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Osteological characterization of four putative species of the genus *Adenomera* (Anura: Leptodactylidae), with comments on intra- and interspecific variation

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Abstract

Previous osteological characterizations of members of the frog genus *Adenomera* have been based on selected features. The purpose of this study is to establish a detailed osteological description of four putative species of the genus *Adenomera*. There is considerable variation, which we postulate corresponds to both intra- and interspecific variation. Osteology should provide a set of characters appropriate for cladistic analyses to help elucidate relationships within the Subfamily Leptodactylinae. Much of the adult osteological variation has a high probability of having evolved through the process of heterochrony.

Key words: Leptodactylidae, osteology, *Adenomera*, taxonomy

Resumen

Caracterizaciones osteológicas previas de los miembros del género *Adenomera* han sido basadas en algunos caracteres particulares. La propuesta de este estudio es establecer una detallada descripción de cuatro especies putativas del género *Adenomera.* El material estudiado presentó considerable variación, tanto intra como interespecífica. De manera que la osteología aportaría un set de caracteres útiles para un análisis cladístico que contribuya a elucidar las relaciones dentro de la subfamilia Leptodactylinae. Mucha de la variación osteológica tiene una alta probabilidad de haber evolucionado a través de procesos de heterocronía.

Introduction

The genus *Adenomera* currently includes eight recognized species (Heyer, 1975; De la Riva, 1996; Kwet & Angulo, 2002; Frost, 2006) that occur in South America east of the Andes (Heyer, 1973; Frost, 2004). Heyer (1973; 1974; 1977) studied the systematics of the *marmoratus* group of the genus *Leptodactylus* and revalidated the genus *Adenomera*, which exhibits extensive morphological variation within and among populations of species (De la Riva, 1996). Preserved specimens are difficult to diagnose, and the external morphological variation is such that it is difficult to delimit one species from another. Historically, it has been difficult to resolve the taxonomic problems of the genus and it has been suggested that the resolution of the intrageneric relationships may require molecular and advertisement call data (Heyer, 1975; 1984). In fact, advertisement calls have been effectively used to identity species and recently, researchers have identified new species based on such data (Kwet & Angulo, 2002; Angulo *et al.*, 2003; Angulo & Icochea, 2003). Morphological studies

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are an important complement to acoustic data; for example, in four species of *Adenomera* from southern Peru, subtle morphological differences associated with each call type provided evidence that the different call types are not different signals in the repertoire of the same species (Angulo *et al.*, 2003). The reproductive mode is also useful in discerning species limits in the genus (Angulo *et al.*, 2003). *Adenomera* has the most terrestrial reproductive biology in the Subfamily Leptodactylinae; in some species eggs are deposited in foam nests away from standing water, and tadpoles complete development in the nest (Heyer, 1974). A second reproductive mode was inferred (Heyer, 1973) and recently confirmed — eggs are deposited in a foam nest, but tadpoles do not complete their development in the nest and have a free swimming aquatic stage (De la Riva, 1995; Almeida & Angulo, 2002).

Frost *et al*. (2006) re-evaluated phylogenetic relationships of amphibians based primarily on molecular data. Those authors proposed a new taxonomy for the genus *Adenomera*, placing it, *Lithodytes*, and *Vanzolinius* in the synonymy of *Leptodactylus* and recognizing the clade *Adenomera* + *Lithodytes* as the subgenus *Lithodytes*. The Frost *et al*. (2006) proposal should be tested with greater taxon sampling and other suites of characters to confirm whether the taxonomic arrangement they propose is robust or not. Until such studies are accomplished, we prefer to recognize *Adenomera*, *Leptodactylus*, and *Lithodytes* as distinct genera, but we concur with placing *Vanzolinius* in the synonymy of *Leptodactylus* (de Sá *et al.*, 2006).

The purpose of this paper is to provide a detailed osteological description of the genus *Adenomera*, which can serve as a source of information for analyses of relationships involving the genus. Our results indicate that osteological characters may be of use in analyzing relationships among the species of *Adenomera*, as well.

Materials and methods

Cleared and stained specimens were prepared following the protocol of Wassersug (1976). The osteology of adults and juveniles (Stage 46 of Gosner, 1960) was compared. Osteological terminology is that of Heyer (1975), Lynch (1971), Trueb (1973, 1993) and Trueb *et al.* (2000); phalangeal and carpal osteology follow Fabrezi (1992); tarsal osteology is that of Fabrezi (1993); laryngeal morphology follows Trewavas (1933). Fingers are numbered II–V following Fabrezi and Alberch (1996), who concluded that digit I is lost in anurans.

Specimens examined are listed in Appendix 1. Museum abbreviations follow Leviton *et al.* (1985), with the exception of QCAZ = Museo de Zoología de la Pontificia Universidad Católica del Ecuador, Quito. As mentioned above, the genus *Adenomera* is taxonomically difficult due largely to morphological variation both within and between species. It is even possible to find two or more cryptic species of this genus living in sympatry and even in syntopy (Angulo *et al*., 2003). Identification of the specimens based on external morphology is difficult. Of the examined specimens, only those from Boracéia are unequivocally assigned to *A. marmorata*, because there is only one call type at this locality and the site has been extensively worked herpetologically. To avoid mistakes in identification, the other specimens are treated as operational taxonomic units (OTUs). No juveniles were available from the Ecuador or Fazenda Unacau localities. Both museum specimens from Ecuador were identified as *Adenomera andreae* and all four museum specimens from Fazenda Unacau, Brazil were identified as *Adenomera marmorata*. Our studies indicate the specimens represent two distinct species from both the Ecuadorian site and Fazenda Unacau (see discussion).

Results

All the studied specimens show an osteologically common pattern described as follows:

Cranial osteology of adults

(Figs. 1 and 2)

Maxillary Arcade (Fig. 1b). The maxillary arcade is complete. The upper jaw is composed of premaxillae, maxillae and quadratojugals.

Each premaxilla bears 10 or 11 teeth. The lateral end is juxtaposed with the adjacent maxillae. The alary process is subrectangular, perpendicular to the longitudinal axis of skull and it is oriented dorsally and curved backwards. The alary process has a narrow base and the processes are parallel to each other. The pars palatina is subrectangular, its posterior side is concave.

The pars facialis of the maxilla overlaps the lateral end of the premaxilla; the narrow posterior end articulates with the quadratojugal. The teeth are straight, cylindrical, with blunt tips. The teeth extend from the middle of the space between the extreme of the quadratojugal and the extreme of the anterior ramus of the pterygoid. Each maxilla bears from 41–54 teeth. The pars facialis ends at the level of the neopalatines and is separated from the nasals.

The quadratojugal is a completely ossified rod-shaped bone. It is in dorsal contact with the descendent ramus of the squamosal and overlaps the maxilla.

Mandible (Fig. 2). The mentomeckelian is L-shaped, with the shorter rami perpendicular to horizontal plane of the skull. The articulate middle side of this bone is subcircular-concave. The dentary is narrow, curved anteriorly. Transversally, it has the form of a thin plate, not excavated; laterally it partially covers Meckel's cartilage. The angulosplenial is the long main bony component of the mandible, separated from the mentomeckelian by a space shorter than the length of the latter and covering the inner side of Meckel's cartilage. The articular region of the mandible is cartilaginous. The coronoid process is rectangular.

Nasal capsule (Fig. 1a) The tectum nasi is straight anteriorly, extending behind or to the same level of the premaxillae.

Endocranial Braincase (Fig. 1a). The endocranial braincase is composed of pairs of cartilage replacement bones — sphenethmoid, prootics, and exoccipitals — that are partially covered by frontoparietals dorsally and the parasphenoid ventrally.

The sphenethmoid forms the floor, edges of the roof and the anterolateral wall of each side of the braincase. The region between the nasals and vomers is calcified. It is dorsally visible in the space between the nasals and frontoparietals. Its ventral face overlaps the inner one-third of the neopalatines. The sphenethmoid borders are separated from the optic foramen. The posterior half of the nasals and the anterior half of the frontoparietals overlap the sphenethmoid. Ventrally the vomers overlap the sphenethmoid (Fig. 1b). An orbitonasal foramen is visible, anterodorsal, and enclosed by the sphenethmoid.

Sometimes the limits between the sphenethmoid, the orbitosphenoid and prootic are distinct, but these elements can be fused to form a single lateral wall on each side of the posterior braincase (Fig. 1b).

The prootics are fused with the exoccipitals; both elements form the posterior region of the braincase and each one contributes to the otic capsules. They are not fused with the frontoparietals. They lack dorsal ornamentation, although they have protuberant crests. There is an enclosed occipital canal. The prootic foramen of the orbital region of the prootics is totally bordered by bone. The oculomotor foramen is enclosed by the prootic and the orbitosphenoid. The prootic foramen is enclosed by the prootic.

The exoccipitals form the posteromedial walls of the otic capsules, the margins of the foramen magnum and the occipital condyle. There is mineralized cartilage between them. They are ventrally covered by the parasphenoid.

The occipital condyles are widely separated, diverging upwards, half-moon-shaped, protuberant. They are behind an imaginary line that joins the posterior borders of the quadrates.

The jugular foramen is lateral to the occipital condyles, entering the posterior wall of the otic capsule.

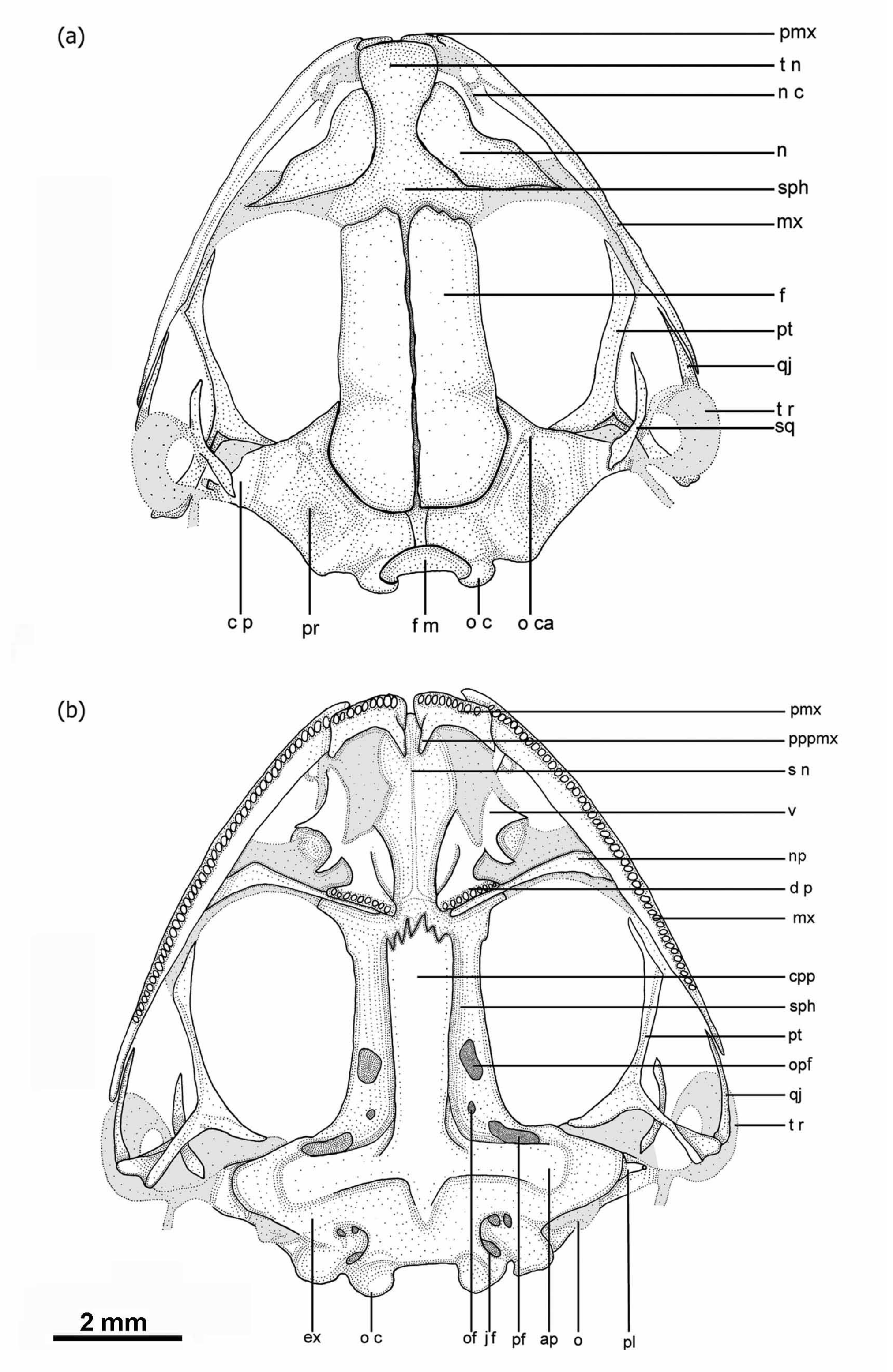

FIGURE 1. Skull of OTU 1, QCAZ 6192 from Ecuador (male specimen) (a) Dorsal view; (b) Ventral view. a–p: ala of parasphenoid; c–p: crista parotica; c–p–p: cultriform process of parasphenoid; d–p: dentigerous process of vomer; ex: exoccipital; f: frontoparietal; f–m: foramen magnum; j–f: jugular foramen; mx: maxilla; n: nasal; n–c: nasal cartilages; np: neopalatines; o: operculum; o–c: occipital condyle; o–ca: occipital canal; o–f: orbitonasal foramina; op–f: optic foramen; p–f: prootic foramina; pl: plectro; pmx: premaxilla; p–p–pmx; palatine process of premaxilla; pr: prootic; pt: pterygoid; qj: quadratojugal; s–n: septum nasi; sph: sphenethmoid; sq: squamosal; t–n: tectum nasi; t–r: tympanic ring;. v: vomer.

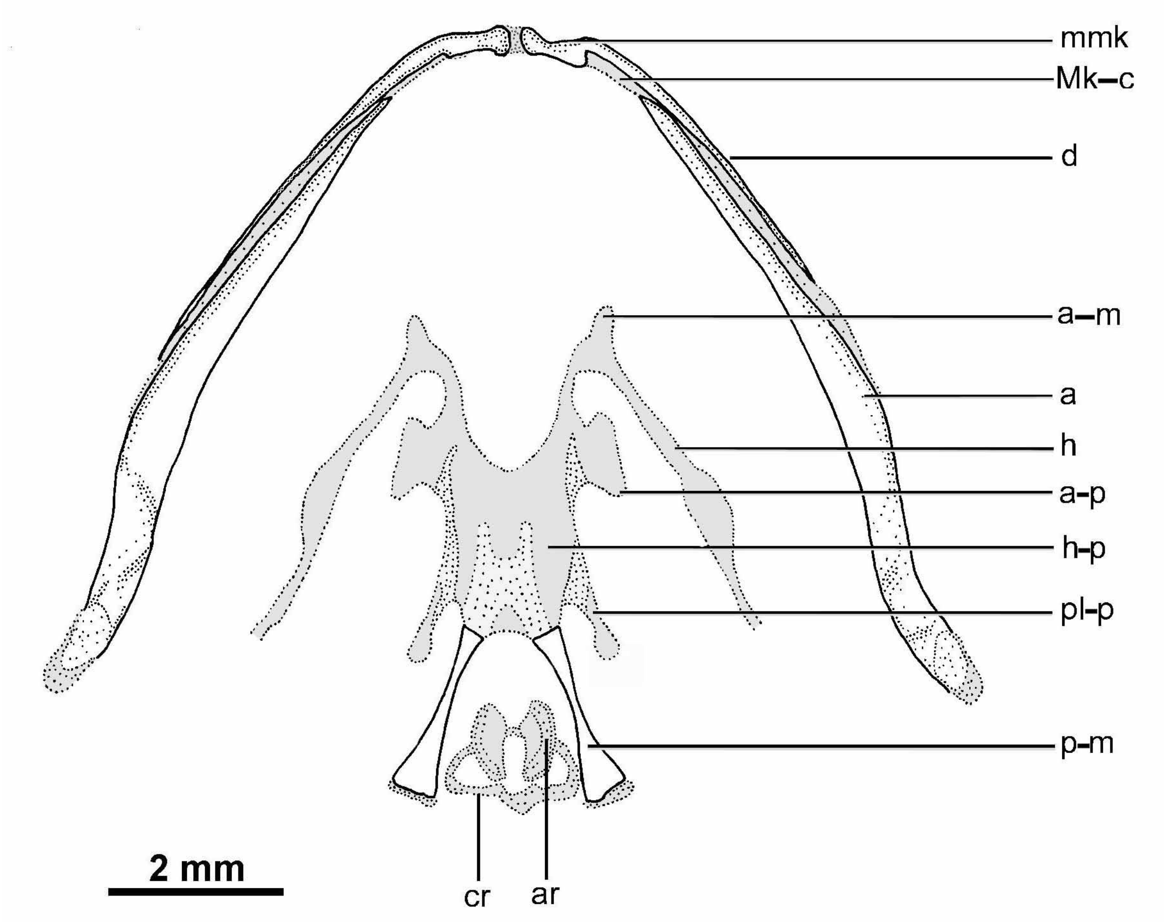

FIGURE 2. Hyoid of OTU 1, QCAZ 6192 from Ecuador. a–m: anteromedial process; a–p: alary process; ar: arytenoids; cr: cricoid; h: hyale process; h–p: hyoid plate; pl–p: posterolateral process; p–m: posteromedial process. Mandible. a: angulosplenial; d: dentary; Mk–c: Meckel's cartilage; mmk: mentomeckelian.

Plectral Apparatus. The pars externa plectri is approximately globe-shaped, slightly elongated. The pars interna plectri is expanded; it lies adjacent to the operculum. The angle of the pseudoperculum is very prominent in a lateral view. The apophysis of the plectro is short, conical. The pars media plectri is a slightly curved ossified stylus.

The operculum is mineralized, elliptic, and externally convex. It is adjacent to the dorsal posterior edge of the pars interna plectri. It covers almost completely the oval window. The opercular crest is well developed.

Dermal Investing Bones. The frontoparietals (Fig. 1a) are paired. The external borders are parallel to each other. The posterior sides are expanded and rounded. The posterior margin is convex. Posteriorly, the frontoparietals overlap the prootics, but they do not reach the foramen magnum. They cover the sphenethmoid for half of its length, but in some specimens it is not possible to determine the percentage of overlap because the limit between the sphenethmoid and septum nasi is indistinct (Table 1). The lateral margins are not fused with the prootics. The frontoparietal fontanelle is completely covered by the frontoparietals. The frontoparietal fenestra is oval-shaped, with a posterior and anterior small "tongue". The frontoparietal fenestra extends forward starting at mid skull.

The nasals (Fig. 1a) are paired subtriangular bones, transversally oriented, with curved borders. They are superimposed on the sphenethmoid. A small space separates the nasals from the cartilage of the nasal capsule. The maxillary process is well differentiated from the nasal body. It has sharp ends and straight borders. The irregular posterointernal angle is separated from the frontoparietals. The anterior border is deeply or slightly concave. The pars facialis of the maxilla is separated from the nasals.

TABLE 1. Characterization of osteological variation observed in *Adenomera* specimens examined. ? = missing data; / = both states are present. * The state “ossified” was differentiated from “mineralized” because in the first case the structure is completely stained red with alizarin, and in the second case, on a blue cartilaginous structure, some dispersed osseous depositions are discernible. ** This character presents three states because one of the two specimens is asymmetric and shows both states. Only the *Adenomera marmorata* specimens from Boracéia can be confidently identified to species (see Materials and Methods and Discussion).

	OTUs								
Characters	Ecuador OTU 1	Ecuador OTU 2	Fazenda Unacau. Brazil. OTU 1	Fazenda Unacau.Brazil. OTU 2	Fazenda Unacau. Brazil. OTU 3	Fazenda Unacau. Brazil. OTU 4	Boracéia. Brazil (juveniles, n=4)	Boracéia. Brazil (advanced juveniles, n=2)	Boracéia. Brazil (adult)
1- Septum nasi, tectum nasi and solum nasi *	ossified	mineralized	mineralized	mineralized	mineralized	mineralized	cartilaginous	cartilaginous	mineralized
2- Lateral borders of the cultriform process of parasphenoid	straight and parallel (Fig. 1b)	broadened medially, curved, convergent anteriorly	broadened medially, straight with sharp angle at broadest point, convergent anteriorly	broadened medially, straight with sharp angle at broadest point, convergent anteriorly	broadened medially, straight with sharp angle at broadest point, convergent anteriorly	broadened medially, straight with sharp angle at broadest point, convergent anteriorly	straight and parallel	straight and parallel	broadened medially, straight with sharp angle at broadest point, convergent anteriorly
3- Zygomatic ramus of each squamosal	parallel (Fig. 1a)	oriented slightly to the outside	oriented slightly to the outside	oriented slightly to the outside	oriented slightly to the outside	oriented slightly to the outside	oriented slightly to the outside/ parallel	oriented slightly to the outside/ inside/ parallel **	oriented slightly to the outside
4- Tips of the posterolateral processes of hyoid	rounded and expanded (Fig.2)	flattened	flattened	flattened	flattened	flattened	acute/ flattened	rounded	rounded
5- Dentigerous process of vomer	straight series (Fig. 1b)	arched series	arched series	arched series	straight series	straight series	straight series	straight series	straight series
6- Epicoracoid	left overlaps the right (Fig. 3)	left overlaps the right	left overlaps the right	right overlaps the left	left overlaps the right	left overlaps the right	left overlaps the right/ right overlaps the left	left overlaps the right	left overlaps the right

	OTUs								
Characters	Ecuador OTU 1	Ecuador OTU 2	Fazenda Unacau. Brazil. OTU 1	Fazenda Unacau.Brazil. OTU 2	Fazenda Unacau. Brazil. OTU 3	Fazenda Unacau. Brazil. OTU 4	Boracéia. Brazil (juveniles, n=4)	Boracéia. Brazil (advanced juveniles n=2)	Boracéia. Brazil (Adult)
7- Mineralized element between scapula and coracoid	absent	absent	present	present	absent	absent	present, cartilaginous	absent	absent
8- Nasal shape	subtriangular, transversely oriented (Fig. 1a)	rectangular-shaped, transverse to diagonal orientation	subtriangular, transversely oriented	subtriangular, transversely oriented	subtriangular, transversely oriented	subtriangular, transversely oriented	stick-shaped, diagonally oriented	subtriangular, transversely oriented	subtriangular, transversely oriented
9- Nasal extent	broadly separated (Fig. 1a)	contiguous	broadly separated	broadly separated	contiguous	broadly separated	broadly separated	broadly separated	broadly separated
10- Number of carpal elements	six	six	six	seven	six	six	?	six	six
11- The esophageal process of the cricoid with an inferior slope	no	no	no	yes	no	?	?	no	yes
12- The esophageal process of the cricoid with a superior slope	yes	yes	yes	yes	no	?	yes	no	yes
13- Posterolateral margins of the sphenethmoid	concave	convex	concave	?	convex	convex	concave	?	convex
14- Anterior expansion of omosternum	fan-shaped (Fig. 3)	fan-shaped	ovoid	ovoid	ovoid	fan-shaped	ovoid/ fan-shaped	?	fan-shaped

The parasphenoid (Fig. 1b) is an unpaired, T-shaped bone. It is not fused with the subjacent bones. The cultriform process is long, not keeled and serrated anteriorly. The cultriform process rests on the sphenethmoid. The anterior border is very close to the neopalatines. The posteromedial process of the parasphenoid is acuminate and terminates well short of the margin of the foramen magnum. The alae of the parasphenoid are not fused with the otoccipitals, their orientation deflects posteriorly, and they are slightly expanded distally.

The neopalatines (Fig. 1b) are curved bones, posteriorly concave. The lateral portion of the neopalatine is covered dorsally by the planum antorbitale cartilage. The edges are sharp without ornamentation or odontoids. They have an inferior ridge. They are oriented perpendicular to the axial axis of the skull. One-third of its inner length overlaps with the sphenethmoid. The outer edge is gradually expanded and contacts the pars palatina of the maxilla. The vomers cover the inner projections of the neopalatines, which are separate from each other.

The vomers (Fig. 1b) are paired bones that overlap the sphenethmoid. A dentigerous process and three alae that constitute the anterior and medial margins of choanae form them. The anterior ramus is shorter or equal to the middle ramus; the latter is longer or equal to the posterior ramus. The angle between the anterior and middle ala is greater than 90º; between the middle and posterior ala angle is less than 90º. Each dentigerous process has an oblique series of 9–14 teeth. The end of the anterior ala is spine-like, in contact with the maxilla. Contact between the anterior ala and the maxilla is not clearly visible in Figure 1b because of cartilaginous element overlap in the contact area.

Suspensorium. Each pterygoid has well-developed anterior, medial and posterior rami (Fig. 1b). The anterior ramus is expanded anteriorly; it does not extend to the neopalatines, but it does extend to the anterior margin of the orbit. The pterygoid articulates with the inner side of the maxilla. The medial ramus rests on the prootic with cartilage in between. The anterior and posterior rami form an elongated S-shape. The posterior ramus is laminar, curved, and joins the quadratojugal-squamosal complex.

The zygomatic ramus of the squamosal (Fig. 1a) is curved and subtriangular. The otic ramus is subtriangular or subrectangular. It does not contact the frontoparietal. It reaches or overlaps the border of crista parotica. The length of the otic ramus is approximately equal to that of the zygomatic ramus. The descendent ramus has a medial canal. The inferior end of the descendant ramus is expanded, forming an angle greater than 45º with the horizontal plane of the skull. The angle between the squamosal and maxilla is less than 45º. The crista parotica is cartilaginous with calcifications. The tympanic ring is dorsally opened, cartilaginous, and joined to the zygomatic ramus.

Hyoid Apparatus (Fig. 2). The hyoid plate is cartilaginous, mineralized at the base of the alary, hyale, posterolateral and posteromedial processes. The margins are parallel, sometimes slightly divergent.

The alary processes are perpendicular to the axial axis of the hyoid plate, slightly oriented forward, with a distal expansion. The bases of the alary processes are broad.

The hyales are thin, curved, and generally expanded at the level or close to the level of the alary processes. The distal extremes are joined by cartilage with the otic capsule anteriorly and under the columella. Anteromedial processes are present.

The posterolateral processes are thin. The base arises on the posterolateral side of the hyoid plate and is oriented postero-laterally. The posterior end extends well beyond the level of the posterior edge of the hyoid plate.

The posterior ends of the bony posteromedial processes are cartilaginous. Both anterior and posterior ends are expanded.

Laryngeal Cartilages (Fig. 2). The arytenoids consist of a pair of valve-shaped cartilages, triangular in lateral view. The cricoid forms a complete ring, not mineralized. An esophageal process is differentiated and trapezoidal. The bronchial process is differentiated, noticeably thin.

Postcranial osteology

Vertebral Column (Fig. 5). The column is composed of eight procoelous presacral vertebrae, the sacrum and the urostyle. The vertebrae are imbricated. The relative length of transverse processes are variable, but most specimens have the pattern: III > IV = V = VI = VII = VIII = II. Ventrally the relative lengths of the vertebral centra are: 2 < 3 = remaining vertebrae. The neural arch of each vertebra has a well-developed dorsal ridge, and a pair of parasagital processes extending laterally. The neural arches are not imbricated.

The atlas is not fused to the adjacent vertebra. The anterior edge is convex. The atlas is not dorsally divided. The centrum of the atlas (in ventral view), is wider than the other vertebrae.

The cotylar arrangement of the atlas resembles Type I of Lynch (1971). The cervical cotyles are oriented anteriorly or lateroanteriorly and are separated from each other and semilunar in shape. The intercotylar region is concave.

The moderately dilated sacral diapophyses are oriented toward the back, and they are ovoid in cross-section.

The ilio-sacral articulation is type II B of Emerson (1982). There is a mineralized sesamoid element between the ilio-sacral articulations. The sacral-coccygeal articulation is bicondylar.

The urostyle is compressed, with a well-developed dorsal spine, and an anterior protuberance. The urostyle is not fused to the sacrum. The dorsal ridge is highest anteriorly and diminishes in height posteriorly.

Pectoral girdle (Fig. 3). The girdle is arciferous.

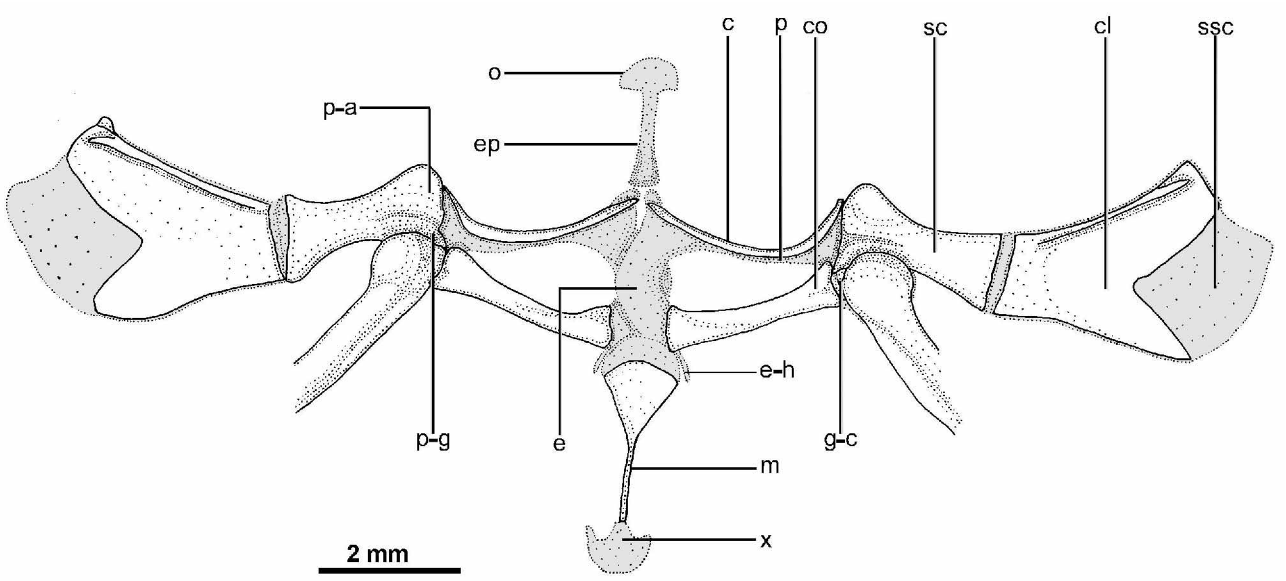

FIGURE 3. Pectoral girdle of OTU 1, QCAZ 6192 from Ecuador (ventral view). c: clavicle; cl: cleithrum; co: coracoid; e: epicoracoid; e–h: epicoracoidal horns; ep: episternum; g–c: glenoid cavity; m: mesosternum; o: omosternum; p: procoracoid; p–a: pars acromialis; p–g: pars glenoidalis; sc: scapula; ssc: suprascapula; x: xiphisternum.

The omosternum is cartilaginous. The distal end is expanded.

The episternum is cartilaginous and stick-like, slightly expanded posteriorly.

The mesosternum is ossified, with the anterior end cartilaginous. The edges are convergent backward to the middle of its length; from this point they continue parallel to each other.

The semicircular xiphisternum is cartilaginous, expanded and mineralized anteriorly.

The procoracoid is present and extends to the level of the sharp medial ends of the clavicles, in a way that the clavicles do not touch each other.

The epicoracoid is cartilaginous with the anterior and inner lateral edges mineralized. The pectoral fenestra is wider than long, tear-drop-shaped, with the principal axis transverse to the vertebral column. The inner margin is concave; the outer is extremely narrow. Each pectoral fenestra is anteriorly bordered by the procoracoid cartilage, medially by the epicoracoid cartilage, and posteriorly by the coracoid. Epicoracoidal horns are present.

Each clavicle is curved, bow-shaped, with the anterior side concave. The glenoid end of the clavicle is expanded dorsolaterally into a wedge-shaped process that articulates with the pars acromialis of the scapula. The inner ends are acuminate and do not touch each other because of the interposed procoracoid. The clavicles do not reach the glenoid fossa.

The scapula is rectangular-shaped. It is almost equal in length to the coracoid, but the width of the scapula is twice that of the coracoid. The anterodorsal portion of the scapula is composed of a posteriorly convex plate, the pars acromialis; whereas a posteriorly concave plate, the pars glenoidalis, represents the posterodorsal part. The pars acromialis is larger than the pars glenoidalis. The area of contact with the cleithrum is cartilaginous and mineralized. The joints with the upper portion of the pars glenoidalis and the clavicle, and the inferior portion and the coracoid are cartilaginous. A prolongation of the procoracoid separates the pars acromialis from the clavicle.

The coracoid is subrectangular. The extremes are distally expanded.

The glenoid cavity is composed by the scapula and coracoid.

The outer edge of the suprascapular cartilage is cartilaginous and mineralized. The suprascapular cartilage has an obvious triangular projection on the outer anterior portion, just beyond the superior spur of the cleithrum.

The cleithrum is very mineralized, continuous with the suprascapula. It is an ossified, very thin and bifid lamina, with the posterior ramus shorter or equal to the anterior ramus. On the anterior side it has a ledge forming a longitudinal crest.

Forelimb and manus. The anterior end of the humerus has two humeral crests, which are separated by a groove, one more developed than the other. The distal head, eminentia capitata, is broadly expanded and partially ossified. The glenoid head, caput humeri, is rounded and slightly compressed; it inserts like a wedge in the glenoid cavity; it is equal in size to the eminentia capitata. The radial-ulnar has a sulcus intermedius indicated by a shallow indistinct groove. The radius and ulna are completely fused; at best, a groove indicates the junction of the two elements.

Six carpal elements are present (Fig. 4), representing the Type C morphology of Fabrezi (1992): ulnare, distal carpal 5–4–3, element *y*, distal carpal 2, radial and proximal element of prepollex. Sub-quadrangular or sub-circular sesamoids are present on the ventral face of the distal epiphysis of metacarpus; also at the distal epiphysis of proximal phalanges of digits IV to V, the sesamoids are either two separate or medially fused elements. The epiphyses of the metacarpus and some phalanges have small lateral projections. The inner metacarpus lacks nuptial excrescences.

The phalangeal formula is 2–2–3–3. The relative lengths of the digits are: IV $>$ II $\cong$ III $\cong$ V. The terminal phalanges are T-shaped. The prepollex is present, lacking nuptial excrescences. The prepollex consists of three mineralized segments in addition to the basal segment; there are three smaller segments beside each respective segment.

Pelvic Girdle. The pelvic plate is semicircular. The angle between the ilial shaft and preacetabular expansion is less than 90º. The middle joint of ilia, that corresponds to the preacetabular area, projects anteriorly like a wedge, but with a round tip. The edge of the ischium is above the level of the ilium. The acetabular portions of the ilium and ischium are almost equal in size. The internal margins of the ilia form a U-shape. The ilia shaft is rounded in cross section. The pubis is localized as a wedge between the ilium and ischium. The ilia are firmly united to each other medially and the ischia posteriorly; sometimes the sutures are not distinct because of fusion between the elements.

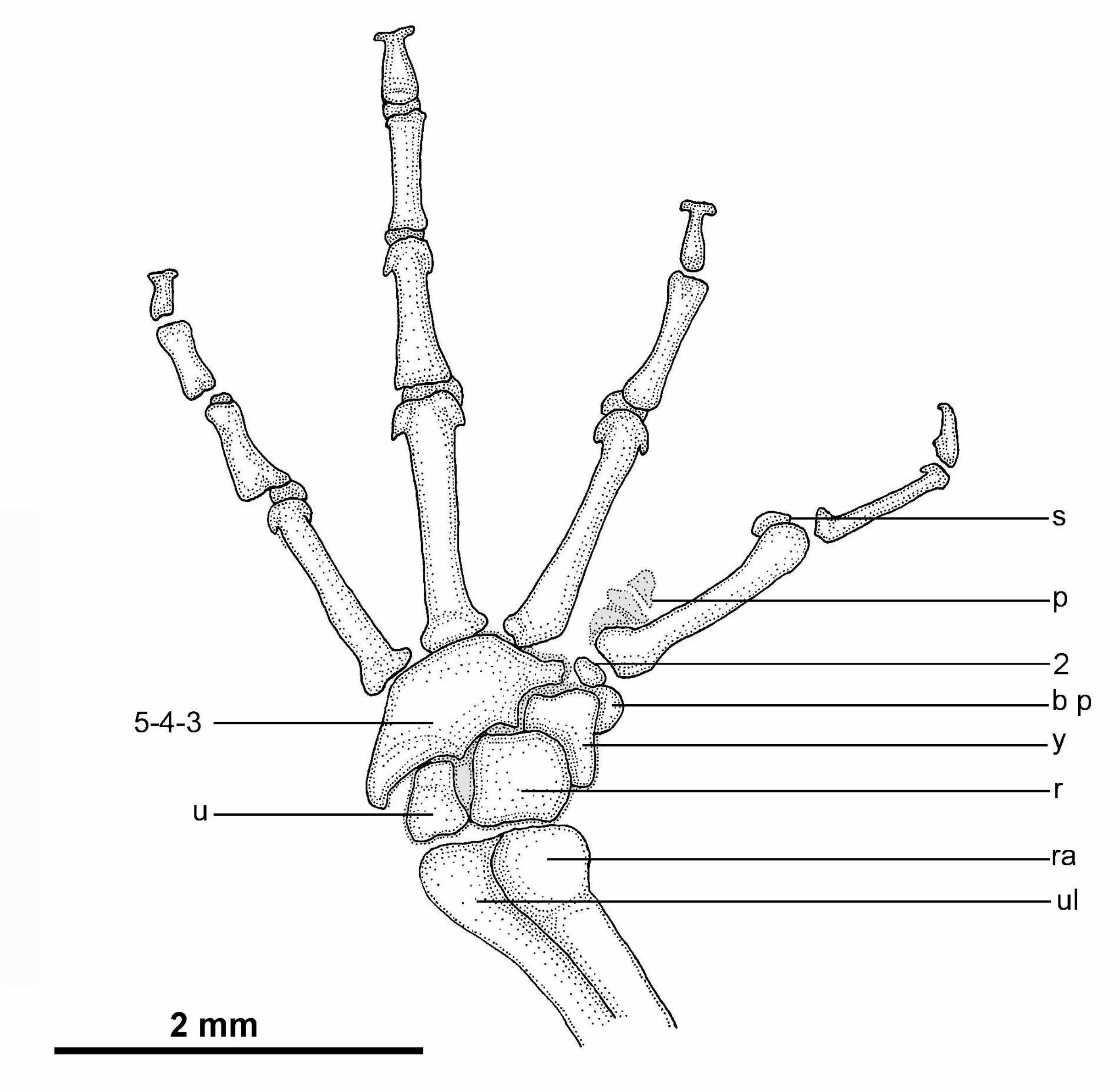

FIGURE 4. Manus of OTU 1, QCAZ 6192 from Ecuador. b–p: base of the prepollex; p: prepollex; r: radiale; ra: radius; s: sesamoid; u: ulnare; ul: ulna; y: element y; 2: distal carpal 2; 5–4–3: distal carpal 5 + distal carpal 4 + distal carpal 3.

The dorsal protuberance of each ilium is elongated or drop-shaped, of normal development (height equal to the dorsal acetabular expansion). The posterior edge of the dorsal protuberance is slightly inclined. The dorsal crest is well developed; anteriorly the dorsal crest rises sharply to its greatest height (about equal to the diameter of the ilial shaft) and diminishes gradually for about ½–¾ the length of the crest at which point it rises gradually to its articulation with the sacral diapophyses.

Hind Limb and Pes. The femur is sigmoid-shaped. Both the caput femoris and the distal head of the femur are partially calcified. The tibiofibula is longer than the femur. A distinct sulcus intermedius marks the medial union of the tibia and fibula on both sides of the bone. The proximal head is almost equal to the distal head. The tibiale and fibulare are shorter than the femur, widely separated from each other at their midpoint, but both the proximal and distal heads of the tibiale and fibulare are fused. Spherical sesamoids are present at the femur-tibiofibular and tibiofibular-tibiale/fibulare articulations.

Three tarsal elements are present: element *y*, distal tarsal 1 and distal tarsal 2–3. The element *y* articulates with the base of the prehallux, distal tarsal 1, tibiale, and metatarsal 1. Distal tarsal 1 is the smallest and articulates with element *y*, distal tarsal 2–3 and metatarsal I and II. Distal tarsal 2–3 articulates mainly with metatarsal III, also with metatarsal II and IV and with distal tarsal I. There are 2 or 3 small sesamoids under the tarsals. The digital phalangeal formula is: 2–2–3–4–3. The terminal phalanges are T-shaped. Subarticular sesamoids are present. The distal epiphyses of some phalanges and metatarsals have small lateral excrescences. The prehallux has three or four segments, with mineralization that decreases from the base to the distal segment.

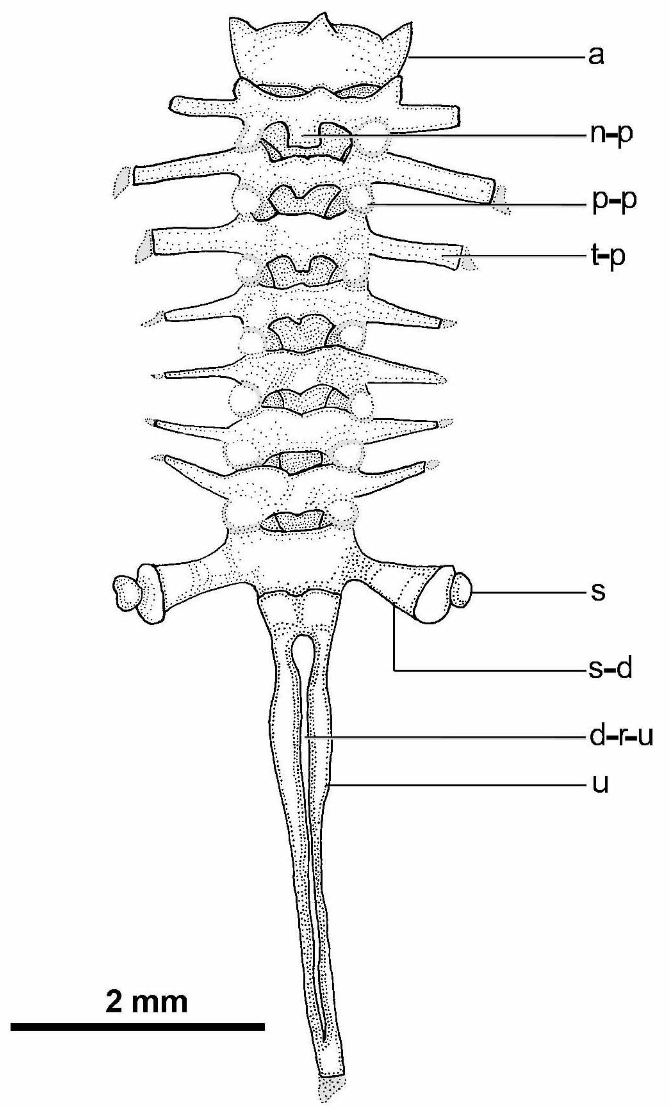

FIGURE 5. Vertebral column of OTU 1, QCAZ 6192 from Ecuador (dorsal view). a: atlas; d–r–u: dorsal ridge of urostyle; n–p: neural process; p–p: parasagital process; s: sesamoid; s–d: sacral diapophysis; t–p: transverse process; u: urostyle.

Osteology of juveniles of *Adenomera marmorata* from Boracéia

There are two classes of juveniles in the available materials – juveniles (MZUSP specimens, Appendix) and near adult-size individuals (USNM specimens, Appendix). The classification of the latter individuals is based on the state of ossification of some elements – the specimens are near-adult size.

In general the osteology of juveniles shows little difference with the adults. These differences are associated mainly with the absence of calcification, fusion and the development of structures in young individuals. The differences are described for the MZUSP specimens (n = 4).

Frontoparietals. The frontoparietal fenestra is not covered by the frontoparietals anteriorly from the midpoint of the frontoparietals forward. In the parietal otic portion, the frontoparietals do not touch medially.

Nasals. The nasals are broadly separated from each other and diagonally oriented. The postero-internal angles are not developed and are stick-shaped. In some specimens the borders are straight.

Parasphenoids. The anterior end of the parasphenoid is variably cartilaginous and the borders are parallel. The triangular end of the posteromedial process is absent or cartilaginous. The alae may or may not be fully ossified.

Neopalatines. The neopalatines are fully covered by the planum antorbitale cartilage. They do not contact the pars palatina of the maxilla. The neopalatines are scarcely developed and the inferior ridge is indistinct; the external end is not expanded.

Vomer. The teeth of the dentigerous process are arranged in a straight line and there are no more than four teeth. The tip of the anterior ala is spine-like and it does not reach the maxilla.

Sphenethmoids. The sphenethmoids are completely cartilaginous. The prootic foramen is not completely bordered by bone.

The tectum nasi and solum nasi are cartilaginous without calcification.

Squamosal. The descendent ramus is variably perpendicular to the horizontal plane of the skull, forming a right angle to it. The zygomatic ramus is scarcely developed and sharp. The end of the otic ramus is cartilaginous.

Pterygoids. The pterygoid is totally differentiated or not and the posterior ramus has a position more perpendicular to the horizontal plane of the skull than in adults.

Prootic. The prootic is partially ossified and variably fused with the exoccipital. The exoccipital is cartilaginous.

Crista parotica. The crista parotica is cartilaginous.

Mentomeckelian. The mentomeckelian is not completely differentiated.

Angular. The angular is separated from the mentomeckelian by a distance equal to the length of the latter.

Premaxilla. The bone bears 7–8 teeth.

Maxilla. The bone bears 24–27 teeth. The pars facialis of the maxilla does not reach the neopalatines.

Mesosternum. The mesosternum is not divided but it has a medial groove.

Vertebrae. The neural arches of vertebrae are not developed.

Ilium. The ilial dorsal protuberance is always elongate.

Variation

All variation observed among the specimens studied is summarized in Table 1.

Discussion

Most systematic studies of variation in osteology of frogs are undertaken to understand higher relationships among taxa, such as families, genera, or species groups (e.g. Lynch, 1971). The primary reason for not studying variation in osteology to understand relationships at the species level is the assumption that because of the reduced number of skeletal elements (compared to fishes, for example), there are few characters to begin with and closely related species would not be expected to demonstrate much variation in them.

This study has identified a considerable amount of osteological variation among the specimens of *Adenomera* examined (Table 1).

We are able to discern intraspecific variation for characters 2 (Lateral borders of the cultriform process of parasphenoid); 3 (Zygomatic ramus of squamosal); 4 (Tips of the posterolateral processes of hyoid); 6 (Epicoracoid); 7 (Mineralized element between scapula and coracoid); 11 (The esophageal process of the cricoid with an inferior slope); 12 (The esophageal process of the cricoid with a superior slope) and 14 (Anterior expansion of omosternum)(Table 1).

Based on our experience with evaluating osteological variation in leptodactylid frogs and species of the closely related genus *Leptodactylus* in particular, we predict that the following taxonomic conclusions will be supported if and when new independent data become available for the species identifications of all the specimens analyzed as OTUs in this study.

- OTU 1 and OTU 2 from Ecuador represent distinct species. The numbers and kinds of osteological characters that differ between these two specimens (Table 1) are entirely consistent with interspecific variation. We are confident in this prediction.
- There are two species represented by the specimens from Fazenda Unacau – OTU 1 and OTU 2 represent one species and OTU 3 and OTU 4 represent a second species. The evidence for this prediction is not as strong as that for the Ecuadorian specimens, but the variation strikes us as being more consistent with interspecific rather than intraspecific variation.
- *Adenomera marmorata* specimens from Boracéia share the most meaningful character states with OTUs 3 and 4 from Fazenda Unacau. We interpret the levels of variation involved as intraspecific. However, the osteological data should not be used as the sole basis for species indentification until much broader osteological surveys of *Adenomera* species are accomplished.

The osteological features that demonstrate variation are not associated with sexual dimorphism in frogs in general and the specimens of this study in particular. Sexual dimorphism in osteological characters involves features such as the length of the femur and humerus; occurrence of humeral crests; morphology of the hyoid, clavicle, and scapula; degree of ossification of the metacarpus, prepollex, prehallux, etc. None of these sexually dimorphic features are evident in OTU 1 (male) and OTU 2 (female) from Ecuador or OTU 3 (female) and OTU 4 (male) from Fazenda Unacau.

Problems of identification at the species level confound comparing our results with those of an earlier study by one of us (Heyer, 1974). In that earlier study, 20 osteological characters were reported, with the main differences in characters between that study and this shown in Table 2. The absence of an anterior process of the hyale in Heyer, 1974 could be a consequence that the observations of the hyoid apparatus were based on examination of the structure before it was cleared and stained (alizarin only). However, the three largest specimens from Boracéia have incipient processes. The differences in character 38 for *A. andreae* in Heyer, 1974 are due to a coding error. WRH’s original drawing made for the work of 1974 shows that the vomer articulates with the maxilla and in another section WRH mentioned that in *Adenomera*, “vomer bones articulate with the premaxilla and/or maxilla” (page 35). The differences in the remaining characters listed in Table 2 could be due to observer error, different observer interpretation, intraspecific variation, or interspecific variation. None of the specimens examined in the previous study are from the same localities as those reported in this paper. The two specimens identified as *A. andreae* in Heyer (1974) are from Brazil, Amazonas, Teresina (WRH 16 = USNM 227567) and Ecuador, Pastaza, Canelos (KU 119331); the two specimens identified as *A. marmorata* are from Brazil, Rio de Janeiro, Tijuca (WCAB 30563 = USNM 227568; WCAB 30567 presently unlocatable). At least some of these specimens from the earlier study could represent species different from those included in this study.

The osteology of the *Adenomera* specimens presented here and the genus *Leptodactylus* have a lot in common (Gallardo, 1964; Lynch, 1971; Limeses *et al.*, 1972; Heyer, 1974; Heyer, 1998; Ponssa, 2003). This is not unexpected because the genus *Adenomera* used to constitute the *marmoratus* group of the genus *Leptodactylus* (Heyer, 1974). Previously reported data suggest that unique character state distributions of (1) the relationship of the frontoparietal and prootic ridge (Heyer, 1969), (2) anterior processes of the hyale (Heyer, 1974), and (3) terminal phalangeal shape (Heyer, 1974) differentiate *Adenomera* from *Leptodactylus* osteologically. These characters and several others are currently being assembled and evaluated by MLP. The currently known differences between the two genera are: (1) lateral borders of the cultriform process of parasphenoid (character 2, table 1): the state straight and parallel was not observed in *Leptodactylus*; (2) the bases of the alary processes are broad in *Adenomera* and narrower in *Leptodactylus*; (3) orientation of dentigerous processes of vomers is oblique in *Adenomera* and horizontal in *Leptodactylus*; (4) terminal phalanges T-shaped in *Adenomera* (specimens observed herein) and either rounded and bifurcated, dilated with a medial indentation, or T -shaped (observed thus far only in *L. validus*) in *Leptodactylus.*

TABLE 2. Osteological differences between the observations of Heyer (1974) and this study.

Character	Heyer (1974)	This study
Anterior processes of hyale	Absent (character 43)	Present, except incipient in some Boracéia specimens
Anterior articulation of vomer in *A. andreae*	Not in contact with premaxilla or maxilla (character 38)	Anterior tip of vomer articulates with the maxilla
Relationship of sphenethmoid with the optic foramen in *A. marmorata*	Posterior end borders the optic foramen (character 39)	Posterior end separated from the optic foramen
Relationship between sphenethmoid and vomer in *A. marmorata*	Sphenethmoid extends beyond the middle of vomers (character 40)	Sphenethmoid reaches half or less of the length of vomers
Terminal phalanges	T-shaped in both species, expanded in *A. marmorata* (character 47)	T-shaped, not expanded in all specimens

Some of the characters and character states considered by Heyer (1974) as shared between *Adenomera* and members of the *fuscus* species group deserve further comments. The inclusion of the sphenethmoid in the border of the optic foramen was considered a shared condition between *Adenomera* and the species of the *fuscus* group, but it was shown to be a variable character in the latter set of species (Ponssa, 2003) and the condition is not present in all the specimens of *Adenomera* studied herein. Another character that Heyer (1974) considered as shared between *Adenomera* and the species of the *Leptodactylus fuscus* group was the sphenethmoid extending anteriorly beyond the middle of the vomerine bones. However, in almost all the species included in the *fuscus* group of the genus *Leptodactylus*, the sphenethmoid and the septum nasi are co-ossified; the only exceptions are *L. labrosus* and *L. ventrimaculatus*, where the septum nasi remain cartilaginous. In these latter two species of *Leptodactylus,* the sphenethmoid extends not far beyond the middle of the vomerine bones (Ponssa, 2003), similar to the condition found in *Adenomera* observed in this study.

The small size of the species of *Adenomera* examined herein could be indicative of a heterochronic pattern. Characters numbered 2, 5, and 9 in Table 1 and the absence of imbrications of the neural arches in the adults could be paedomorphic features, because the adults present the same character state as the juveniles examined in this study and juveniles in the closely related genus *Leptodactylus* (Ponssa, 2003). However, a phylogeny is needed to polarize the directions of heterochronic changes among species (Fink, 1982, 1988, in Reilly *et al*., 1997). Heterochrony is a relative concept, because the fact that in some species the development of some structures is retarded implies that the development of these structures is accelerated in related species. The optimization of the features proposed as heterochronic, on a cladogram, would allow distinguishing whether the characters have passed through paedomorphosis or peramorphosis (which results in traits produced by extended development).

The osteological characterization presented in this paper will serve as a source of information for determining character state variation in *Adenomera* osteology at the genus level. The results of this paper also indicate that there is appropriate interspecific variation among species of *Adenomera* to use osteological features in part of a suite of characters to address relationships within the genus. However, much more work needs to be done to characterize osteological variation for most of the species of *Adenomera* based on specimens identified on either the basis of advertisement call or molecular data for the taxa that are problematic to identify based solely on external morphology.

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Appendix 1: specimens examined

Adenomera andreae: QCAZ 6192 (Ecuador OTU 1) (male), 6209 (Ecuador OTU 2) (female), Reserva de Producción Faunística Cuyabeno, Estación Científica de la Pontificia Universidad Católica del Ecuador, 220 m, Provincia Sucumbios, Ecuador.

Adenomera marmorata: Juveniles: MZUSP 24304–24305, 24307, 24309, Boracéia, São Paulo, Brazil. Advanced juveniles: USNM 292479–292480, Boracéia, São Paulo, Brazil. Adults: MZUSP 63549 (Fazenda Unacau OTU 1) (female), MZUSP 63551 (Fazenda Unacau OTU 2) (female), MZUSP 63550 (Fazenda Unacau OTU 3) (female), MZUSP 63552 (Fazenda Unacau OTU 4) (male), São José (Fazenda Unacau), Bahía, Brazil; USNM 292478, Boracéia, São Paulo, Brazil (male).