphology within a comparative and evolutive framework shows us that these structures are a substantial source of systematic and phylogenetic information, which could be useful even to functional studies. These structures should be studied deepest to complete as much as possible the gap of the information, especially within lizards' groups that have the four extracolumellar processes, which may present a considerable morphological variation.

The morphological variation of both the columella and extracolumella may have a distinctive role associated with their efficiency in transmitting the sound, and with the vocalizations produced by some clades. Also, the variation of the extracolumellar structures probably is correlated with the different morphological patterns of the outer ear, which at the same time are related to the specific habitats of each lizard's group. These correlations should be established by studying the morphological and functional association between the middle and outer ear with the vocalizations within an ecological context.

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Table 1(on next page)

Species and number of specimens examined.

Species and number of specimens examined.

Group	Family	Genus	Species	Number of Specimens
Gekkota	Gekkonidae	*Hemidactylus*	*H. brasilianus*	1
		Phelsuma	*P. madagascariensis*	1
	Phyllodactylidae	*Tarentola*	*T. mauritanica*	1
		Thecadactylus	*T. rapicauda*	1
	Pygopodidae	*Lialis*	*L. jicari*	1
	Sphaerodactylidae	*Gonatodes*	*G. albogularis*	1
			G. concinnatus	1
Iguania	Agamidae	*Acanthocercus*	*A. atricollis*	1
		Leiolepis	*L. belliana*	1
		Stellagama	*S. stellio*	1
	Dactyloidae	*Anolis*	*A. antonii*	2
			A. auratus	2
			A. chrysolepis	2
			A. fuscoauratus	1
			A. maculiventris	4
			A. mariarum	3
			A. tolimensis	2
			A. trachyderma	2
			A. ventrimaculatus	3
	Hoplocercidae	*Hoplocercus*	*H. spinosus*	1
		Morunasaurus	*M. groi*	1
	Tropiduridae	*Stenocercus*	*S. erythrogaster*	1
		Tropidurus	*S. trachycephalus*	2
			T. pinima	1
Lacertoidea	Gymnophthalmidae	*Anadia*	*A. bogotensis*	4
		Gelanesaurus	*G. cochranae*	1
		Loxopholis	*L. rugiceps*	1
		Neusticurus	*N. medemi*	1
		Pholidobolus	*P. montium*	2
			P. vertebralis	1
		Riama	*R. striata*	3
		Tretioscincus	*T. bifasciatus*	1
	Teiidae	*Cnemidophorus*	*C. lemniscatus*	1
	Lacertidae	*Acanthodactylus*	*A. cf. schmidti*	1
Scincoidea	Scincidae	*Mabuya*	*M. falconensis*	1
			M. nigropunctatum	2
			Mabuya sp. 1	2
			Mabuya sp. 2	3

The taxonomic classification follows Zheng and Wiens (2016).

Table 2(on next page)

Characterization of the morphological variation of the columella, and the joint with the extracolumella.

Characterization of the morphological variation of the columella, and the joint with the extracolumella.

Species	Columella			Joint of stapes
	Stapedial foramen	***Length of the columella**	**Widening of the osseous distal end**	**Connective tissue**
GEKKOTA				
Gekkonidae				
Hemidactylus brasilianus	present	equal	absent	absent
Phelsuma madagascariensis	present	equal	present	absent
Phyllodactylidae				
Tarentola mauritanica	present	longer	absent	surrounding the joint
Thecadactylus rapicauda	absent	equal	absent	absent
Pygopodidae				
Lialis jicari	absent	shorter	present	absent
Sphaerodactylidae				
Gonatodes albogularis	present	shorter	absent	absent
Gonatodes concinnatus	present	shorter	absent	absent
IGUANIA				
Agamidae				
Acanthocercus atricollis	?	longer	present	surrounding the joint
Leiolepis belliana	?	?	absent	absent
Stellagama stellio	?	?	?	?
Dactyloidae				
Anolis antonii	absent	equal	present	between the joint
Anolis auratus	absent	equal	present	absent
Anolis chrysolepis	absent	equal	present	between the joint
Anolis fuscoauratus	absent	equal	present	between the joint
Anolis maculiventris	absent	equal	present	between the joint
Anolis mariarum	absent	equal	present	absent / between the joint
Anolis tolimensis	absent	equal	present	surrounding the joint
Anolis trachyderma	absent	equal	present	between the joint
Anolis ventrimaculatus	absent	equal	present	absent between the joint
Hoplocercidae				
Hoplocercus spinosus	absent	shorter	absent	between the joint
Morunasaurus groi	absent	shorter	present	absent
Tropiduridae				
Stenocercus erythrogaster	absent	?	absent	absent

Stenocercus trachycephalus	absent	equal	present	surrounding the joint
Tropidurus pinima	absent	shorter	present	absent
LACERTOIDEA				
Gymnophthalmidae				
Anadia bogotensis	absent	shorter	absent present	absent
Gelanesaurus cochranae	absent	shorter	absent	?
Loxopholis rugiceps	absent	shorter	present	absent
Neusticurus medemi	absent	shorter	absent	absent
Pholidobolus montium	absent	shorter	absent	?
Pholidobolus vertebralis	absent	shorter	present	absent
Riama striata	absent	equal	absent	surrounding the joint
Tretioscincus bifasciatus	absent	longer	present	surrounding the joint
Teiidae				
Cnemidophorus lemniscatus	absent	?	absent	absent
Lacertidae				
Acanthodactylus cf. schmidti	absent	equal	present	surrounding the joint
SCINCOIDEA				
Scincidae				
Mabuya falconensis	absent	equal	present	absent
Mabuya nigropunctatum	absent	longer	present	between the joint
Mabuya sp. 1	absent	equal	present	absent
Mabuya sp. 2	absent	equal	present	between the joint

(*) Length of the columella relative to that of the vertical axis of the extracolumella; (?) the condition of the specimen negated the ability to define this feature.

Table 3(on next page)

Characterization of the morphological variation of the extracolumella.

Characterization of the morphological variation of the extracolumella.

Species	Pars superior	Pars inferior	Anterior process	Posterior process	Internal process
GEKKOTA					
Gekkonidae					
Hemidactylus brasilianus	- posterior extension downward - straight upper edge	thick with projections	long with small projections	short and pointed	absent
Phelsuma madagascariensis	- posterior extension downward - straight upper edge	sharp	long with small projections	extended and thin	absent
Phyllodactylidae					
Tarentola mauritanica	- posterior extension downward - straight upper edge	sharp	long with small projections	extended and thin	absent
Thecadactylus rapicauda	- posterior extension downward - straight upper edge	thick with projections	long with small projections	extended and thin	absent
Pygopodidae					
Lialis jicari	- posterior extension straight - straight upper edge	sharp	long pointed, downward	long and thick turned upward	absent
Sphaerodactylidae					
Gonatodes albogularis	- posterior extension downward - straight upper edge	thick with projections	short, downward	short and pointed	absent
Gonatodes concinnatus	- posterior extension downward - straight upper edge	thick with projections	short, downward	short and pointed	absent
IGUANIA					
Agamidae					
Acanthocercus atricollis	- no extension	sharp	long pointed and	extended and thin	present

	- straight upper edge		straight		
Leiolepis belliana	- no extension - straight upper edge	sharp	long pointed and straight	short and pointed	present
Stellagama stellio	- no extension - rounded upper edge	sharp	absent	absent	present
Dactyloidae					
Anolis antonii	- no extension - straight upper edge	sharp	short and pointed	short and pointed	present
Anolis auratus	- no extension - straight upper edge	sharp	short and pointed	short and pointed	present
Anolis chrysolepis	- no extension - straight upper edge	sharp	short and pointed	short and pointed	present
Anolis fuscoauratus	- no extension - straight upper edge	sharp	short and pointed	short and pointed	present
Anolis maculiventris	- no extension - straight upper edge	sharp	short and pointed	short and pointed	present
Anolis mariarum	- no extension - straight upper edge	sharp	short and pointed	short and pointed	present
Anolis tolimensis	- no extension - straight upper edge	sharp	short and pointed	short and pointed	present
Anolis trachyderma	- no extension - straight upper edge	sharp	short and pointed	short and pointed	present
Anolis ventrimaculatus	- no extension - straight upper edge	sharp	short and bifurcated	extended and thin	present
Hoplocercidae					
Hoplocercus spinosus	- no extension - rounded upper edge	sharp	long pointed and straight	extended and thin	present
Morunasaurus groi	- no extension - rounded upper edge	sharp	long pointed and straight	short and pointed	present
Tropiduridae					
Stenocercus erythrogaster	- no extension straight upper edge	sharp	long pointed and straight	extended and thin	present
Stenocercus trachycephalus	- no extension	sharp	long pointed and	extended and thin	present

	- straight upper edge		straight		
Tropidurus pinima	- anterior extension straight - straight upper edge	sharp	long pointed and straight	extended and thin	present
LACERTOIDEA					
Gymnophthalmidae					
Anadia bogotensis	- no extension - straight upper edge	sharp	absent	short and pointed	absent
Gelanesaurus cochranae	- no extension - straight upper edge	sharp	absent	short and pointed	absent
Loxopholis rugiceps	- no extension - straight upper edge	sharp	absent	absent	absent
Neusticurus medemi	- no extension - straight upper edge	sharp	absent	extended and thin	absent
Pholidobolus montium	- no extension - straight upper edge	sharp	absent	short and pointed	absent
Pholidobolus vertebralis	- no extension - straight upper edge	sharp	absent	absent	absent
Riama striata	- no extension - straight upper edge	sharp	absent	short and pointed	absent
Tretioscincus bifasciatus	- no extension - straight upper edge	sharp	absent	short and pointed	absent
Teiidae					
Cnemidophorus lemniscatus	- no extension - straight upper edge	sharp	absent	absent	present
Lacertidae					
Acanthodactylus cf. schmidti	- no extension - straight upper edge	sharp	long pointed and straight	short and pointed	present
SCINCOIDEA					
Scincidae					
Mabuya falconensis	- no extension - tridentate upper edge	sharp	absent	absent	absent
Mabuya nigropunctatum	- no extension	sharp	absent	absent	absent

	- tridentate upper edge				
Mabuya sp. 1	- no extension - tridentate upper edge	sharp	absent	absent	absent
Mabuya sp. 2	- no extension - tridentate upper edge	sharp	absent	absent	absent

Table 4(on next page)

Sources of the published data used to score the character states of the middle ear.

1 **Sources of the published data used to score the character states of the middle ear.**

Group	Family	Species	Reference
Rhincocephalia	Sphenodontidae	*Sphenodon punctatus*	Gray (1913), Baird (1970), Gans & Wever (1976), Wever (1978)
	Dibamidae	*Anelytropsis papillosus*	McDowell (1967), Greer (1976), Wever (1978)
Anguimorpha	Anguidae	*Anguis fragilis*	Versluys (1898), Wever (1973, 1978)
		Anniella pulchra	Wever (1973, 1978)
		Ophisaurus	Baird (1970)
	Helodermatidae	*Heloderma suspectum*	Versluys(1898)
	Lanthanotidae		Wever (1978)
		Lanthanotus borneensis	McDowell (1967), Baird (1970)
	Varanidae	*Varanus bengalensis*	McDowell (1967)
		Varanus niloticus	Versluys(1898)
		Varanus salvator	Han & Young (2016)
	Xenosauridae	*Xenosaurus grandis*	Wever (1973, 1978)
Gekkota	Eublepharidae	*Coleonyx variegatus*	Posner & Chiason (1966)
		Eublepharis macularius	Wever (1978), Werner et al. (2005, 2008)
	Gekkonidae	*Chondrodactylus bibronii* (= *Pachydactylus bibronii*)	Versluys (1898)
		Gekko gecko (= *Gecko verticillatus*)	Versluys (1898), Iordansky (1968), Wever (1978), Werner & Wever (1972)
		Hemidactylus garnotti	Kluge & Eckardt (1969)
		Narudasia festiva	Daza, Aurich & Bauer (2012)
		Uroplatus fimbriatus	Versluys(1898)
	Pygopodidae	*Aprasia sps*	Baird (1970), Wever (1978)
		Lialis burtonis	Wever (1974)

	Sphaerodactylidae	*Teratoscincus scincus*	Underwood (1957), McDowell (1967), Baird (1970), Greer (1976)
Iguania	Agamidae	*Bronchocela jubata* (= *Calotes jubatus*)	Versluys (1898)
		Ceratophora stoddarti	Wever (1973, 1978)
		Ceratophora tennenti	Wever (1973, 1978)
		Draco Volans	Versluys (1898), Wever (1973, 1978)
		Phrynocephalus maculatus	Wever (1973, 1978)
		Phrynocephalus sp.	Wever (1973)
		Uromastyx aegyptia	Versluys (1898)
	Chamaleonidae	*Chamaeleo*	Versluys (1898), Wever (1968, 1978)
		Rhampholeon	Toerien (1963)
	Crotaphytidae	*Crotaphytus collaris*	Wever and Werner (1970), Wever (1978)
	Iguanidae	*Iguana iguana* (= *Iguana tuberculata*)	Versluys (1898)
	Phrynosomatidae	*Callisaurus draconoides*	Earle (1961c), Wever (1973, 1978)
		Cophosaurus texanus	Wever (1973, 1978)
		Holbrookia	Earle (1961a; 1961c), Baird (1970)
		Holbrookia maculate	Earle (1961a; 1961c), Wever (1973, 1978)
		Phrynosoma coronatum	Wever (1973)
		Phrynosoma platyrhinos	Wever (1973, 1978)
		Sceloporus magister	Wever (1967, 1973, 1978)
Lacertoidea	Amphisbaenidae	*Amphisbaena*	Gans & Wever (1972), Wever & Gans (1973), Olson (1966), Wever (1973)
		Amphisbaena alba	Wever & Gans (1973)
		Amphisbaena darwini trachura	Wever & Gans (1973)
		Amphishenia manni	Wever & Gans (1973)
		Amphisbaena fuliginosa	Versluys (1898)
		Amphisbaena manni	Wever & Gans (1973)
		Chirindia langi	Wever & Gans (1973)
		Cynisca leucura	Wever & Gans (1973)
		Monopeltis c. capensis	Wever & Gans (1973)

		Zygaspis violacea	Wever & Gans (1973)
	Bipedidae	*Bipes biporus*	Wever & Gans (1972), Wever (1978)
	Blanidae	*Blanus*	Gans & Wever (1975), Wever (1978)
	Lacertidae	*Podarcis muralis* (= *Lacerta muralis*)	Wever (1978)
		Timon lepidus (= *Lacerta ocellata*)	Versluys(1898)
	Rhineuridae	*Rhineura floridana*	Baird (1970), Olson (1966)
	Teiidae	*Aspidoscelis tigris aethiops* (= *Cnemidophorus tessellatus aethiops*)	Peterson (1966)
		Pholidoscelis lineolatus (= *Ameiva lineolata*)	Wever (1978)
		Tupinambis teguixin (= *Tupinambis nigropunctatus*)	Versluys(1898)
	Trogonophidae	*Diplometopon zarudnyi*	Gans & Wever (1975)
		Trogonophis wiegmanni	Wever & Gans (1973)
Scincoidea	Cordylidae		Wever (1978)
	Gerrhosauridae	*Gerrhosaurus m. major*	Wever (1978)
	Scincidae	*Acontias plumbeus*	Wever (1978)
		Eutropis multifasciata (= *Mabuia multifasciata*)	Versluys(1898)
		Feylinia currori	Greer (1976)
		Feylinia polylepis	Greer (1976)
		Scelotes bipes	Torien (1963)
		Trachylepis brevicollis (= *Mabuya brevicollis*)	Wever (1973, 1978)
	Xantusiidae	*Lepidophyma gaigeae*	Greer (1976), Wever (1978)
		Lepidophyma flavimaculatum,	Wever (1978)
		Lepidophyma smithi	Wever (1978)
		Xantusia henshawi	Greer (1976), Wever (1978)
		Xantusia riversiana (= *Klauberrina riversiana*)	Greer (1976)

Serpentes	Berman & Regal (1967), Wever (1978)

2

Table 5(on next page)

Summary of the posterior probabilities estimated for each node by the Bayesian Ancestral State Reconstructions modelled using the models with all rates different (ARD) and equal rates (ER).

Summary of the posterior probabilities estimated for each node by the Bayesian Ancestral State Reconstructions modelled using the models with all rates different (ARD) and equal rates (ER).

	Character 1 ARD model				**Character 1 ER model**			
Node	**-**	**Equal**	**Longer**	**shorter**	-	**equal**	**longer**	**shorter**
2	0,13	0,31	**0,33**	0,23	0,25	0,25	0,25	0,25
4	0,13	0,31	**0,33**	0,23	0,25	0,25	0,25	0,25
7	0,13	0,31	**0,33**	0,23	0,25	0,25	0,25	0,25
17	0,14	0,31	**0,32**	0,23	0,25	0,25	0,25	0,25
18	0,13	0,31	**0,33**	0,23	0,25	0,25	0,25	0,25
19	0,13	0,31	**0,33**	0,23	0,25	0,25	0,25	0,25
20	0,13	0,31	**0,33**	0,23	0,25	0,25	0,25	0,25
25	0,13	0,31	**0,33**	0,23	0,25	0,25	0,25	0,25
26	0,13	0,31	**0,33**	0,23	0,25	0,25	0,25	0,25
27	0,13	0,31	**0,33**	0,23	0,25	0,25	0,25	0,25
29	0,14	0,31	**0,32**	0,23	0,25	0,25	0,25	0,25
30	0,13	0,31	**0,33**	0,23	0,25	0,25	0,25	0,25
33	0,13	0,31	**0,33**	0,23	0,25	0,25	0,25	0,25
34	0,13	0,31	**0,33**	0,23	0,25	0,25	0,25	0,25
35	0,13	0,31	**0,33**	0,23	0,25	0,25	0,25	0,25
38	0,13	0,31	**0,33**	0,23	0,25	0,25	0,25	0,25
39	0,13	0,31	**0,33**	0,23	0,25	0,25	0,25	0,25
42	0,15	0,30	**0,31**	0,24	0,25	0,25	0,25	0,25
43	0,13	0,31	**0,33**	0,23	0,25	0,25	0,25	0,25
47	0,13	0,31	**0,33**	0,23	0,25	0,25	0,25	0,25
50	0,13	0,31	**0,33**	0,23	0,25	0,25	0,25	0,25
51	0,13	0,31	**0,33**	0,23	0,25	0,25	0,25	0,25
55	0,13	0,31	**0,33**	0,23	0,25	0,25	0,25	0,25
60	0,13	0,31	**0,33**	0,23	0,25	0,25	0,25	0,25
73	0,13	0,31	**0,33**	0,23	0,25	0,25	0,25	0,25

Rounded values of the posterior probabilities; the higher values in bold; (**-**) inapplicable characters. See correspondence between the node and the clades in the Results section.

Continuation Table 5

	Character 2 ARD model				**Character 2 ER model**			
node	**absent**	**Expanded**	**extensive**	**reduced**	**absent**	**Expanded**	**extensive**	**reduced**
2	0,01	0,04	0,00	**0,95**	0,00	0,07	0,00	**0,93**
4	0,15	**0,82**	0,01	0,02	0,00	**0,99**	0,00	0,01
7	0,14	**0,85**	0,01	0,00	0,09	**0,91**	0,00	0,00
17	0,00	0,01	0,00	**0,99**	0,00	0,04	0,00	**0,96**
18	0,01	0,03	0,00	**0,96**	0,00	0,06	0,00	**0,94**
19	0,03	0,12	0,01	**0,84**	0,01	0,16	0,01	**0,82**
20	0,14	**0,80**	0,02	0,04	0,00	**0,98**	0,00	0,02
25	0,00	0,00	0,00	**1,00**	0,00	0,00	0,00	**1,00**
26	0,00	0,00	0,00	**1,00**	0,00	0,00	0,00	**1,00**
27	0,00	0,00	0,00	**1,00**	0,00	0,00	0,00	**1,00**
29	0,00	0,00	0,00	**1,00**	0,00	0,00	0,00	**1,00**
30	0,00	0,00	0,00	**1,00**	0,00	0,00	0,00	**1,00**
33	0,00	0,00	0,00	**1,00**	0,00	0,00	0,00	**1,00**
34	0,00	0,00	0,01	**0,99**	0,02	0,00	0,01	**0,97**
35	0,00	0,00	**0,94**	0,06	**0,58**	0,00	0,33	0,09
38	0,00	0,00	**0,96**	0,04	0,30	0,00	**0,70**	0,00
39	0,01	0,07	0,00	**0,92**	0,00	0,08	0,00	**0,92**
42	0,00	0,00	0,00	**1,00**	0,00	0,00	0,00	**1,00**
43	0,00	0,00	0,00	**1,00**	0,00	0,00	0,00	**1,00**
47	0,00	0,00	0,00	**1,00**	0,00	0,00	0,00	**1,00**
50	0,00	0,00	0,00	**1,00**	0,00	0,00	0,00	**1,00**
51	0,00	0,00	0,00	**1,00**	0,00	0,00	0,00	**1,00**
55	0,00	0,00	0,00	**1,00**	0,00	0,00	0,00	**1,00**
60	0,15	**0,80**	0,00	0,05	0,00	**0,95**	0,00	0,05
73	0,18	**0,61**	0,00	0,21	0,00	**0,91**	0,00	0,09

Continuation Table 5

	Character 3 ARD model			**Character 3 ED model**		
node	**-**	**absent**	**present**	**-**	**absent**	**Present**
2	0,00	0,00	**1,00**	0,00	0,36	**0,64**
4	0,00	**0,99**	0,01	0,00	**0,99**	0,01
7	0,00	**0,99**	0,01	0,09	**0,91**	0,00
17	0,00	0,00	**1,00**	0,00	0,21	**0,79**
18	0,00	0,00	**1,00**	0,00	0,21	**0,79**
19	0,00	0,00	**1,00**	0,00	0,18	**0,82**
20	0,00	0,00	**1,00**	0,00	0,24	**0,76**
25	0,00	0,00	**1,00**	0,00	0,02	**0,98**
26	0,00	0,00	**1,00**	0,00	0,02	**0,98**
27	0,00	0,00	**1,00**	0,00	0,09	**0,91**
29	0,00	0,27	**0,73**	0,00	0,35	**0,65**
30	0,00	**0,81**	0,19	0,00	**0,92**	0,08
33	0,00	0,00	**1,00**	0,03	0,02	**0,95**
34	0,10	0,02	**0,88**	0,13	0,02	**0,85**
35	**0,93**	0,01	0,06	**0,89**	0,01	0,10
38	**0,93**	0,01	0,06	**0,89**	0,01	0,10
39	0,00	0,00	**1,00**	0,00	0,00	**1,00**
42	0,00	0,00	**1,00**	0,00	0,00	**1,00**
43	0,00	0,00	**1,00**	0,00	0,00	**1,00**
47	0,00	0,00	**1,00**	0,00	0,03	**0,97**
50	0,00	0,00	**1,00**	0,00	0,00	**1,00**
51	0,00	0,00	**1,00**	0,00	0,00	**1,00**
55	0,00	0,00	**1,00**	0,00	0,00	**1,00**
60	0,00	0,00	**1,00**	0,00	0,00	**1,00**
73	0,00	0,00	**1,00**	0,00	0,00	**1,00**

Figure 1

Schematic representation of the middle ear of lizards.

(A) columella. (B) extracolumella and tympanic membrane. Modified from Mason and Farr (2013).

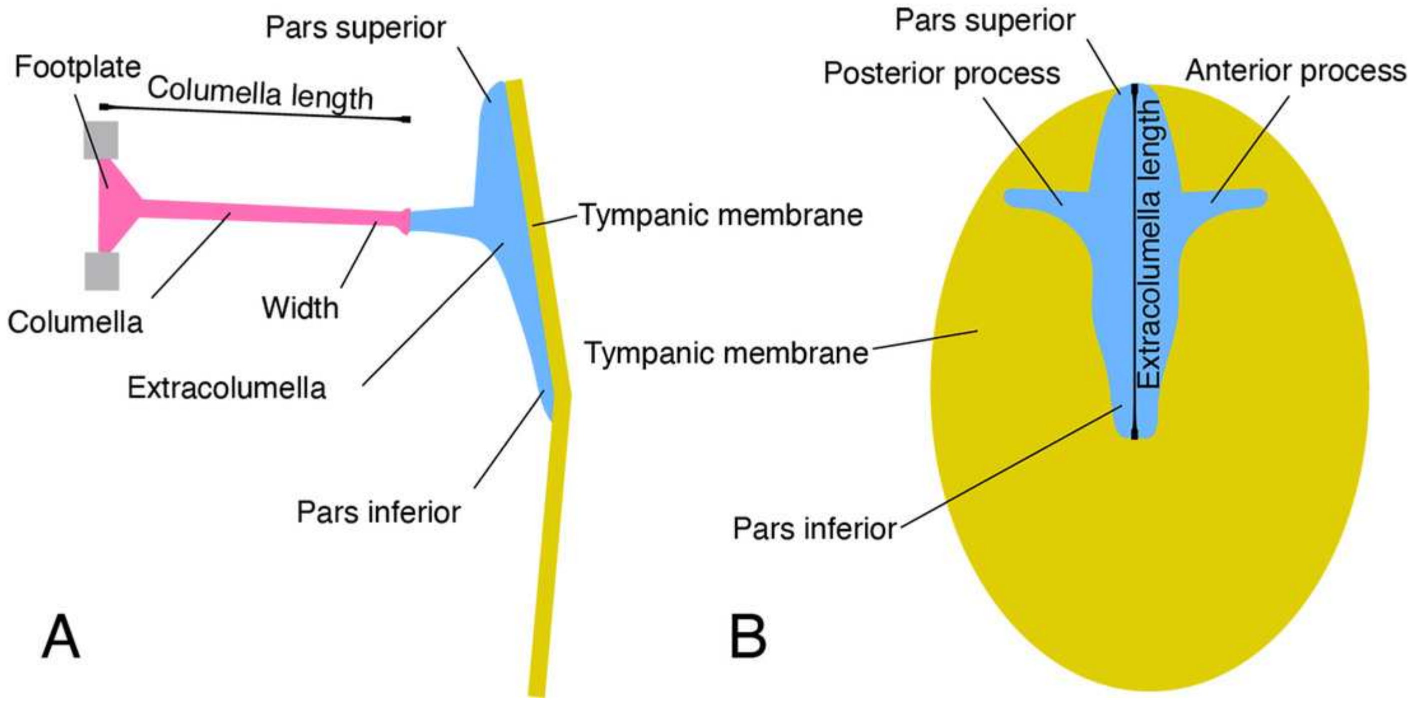


Figure 2

Columella.

(A) *Gonatodes concinnatus* MUJ 733. (B) *Hoplocercus spinosus* MZUSP 92161. (C) *Tretioscincus bifasciatus* ICN 5588.

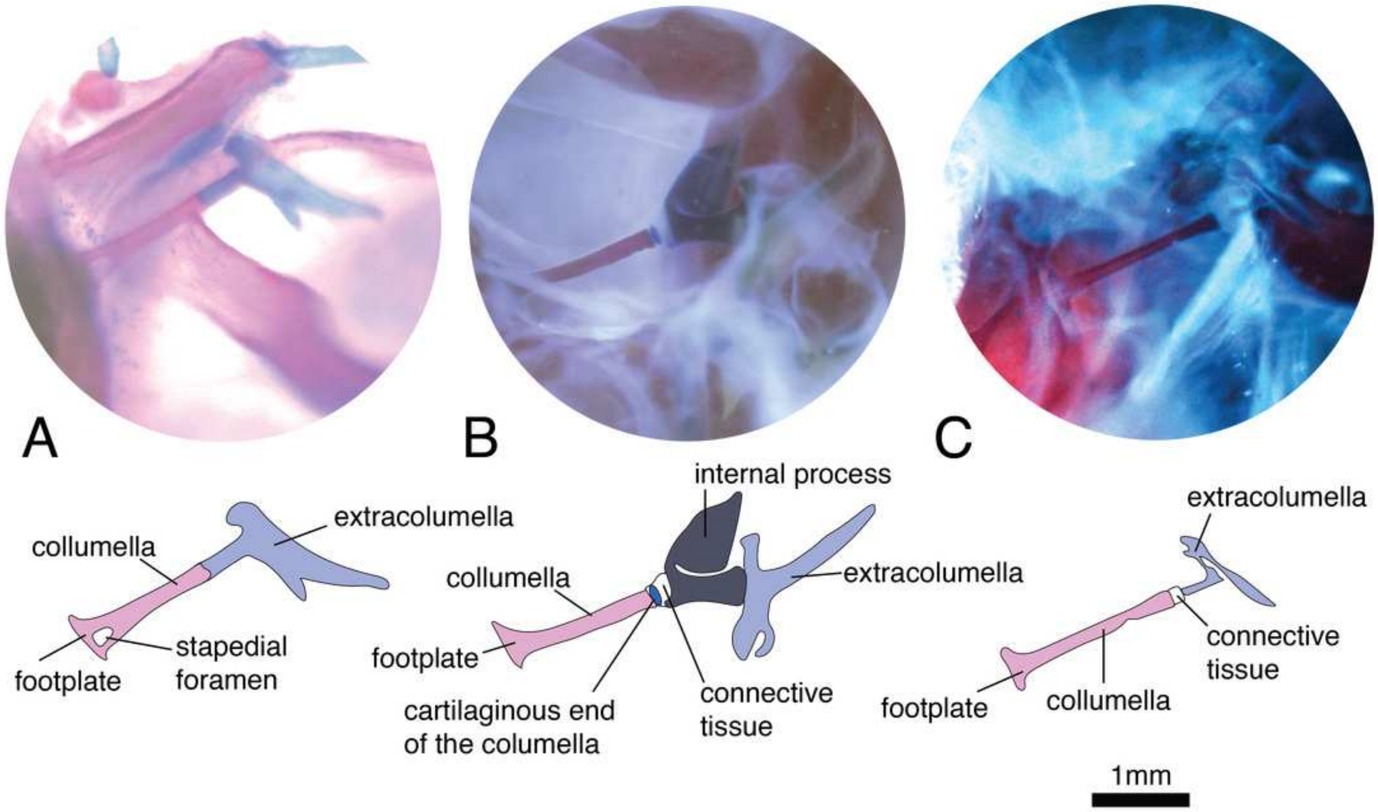


Figure 3

Columella.

(A) *Stenocercus trachycephalus* MUJ 635. (B) *Lialis jicari* MZUSP 67148. (C) *Anolis maculiventris* MHAU 10468.

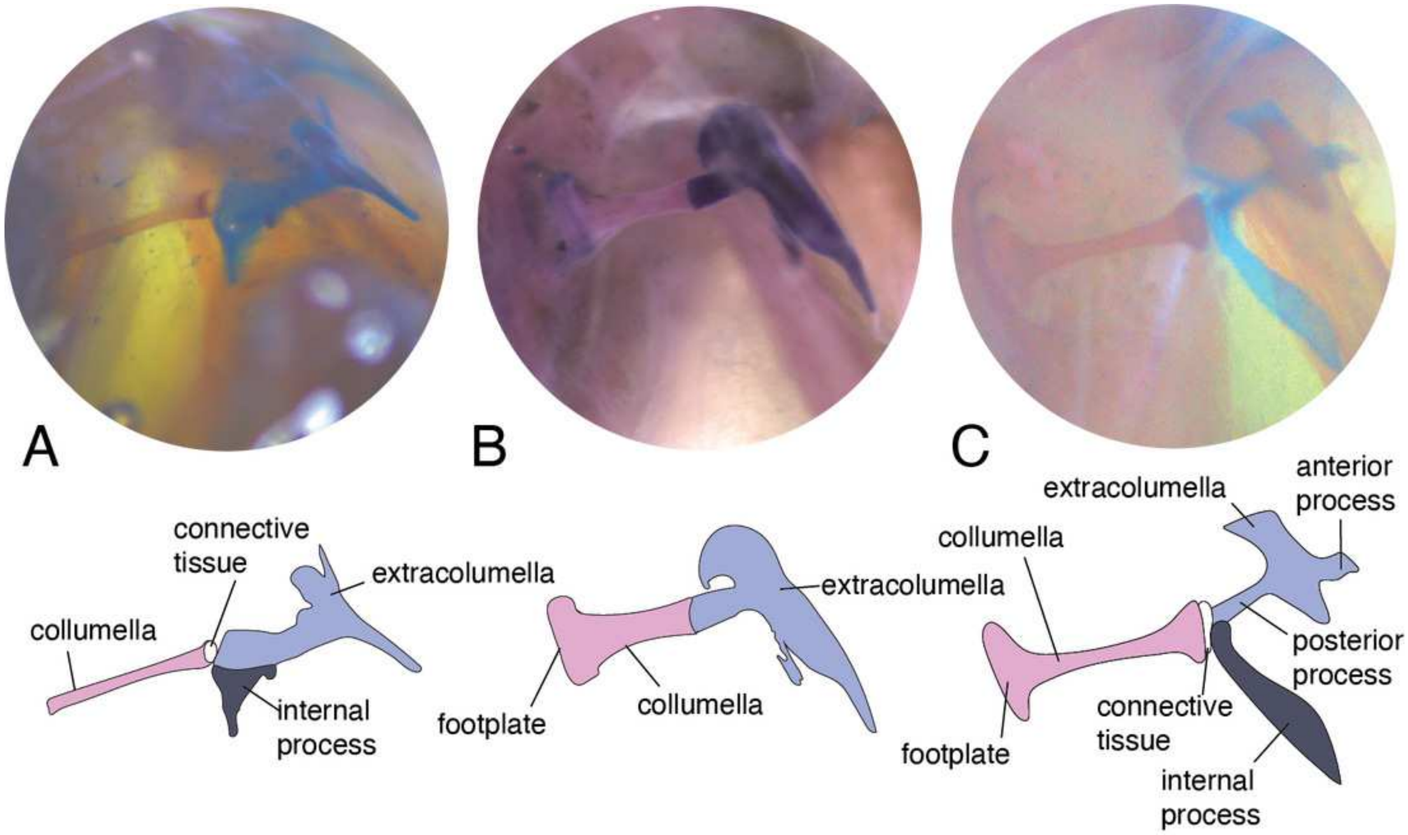


Figure 4

Extracolumella.

(A) *Phelsuma madagascariensis* MZUSP 36938. (B) *Thecadactylus rapicauda* MZUSP 97833. (C) *Lialis jicari* MZUSP 67148.

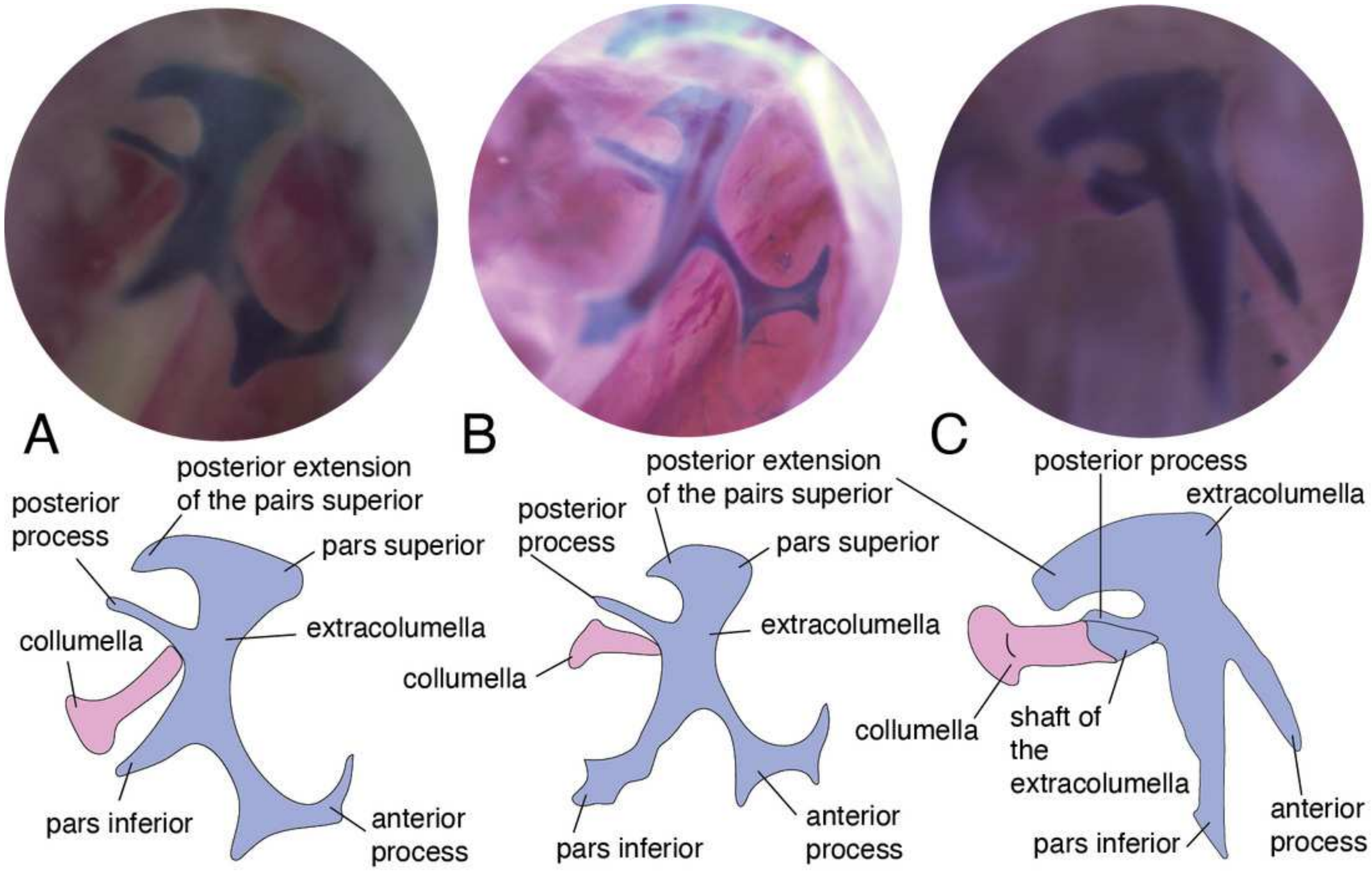


Figure 5

Extracolumella.

(A) *Gonatodes concinnatus* MUJ 733. (B) *Morunasaurus groi* ICN 6270. (C) *Tropidurus pinima* MZUSP 92140.

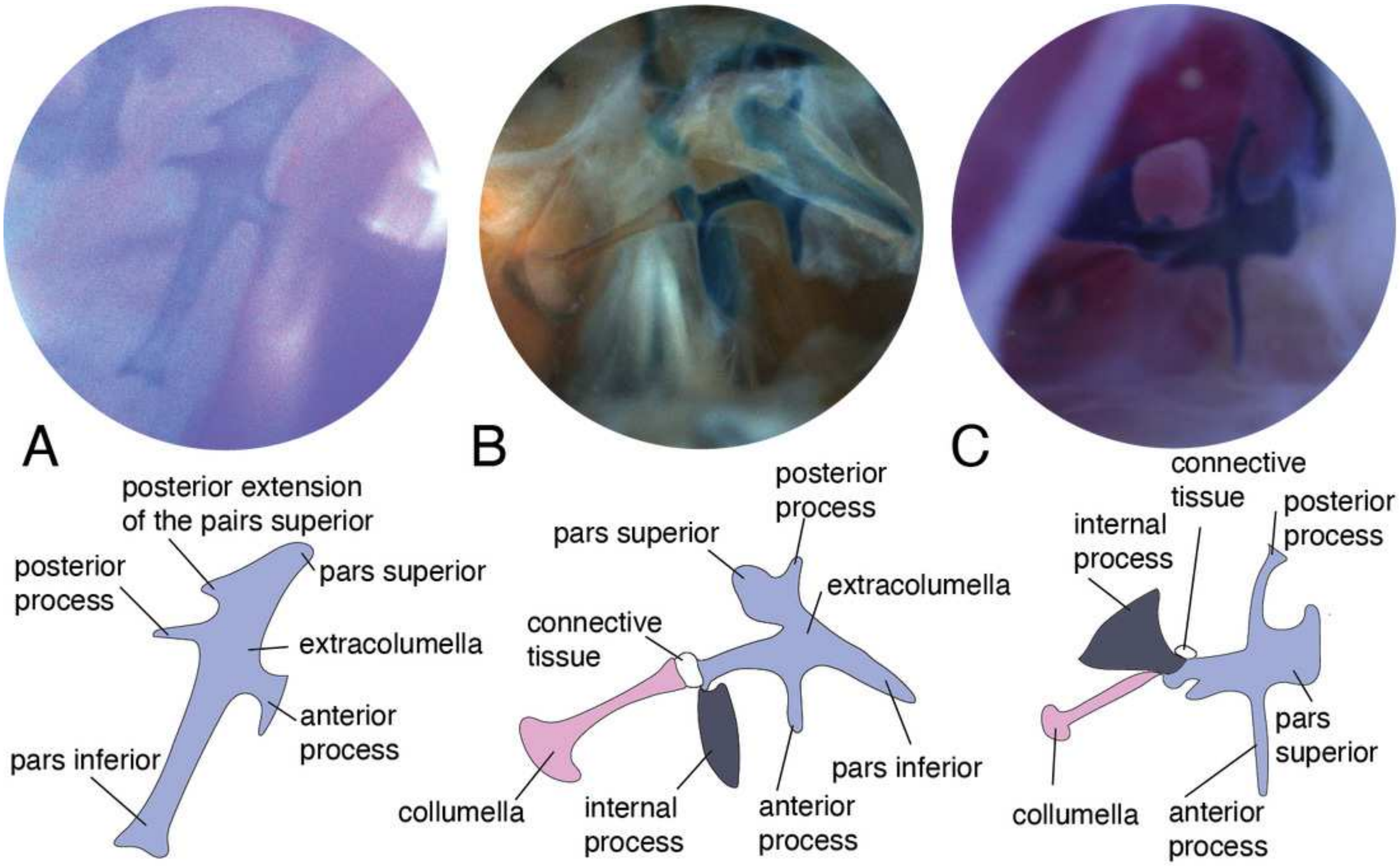


Figure 6

Extracolumella.

(A) *Gelanesaurus cochranae* ICN 9453. (B) *Stellagama stellio* MZUSP 95176. (C) *Mabuya falconensis* ICN 11312.

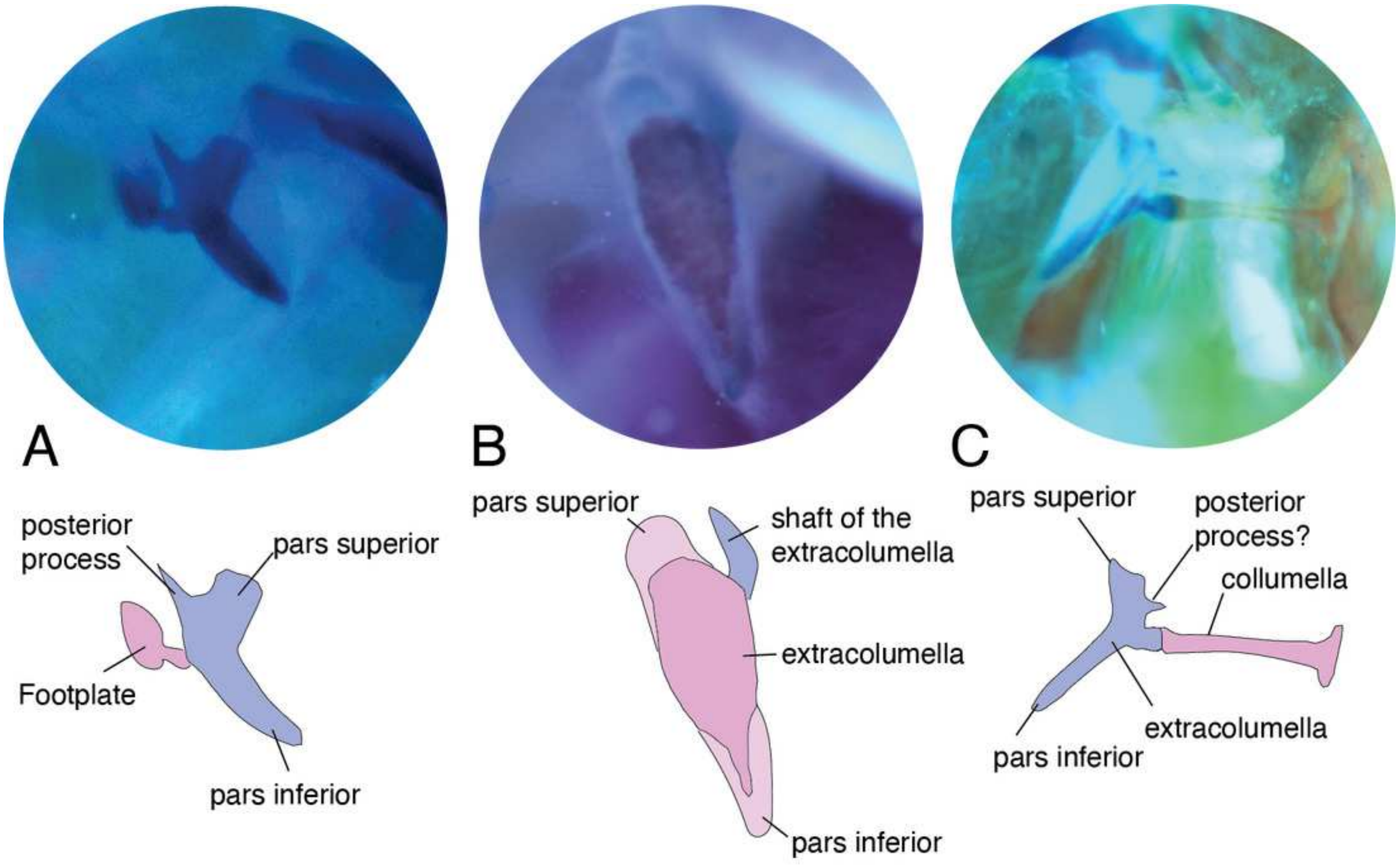


Figure 7

Summary of the mapping of the characters using maximum parsimony (MP).

Character 1. Length of the columella relative to the extracolumella central axis length.

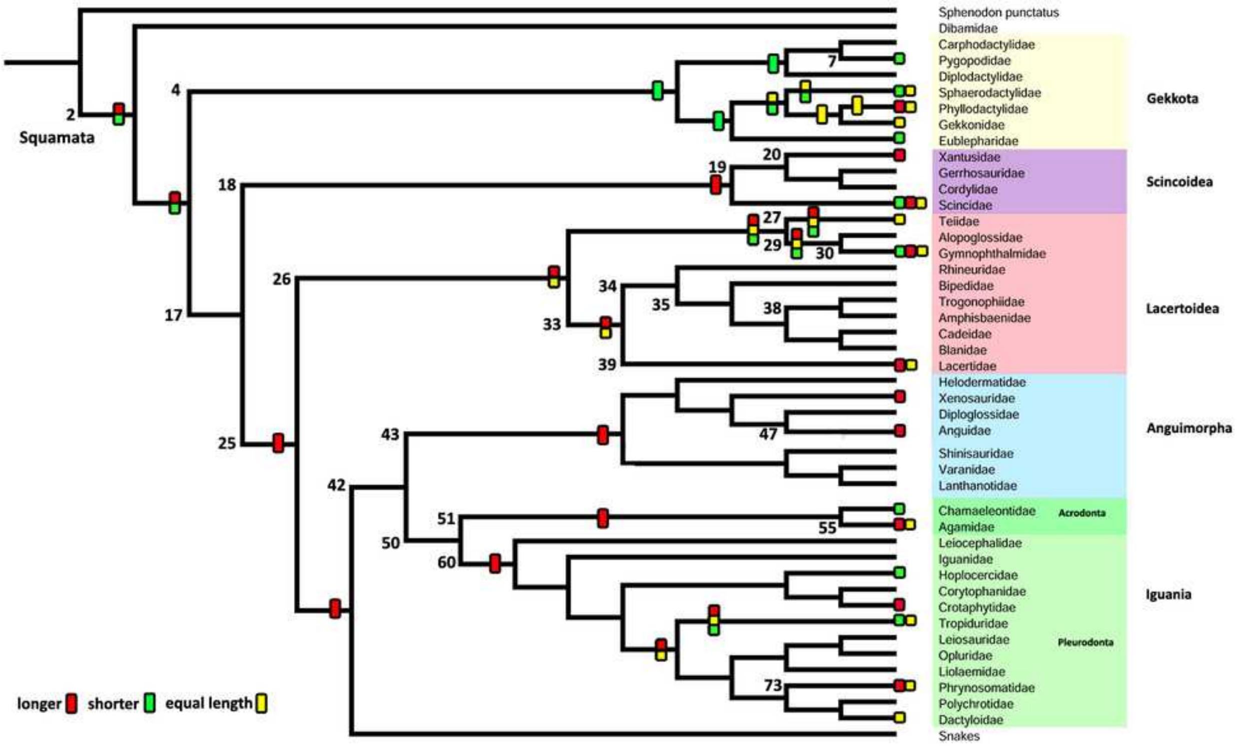
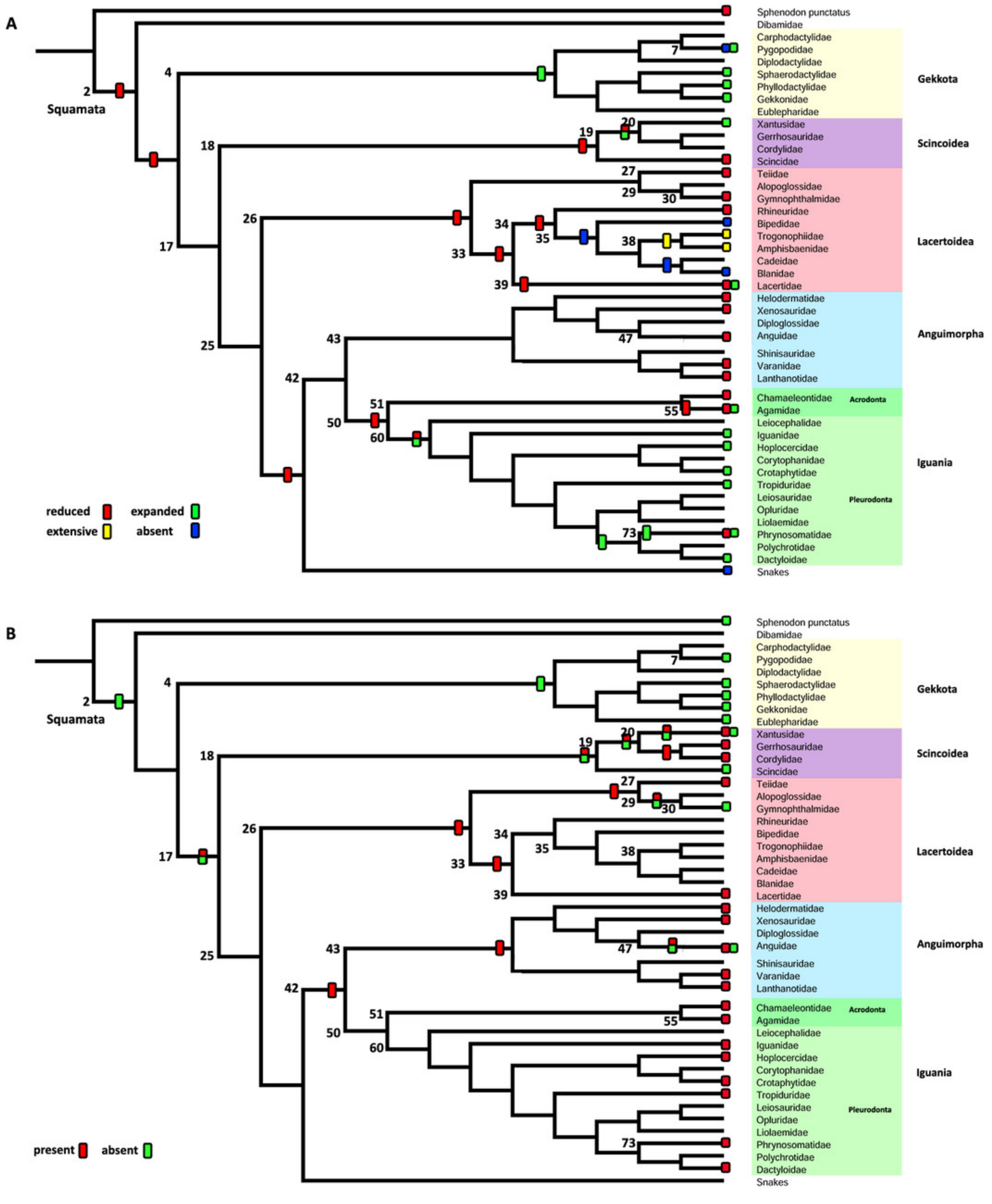


Figure 8

Summary of the mapping of the characters using maximum parsimony (MP).

(A) Character 2. Extracolumella. (B) Character 3. Internal Process.


A
2
Squamata
4
7
17
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27
29
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33
34
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38
39
42
43
47
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51
55
60
73
Sphenodon punctatus
Dibamidae
Carphodactylidae
Pygopodidae
Diplodactylidae
Sphaerodactylidae
Phyllodactylidae
Gekkonidae
Eublepharidae
Gekkota
Xantusidae
Gerrhosauridae
Cordylidae
Scincidae
Scincoidea
Teiidae
Alopoglossidae
Gymnophthalmidae
Rhineuridae
Bipedidae
Trogonophiidae
Amphisbaenidae
Cadeidae
Blanidae
Lacertidae
Lacertoidea
Helodermatidae
Xenosauridae
Diploglossidae
Anguidae
Shinisauridae
Varanidae
Lanthanotidae
Anguimorpha
Chamaeleontidae
Agamidae
Acrodonta
Leiocephalidae
Iguanidae
Hoplocercidae
Corytophanidae
Crotaphytidae
Tropiduridae
Leiosauridae
Opluridae
Liolaemidae
Phrynosomatidae
Polychrotidae
Dactyloidae
Pleurodonta
Iguania
Snakes
reduced 🟥
expanded 🟩
extensive 🟨
absent 🟦
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Squamata
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73
Sphenodon punctatus
Dibamidae
Carphodactylidae
Pygopodidae
Diplodactylidae
Sphaerodactylidae
Phyllodactylidae
Gekkonidae
Eublepharidae
Gekkota
Xantusidae
Gerrhosauridae
Cordylidae
Scincidae
Scincoidea
Teiidae
Alopoglossidae
Gymnophthalmidae
Rhineuridae
Bipedidae
Trogonophiidae
Amphisbaenidae
Cadeidae
Blanidae
Lacertidae
Lacertoidea
Helodermatidae
Xenosauridae
Diploglossidae
Anguidae
Shinisauridae
Varanidae
Lanthanotidae
Anguimorpha
Chamaeleontidae
Agamidae
Acrodonta
Leiocephalidae
Iguanidae
Hoplocercidae
Corytophanidae
Crotaphytidae
Tropiduridae
Leiosauridae
Opluridae
Liolaemidae
Phrynosomatidae
Polychrotidae
Dactyloidae
Pleurodonta
Iguania
Snakes
present 🟥
absent 🟩