New Morphological Synapomorphies for the New World Direct-Developing Frogs (Amphibia: Anura: Terrarana)

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NEW MORPHOLOGICAL SYNAPOMORPHIES FOR THE NEW WORLD DIRECT-DEVELOPING FROGS (AMPHIBIA: ANURA: TERRARANA)

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ABSTRACT: The New World direct-developing frogs (Terrarana) comprise more than 970 species distributed from the southern United States to northern Argentina. Although the composition of this clade has been remarkably stable for many decades, evidence for its monophyly is derived mostly from DNA sequences with putative phenotypic synapomorphies limited to the occurrence of direct development, an embryonic egg tooth (known in few species), and T-shaped terminal phalanges. Based on a survey of the urogenital and vascular systems and the submandibular musculature of hyloid frogs, we report 16 characters that provide putative synapomorphies at a variety of hierarchic levels. Most significantly, they include seven putative synapomorphies for Terrarana that can be observed through simple dissections, including (1) fusion of the Wolffian ducts, resulting in a single, common cloacal opening; (2) Wolffian duct fusion located anteriorly with a single, common duct along the posterior, > 1/3 of the distance between caudal edge of kidneys and cloacal wall; (3) presence of the posterior dorsolumbar vein; (4) absence of the medial dorsolumbar vein; (5) origin of the posterior caval vein in the anterior 1/3 of the kidneys; (6) posterior origin of dorsolumbar arteries; and (7) presence of the pelvic lymphatic septum.

Key words: Submandibular musculature; Systematics; Urogenital system; Vascular system

MOST OF the New World direct-developing frogs are recognized on the basis of DNA sequences as a monophyletic group called either Brachycephalidae (Frost et al., 2006) or Terrarana (Hedges et al., 2008; Heinicke et al., 2009). Prior to the analysis of molecular evidence, and since Lynch (1971) modified earlier proposals by Lutz (1954) and Gallardo (1965), a group of similar composition (with the noteworthy exclusion of *Brachycephalus*) had been recognized as the tribe Eleutherodactylini (later raised to Eleutherodactylinae by Heyer, 1975) of the then-leptodactylid subfamily Telmatobiinae. The systematics of the clade are far from resolved and new species continue to be named every year, but Terrarana currently includes 974 species (Frost, 2013) divided among four families (Pyron and Wiens, 2011) and 26 genera.

Although extensive molecular evidence supports Terrarana, and largely the same group has been recognized taxonomically for decades, the nonmolecular evidence for its monophyly is surprisingly scant. Until DNA sequences were analyzed, the only evidence for the monophyly of Eleutherodactylinae was the occurrence—observed or assumed—of direct development (Lutz, 1954; Gallardo, 1965; Lynch, 1971) and a single, bicuspid, keratinized egg tooth (Sampson, 1904; Noble, 1926). Frost et al. (2006: 197) noted Pombal's (1999) exciting discovery of an egg tooth in *Brachycephalus* and suggested that this is "possibly synapomorphic for the entire Brachycephalidae [=Terrarana]." In an effort to diagnose Terrarana morphologically, Hedges et al. (2008) extracted several more characteristics from the literature but none of them is demonstrably synapomorphic. For example, the arciferal pectoral girdle and absence of Bidder's organ and intercalary elements in the digits are clearly plesiomorphic for these frogs. Heinicke et al. (2009: 5) added that most species of Terrarana have T-shaped terminal phalanges but they only considered this to be "the ancestral condition for Terrarana" without distinguishing between symplesiomorphy and synapomorphy. T-shaped terminal phalanges occur in numerous other groups of Hyloides (e.g., Hylodidae,

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Batrachyla, *Crossodactylodes*, *Leptodactylus*, *Thoropa*, Centrolenidae, Dendrobatoidea; Taylor, 1951; Lynch, 1971). However, in the context of current phylogenetic hypotheses (e.g., Pyron and Wiens, 2011), this character state is an unambiguously optimized synapomorphy of Terrarana, bringing the total number of putative phenotypic synapomorphies to three for this radiation of over 970 species.

In this study we aimed to discover additional morphological synapomorphies of Terrarana, specifically targeting urogenital and vascular anatomy and submandibular musculature. We describe 16 characters, six that are putative synapomorphies of Terrarana and eight that vary within Terrarana and, therefore, might prove useful in delimiting internal clades in future studies. We emphasize that, although all of these characters are internal, the dissections required to score many of them are trivial and can easily be carried out in the course of taxonomic studies.

Materials and Methods

We examined 85 preserved specimens of 60 species from different collections (Appendix 1). Of these, 22 are species of Terrarana representing 12 of the 26 recognized genera, one corresponds to a ranoid, and 37 are included as representatives of hyloid diversity. Information on taxonomic distribution was supplemented with that available in the literature. Collection acronyms follow Sabaj Pérez (2010).

Dissections were performed under a dissecting microscope. A small ventral incision in the pelvic region was made in the skin and carefully continued along both sides of the body. The incision was extended until the first tendinous inscription of the musculus (m.) rectus abdominis or, when present, to the abdominal disc, thereby forming a dissected triangle of skin that followed the perimeter of the underlying m. rectus abdominis. The lymphatic septum associated with the abdominal disc was photographed and preserved for histology. Prior to dissection, methylene blue was injected into the ventral lymphatic sac of each specimen to differentiate its limits. After removal of this structure, an incision was made between the mm. rectus abdominis and

obliquus externus to expose the viscera. Kidneys, testes, Wolffian ducts, and seminal vesicles were exposed by cutting the mesenteries of the small and large intestine and gently deflecting the latter. To study the tributaries of Jacobson's vein (renal portal vein), a longitudinal incision was made in the dorsal peritoneum along the entire length of the kidneys to expose the dorsolumbar veins, if present. In small specimens, in which the vessels were translucent and close to sympathetic nerves, vascular anatomy was examined by serial sections. In specimens that were only examined for the occurrence of ventral lymphatic septa, a sagittal incision was made in the skin from the pelvis to the throat and the skin was gently lifted to reveal the septa.

For histological study, tissues were dehydrated in an ascending series of ethanol, cleared in toluene, and embedded in Paraplast®. Samples were then sectioned at 5 µm and stained with hematoxylin and eosin and Masson-Goldner's trichrome stain (Martoja and Martoja-Pierson, 1970). Transformation series (characters and character states) were delimited following Hawkins et al. (1997), Strong and Lipscomb (1999), and Grant and Kluge (2004).

Results

We describe 16 characters from the urogenital system, vascular system, and submandibular musculature. See Appendix 2 for a survey of the taxonomic distribution of the character states involving the first two character systems discussed below.

Urogenital System

Bhaduri (1953), Bhaduri and Basu (1957), and Bhaduri and Mondal (1967) provided extensive surveys of the anuran urogenital system anatomy. Here we explicitly delimit four characters: (1) fusion of Wolffian ducts, (2) location of Wolffian duct fusion, (3) width of the mesorchium, and (4) occurrence of Müllerian ducts in males.

Wolffian ducts.—In adults, the Wolffian ducts collect urinogenital products from the kidneys and testes and extend from the dorsolateral edge of the kidneys to the cloaca (Fig. 1). In most anurans studied to date, the Wolffian ducts remain separate along their
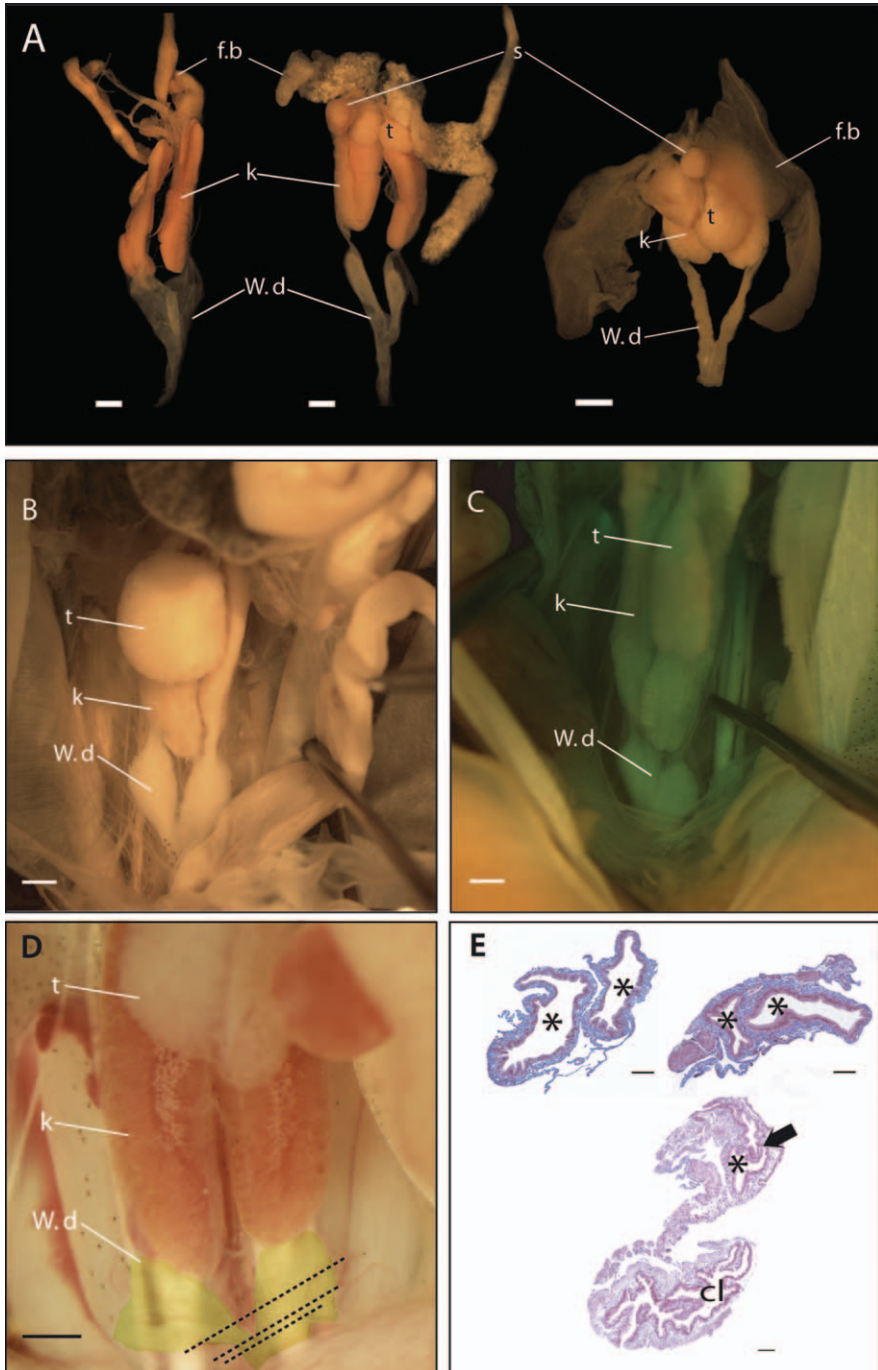
FIG. 1.—Urogenital system of several species of Terrarana showing the fusion of Wolffian ducts well anterior to the cloacal wall (Character 1, State 2). (A) *Eleutherodactylus pinarensis* (AMNH 63008; left), *E. pezopetrus* (AMNH 63458; middle), and *Holoaden bradei* (AMNH 103976; right). (B) *Diasporus gularis* (ICN 36835). (C) *Pristimantis permixtus* (ICN 36132). (D) *Oreobates barituensis* (MACN 40528). Dashed lines indicate sites of sectioning for microscopic analysis. (E) Transverse sections through Wolffian ducts where they expand forming seminal vesicles in *Oreobates*

entire length and open independently into the cloaca. In others, the Wolffian ducts fuse and empty into the cloaca through a single, shared opening. This fusion of the Wolffian ducts can be either terminal (ducts remain separate until reaching the cloacal wall), subterminal (ducts fuse immediately anterior to the cloacal wall), or anterior (a single, common duct accounts for > 1/3 of the length of the Wolffian ducts). Based on this variation, we define the following two characters:

Character 1. Fusion of Wolffian ducts: (0) Absent, Wolffian ducts separate along entire length, each with an independent cloacal opening. (1) Present, Wolffian ducts fused, resulting in single, common cloacal opening. The Wolffian ducts are fused (State 1) in all examined species of Terrarana (Fig. 1A–E) and separate (State 0) in all other hyloids.

Character 2. Location of fusion of Wolffian ducts: (0) Terminal, Wolffian ducts fused within cloacal wall. (1) Subterminal, Wolffian ducts fused immediately anterior to the cloacal wall. (2) Posterior, single, common duct extending along posterior 1/5 of the distance between caudal edge of kidneys and cloacal wall. (3) Anterior, single, common duct extending along posterior, > 1/3 of the distance between caudal edge of kidneys and cloacal wall. The fusion of the Wolffian ducts is anterior (State 3) in all examined species of Terrarana except *Pristimantis fenestratus*, in which the fusion is posterior (see Discussion for comments on fusion in nonhyloids).

Mesorchia.—Bhaduri (1953) noted that in the males of the three *Eleutherodactylus* species he studied (*E. alticola*, *E. nubicola*, and *E. portoricensis*), the testes were tightly bound to the kidneys by narrow mesorchia, making it impossible to discern the vasa efferentia. Bhaduri and Basu (1957) reported the same condition in some ranoids, but all other hyloids and ranoids reported in the literature and examined by us have broad mesorchia that loosely bind the testes to the kidneys, clearly exposing the vasa efferentia

(Appendix 2). Consequently, we define the following transformation series:

Character 3. Width of mesorchia: (0) Narrow, mesorchium tightly binding testis to kidney, vasa efferentia concealed. (1) Broad, mesorchium loosely binding testis to kidney, vasa efferentia visible. Both states occur in Terrarana.

Müllerian ducts in males.—The Müllerian ducts often persist to some degree in male anurans. The taxonomic distribution and degree of development of this structure have been addressed in several studies. Within Terrarana, Lynn (1942) did not report the formation of Müllerian ducts in *Eleutherodactylus nubicola*, even in early stages of development. Bhaduri (1953) confirmed that Müllerian ducts were absent in *E. nubicola* and further reported their absence in *E. alticola* and *E. portoricensis*, and Griffiths (1959) reported their absence in *E. limbatus*. Among the species we examined, Müllerian ducts are absent in all species except *Pristimantis palmeri* and *Strabomantis zygodactylus*, in which they are present as narrow, longitudinal, whitish cords on the dorsal parietal peritoneum, adjacent to the kidneys (for other hyloids see Appendix 2). Based on this variation, we define the following transformation series:

Character 4. Retention of Müllerian ducts in males: (0) Absent. (1) Present.

Vascular System

The anatomical diversity of the anuran circulatory system has barely been addressed in the literature, but superficial surveys are found in the studies of Ecker (1899), Millard (1941), Stephenson and Stephenson (1947), and Szarski (1948, 1951). Here, we report three characters of vascular anatomy concerning tributary veins of the renal portal system, efferent renal veins, and iliac arteries.

Dorsolumbar veins.—The renal portal system is composed chiefly of the veins from the posterior limbs. The ischiadic and external

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barituensis (MACN 40528). Photographs correspond to sections depicted by dashed lines in D. Note the separate Wolffian ducts (top left), a posterior position within the same connective tissue sheet (top right) and fusion outside the cloacal wall (bottom). Asterisk: lumen of Wolffian ducts. Arrow: point of fusion of Wolffian ducts. Abbreviations: cl = cloaca; f.b = fat bodies; k = kidney; s = spleen; t = testis; W.d = Wolffian duct. Masson Goldner's Trichrome. Scale: A–D = 1 mm; E = 100 μm. (A color version of this figure is available online.)

iliac veins (v. ischiadica and v. iliaca externa) fuse to form the common iliac vein (v. iliaca communis), which then connects to the posterolateral edge of the kidney to form the renal Jacobson's vein. Jacobson's vein extends anteriad along the dorsalateral edge of the kidney and lies dorsalateral to the Wolffian duct (Fig. 2). The dorsolumbar vein(s) (v. dorso-lumbaris; Fig. 2) collects blood from the dorsal trunk musculature and spinal cord and merges with Jacobson's vein via one or more vessels. Because these veins occur singly or in varying combinations in different taxa (see Discussion), we treat them as separate, nonhomologous characters. The point(s) at which the dorsolumbar vein joins Jacobson's vein presents the following variation:

Character 5. Posterior dorsolumbar vein: (0) Absent. (1) Present, junction with Jacobson's vein near beginning of Jacobson's vein at posterior end of kidney near junction with common iliac vein (Fig. 3A–D). Present in all examined species of Terrarana, absent in all other studied anurans except *Pleurodema* and *Fritziana*.

Character 6. Medial dorsolumbar vein: (0) Absent. (1) Present, junction with Jacobson's vein approximately midway along kidney (Fig. 2). Absent in all examined species of Terrarana and *Fritziana*, present in all other studied anurans.

Character 7. Anterior dorsolumbar veins: (0) Absent. (1) Present, junction with Jacobson's vein at anterior extreme of Jacobson's vein, along kidney. Present only in the examined species of bufonids and *Bombina* and reported in the literature for *Bombina* (Szarski, 1948), *Xenopus* (Millard, 1941), and *Pelobates* (Szarski, 1948).

Posterior caval vein.—The posterior caval vein (v. cava inferior of Ecker, 1899; Vena cava caudalis of Szarski, 1948) arises sagittally from the union of the renal veins and also collects blood directly from the genital veins, fat body veins (vv. corporis adiposi), and oviductal veins (vv. oviducti) in females. Here we consider the origin of the posterior caval vein as the point where the posterior-most efferent renal veins fuse. Based on variation in the position of this origin relative to the kidneys, we propose the following binary character:

Character 8. Origin of posterior caval vein: (0) In anterior 1/3 of kidneys (Fig. 3F). (1) In posterior 1/2 of kidneys (Fig. 3E). The origin of the caval vein of all examined species of Terrarana, as well as in *Dendropsophus minutus*, *Lysapsus limellum*, *Pseudis minuta*, *Melanophryniscus devincenzii*, and *Proceratophrys avelinoi*, is in the anterior 1/3 of the kidneys (State 0). This condition seems to be associated with the presence of a large efferent renal vein running parallel to the kidney and adjacent to or overlapping the adrenal glands. This anterior origin is also found mainly in species of small body size (e.g., *Dendropsophus minutus*, *Lysapsus limellum*, *Melanophryniscus devincenzii*). Within Terrarana, however, even species of large body size like *Strabomantis anomalus* and *Craugastor raniformis* present the same anterior condition, showing that body size and the posterior caval vein origin are independent.

Dorsolumbar arteries.—Within Anura, the dorsolumbar arteries diverge serially from the dorsal aorta (either directly or indirectly through common ducts shared with the urogenital arteries) and irrigate the dorsal musculature of the trunk and spinal chord. We define the following binary character for the variation in the point of origin of these arteries:

Character 9. Origin of dorsolumbar arteries: (0) Anterior, approximately 2/3 along length of dorsal aorta (e.g., dorsolumbar arteries absent in the vicinity of the iliac arteries; Figs. 2,3E). (1) Posterior, near point where iliac arteries diverge from dorsal aorta (Fig. 3A–F). The dorsolumbar arteries diverge near the point where the iliac arteries originate from the dorsal aorta (State 1) in all examined species of Terrarana. No traces of other dorsolumbar arteries were found in fixed specimens. Although dorsolumbar arteries occur in all specimens examined, Valett and Jameson (1961) did not mention it when describing the dorsal aorta in their study of embryology of *Craugastor augusti*, although they did mention the formation of urogenital arteries. The dorsolumbar arteries diverge anteriorly (State 0) in all other studied anurans.
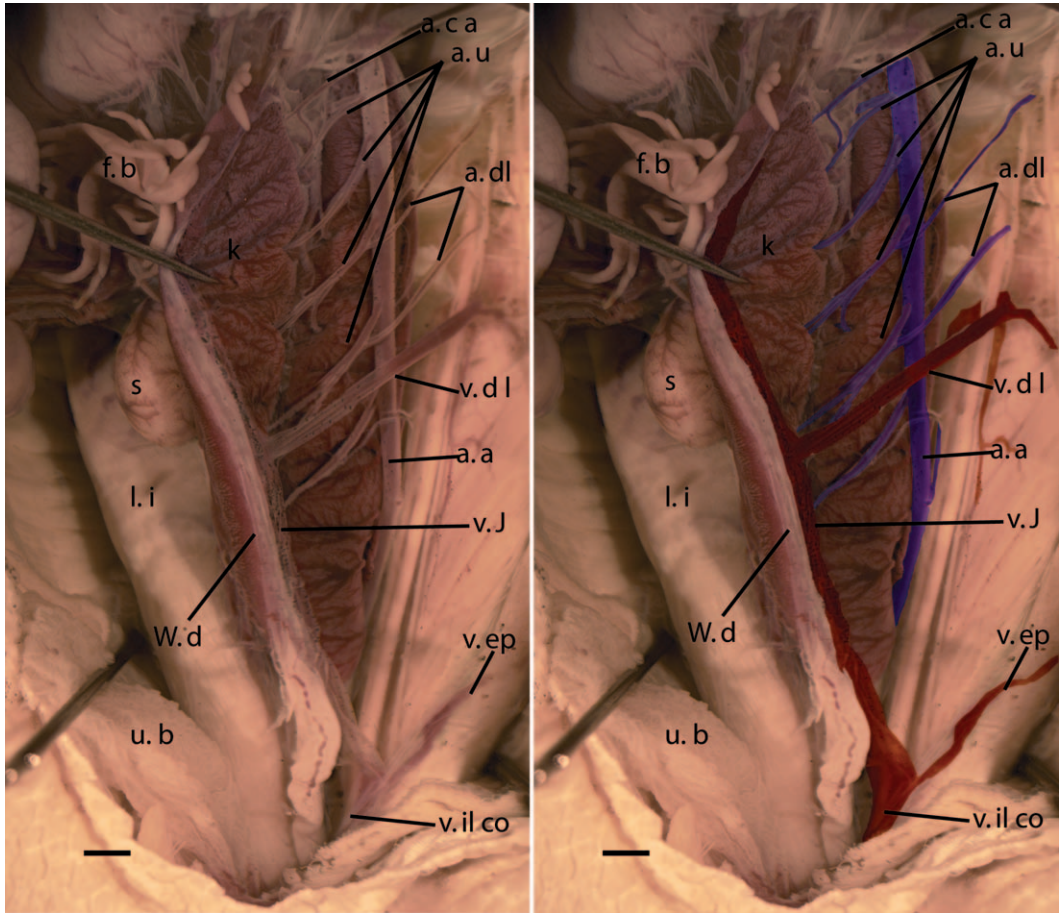
Fɪɢ. 2.—Urogenital system and main vessels of adjacent circulatory system in *Trachycephalus typhonius* (MACN 40545) showing vessels uncolored (left) and colored to enhance contrast (right). Red: veins. Violet: arteries. Abbreviations: a. a = aortal artery; a. ca = fat body artery; a. dl = dorsolumbar arteries; a.u = urogenital arteries; l. i = large intestine; u. b = urinary bladder; v. dl = dorsolumbar vein; v. ep = epigastric vein; v. il co = common iliac vein; v. J = Jacobson's vein. Scale = 2 mm. (A color version of this figure is available online.)

Lymphatic Septa

Lymph sacs, considered an anuran synapomorphy (Trueb and Cloutier, 1991), consist of large subcutaneous spaces divided into discrete compartments by thin connective tissue septa between the muscles and skin and interconnected by one-way valves. Ecker (1899) provided a complete description of the lymphatic system of *Pelophylax esculentus*, Tyler (1971a) provided information on the lymph sacs and septa in the region of the vocal sac for a diverse sample of hylids, and Carter (1979) described variation in 17 anuran species of 7 families (Bufonidae, Hylidae, Leiopelmatidae, Limnodynastidae, Myobatrachidae, Pipidae, Ranidae).

Pelvic lymphatic septum.—In most previously studied anurans, the ventral lymph sac is triangular, delimited anteriorly by the pectoral septum and laterally and posteriorly by the ventral septa. In most species of Terrarana we detected a novel septum that transversely links both ventral septa and divides the ventral sac into two adjacent sacs: an anterior, trapezoidal sac, delimited anteriorly by the pectoral septum and posteriorly by the new septum; and a posterior, triangular sac whose base is delimited anteriorly by the new septum and the sides and apex are formed by the
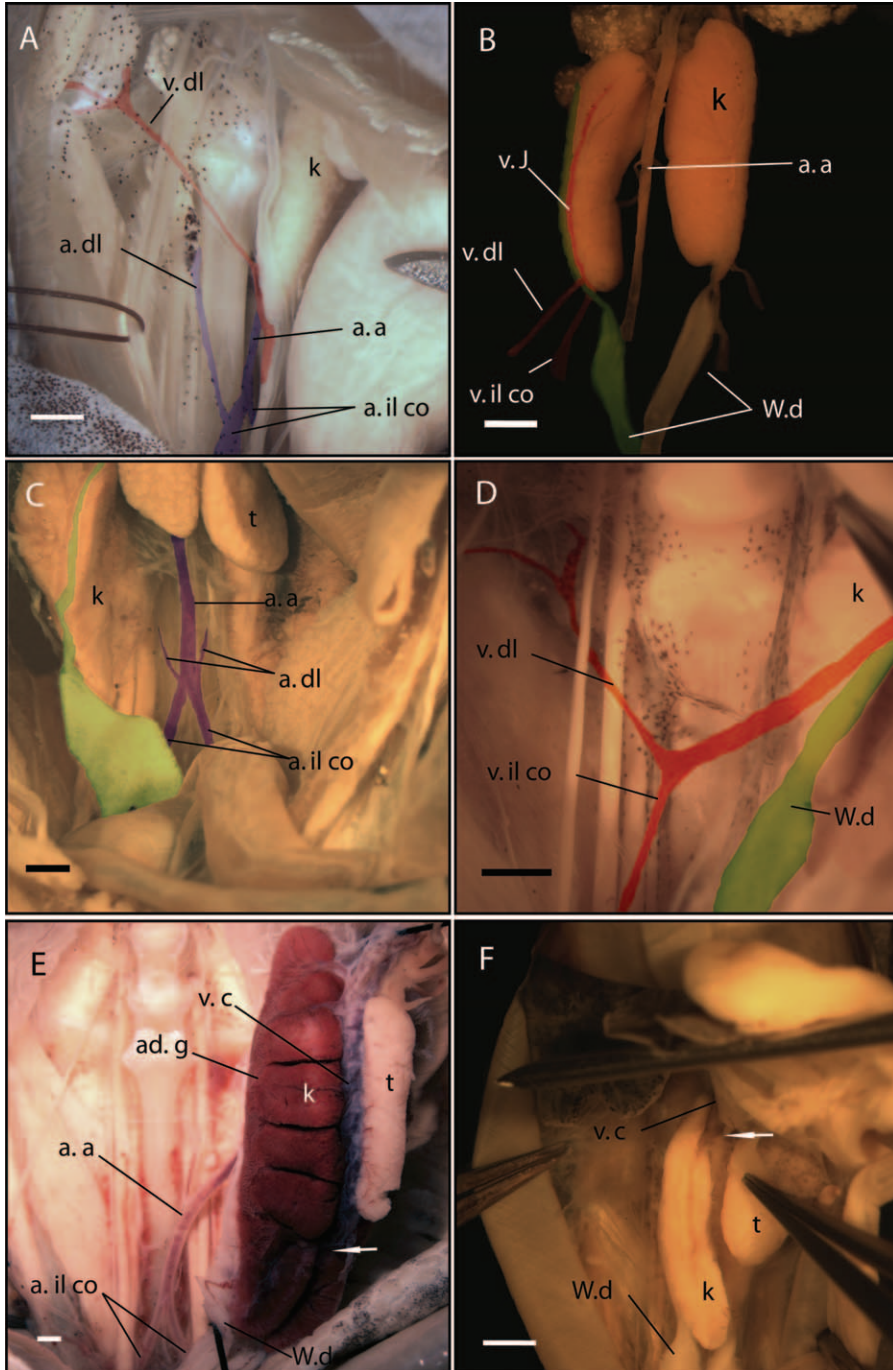
Fig. 3.—Arterial and venous system of some species of Terrarana. (A) *Craugastor fitzingeri* (MACN 39161). Large intestine deflected to expose vessels. Note the junction of the dorsolumbar vein, located in the posterior portion of Jacobson's vein. Dorsolumbar artery originates at the common iliac. (B) *Eleutherodactylus pezopetrus* (AMNH 63458). Dorsal view of urogenital system and associated vessels. Note the posterior junction of the dorsolumbar vein. (C) *Eleutherodactylus johnstonei* (ICN 42263). Kidneys slightly deflected to show arterial system. Note both dorsolumbar

ventral septa (Fig. 4). This additional septum is perpendicular to the longitudinal axis of the m. rectus abdominis and inserts close to its first tendinous inscription. The surface of the septum shows small pores that we interpret to be the one-way valves, whose size varies, presumably an artifact of fixation. Based on its position, we propose the term "pelvic" to denote both the novel lymphatic septum and the posterior lymph sac, and we propose the following transformation series:

Character 10. Pelvic lymphatic septum: (0) Absent. (1) Present (Fig. 4). The pelvic lymphatic septum is present (State 1) in most examined species of Terrarana (single exception *Brachycephalus ephippium*) and Leptodactylidae and absent (State 0) in all other species.

Pectoral lymphatic septum.—The pectoral septum forms the posterior wall of the pectoral lymph sac. Tyler (1971a) pointed out the presence of this septum in many anuran species except for *Brachycephalus* and possibly *Pseudopaludicola*.

Character 11. Pectoral lymphatic septum: (0) Absent. (1) Present. The pectoral lymphatic septum is absent (State 0) in *Brachycephalus* and *Rhinoderma* and present (State 1) in all other examined anurans.

Submandibular Musculature

The submandibular musculature has been surveyed in several groups of anurans (e.g., Trewavas, 1933; Tyler, 1971b, 1979, 1985; Emerson, 1976; Burton, 1986). Burton (1998) studied 87 species of Terrarana, including 84 species then referred to *Eleutherodactylus* and presently referred to *Craugastor*, *Diasporus*, *Eleutherodactylus*, *Haddadus*, *Ischnocnema*, *Pristimantis*, *Strabomantis*, as well as *Barycholos pulcher*, *Euparkerella brasiliensis*, *Geobatrachus ruthveni*, *Holoaden bradei*, *Lynchius flavomaculatus* (as *Phrynopus*), *L. parkeri* (as *Phrynopus*), *Oreobates*

quixensis (as *Ischnocnema*), and *Noblella peruviana* (as *Phrynopus*). Here, we define three characters related to the occurrence of supplementary elements of the m. intermandibularis and two characters involving the morphology of the m. interhyoideus.

The m. intermandibularis can be a single muscle, as described by Burton (1998) for most of species of Terrarana he studied, or can be differentiated into a main and one or more supplementary elements. We observed three morphologically distinct supplementary elements in Terrarana that we describe as three independent, nonhomologous transformation series.

Character 12. Anterolateral supplementary element of m. intermandibularis originating on lingual surface at the level of posterior half of mandible, inserting on the fascia of m. submentalis: (0) Absent. (1) Present. Anterolateral supplementary elements so far are known in *Euparkerella* and *Geobatrachus* (Burton, 1998).

Character 13. Posterolateral supplementary element of m. intermandibularis originating on lingual surface of posterior end of mandible, dorsal to levator musculature, inserting on the fascia of m. submentalis: (0) Absent. (1) Present (Fig. 5A). This supplementary slip was first reported for *Brachycephalus ephippium* by Trewavas (1933), the only genus of Terrarana known to possess this structure.

Character 14. Apical supplementary element of m. intermandibularis originating on lingual surface of anterior end of mandible, inserting posterior to m. submentalis on the aponeurosis of the m. intermandibularis: (0) Absent. (1) Present (Fig. 5B). Among species of Terrarana, we observed this supplementary element in two species of the *Strabomantis biporcatus* series (*S. biporcatus* and *S. sulcatus*) and in one of the two species of the *S. bufoniformis* series (present in *S. anomalus*; absent in *S. zygodactylus*).

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arteries arising from the common iliacs. (D) *Strabomantis zygodactylus* (ICN 27482). Right kidney deflected to show detail of the venous system. Note the dorsolumbar vein fusing directly with the common iliac vein. (E) *Trachycephalus typhonius* (MACN 40545). Right kidney deflected to show overlying aorta and common iliac arteries. Origin of caval vein (arrow) is located in the posterior half of the kidney. (F) *Pristimantis permixtus* (ICN 36132). Right testis deflected to show anterior origin of caval vein (arrow). Red: veins. Violet: arteries. Green: Wolffian duct. Abbreviations: a. il co = common iliac arteries; ad. g = adrenal gland; t = testis. Other abbreviations as in Figs. 1 and 2. Scale = 1 mm. (A color version of this figure is available online.)
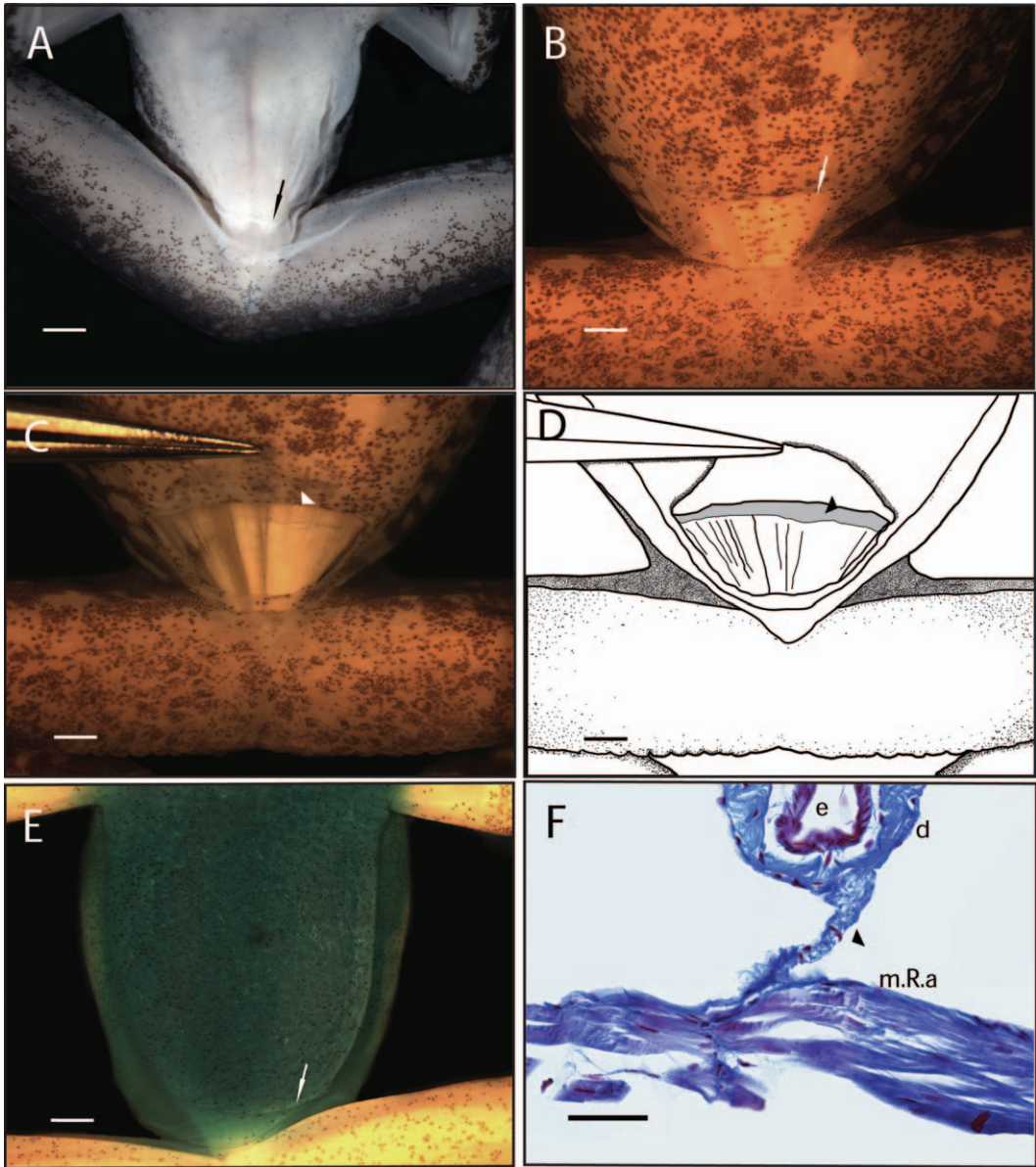
Fɪɢ. 4.—Discoidal fold (arrow) and superjacent pelvic septum (arrowhead) exposed after ventral incision of the skin in some species of Terrarana. (A) *Ischnocnema guentheri* (CFBH 25607). (B–D) *Pristimantis permixtus* (ICN 36132) discoidal fold (B) and pelvic septum exposed (C) and highlighted in the schematic outline drawing (D). (E) *P. nicefori* (ICN 21027). (F) *Haddadus binotatus* (MACN 17043). Transverse section through skin and portion of m. rectus abdominis. Abbreviations: e = epidermis; d = dermis; m.R.a. = m. rectus abdominis. Masson-Goldner's Trichrome. Scale: A–E = 1 mm; F = 50 µm. (A color version of this figure is available online.)

Structure of m. interhyoideus.—The m. interhyoideus has been described for a single species of Terrarana, *Brachycephalus ephippium*, as a single muscle whose fibers meet in a median raphe (Trewavas, 1933).

Burton (1998) made no reference to this muscle in Terrarana. Our observations identified two character states related to the contact between the contralateral portions of the *m. interhyoideus*:
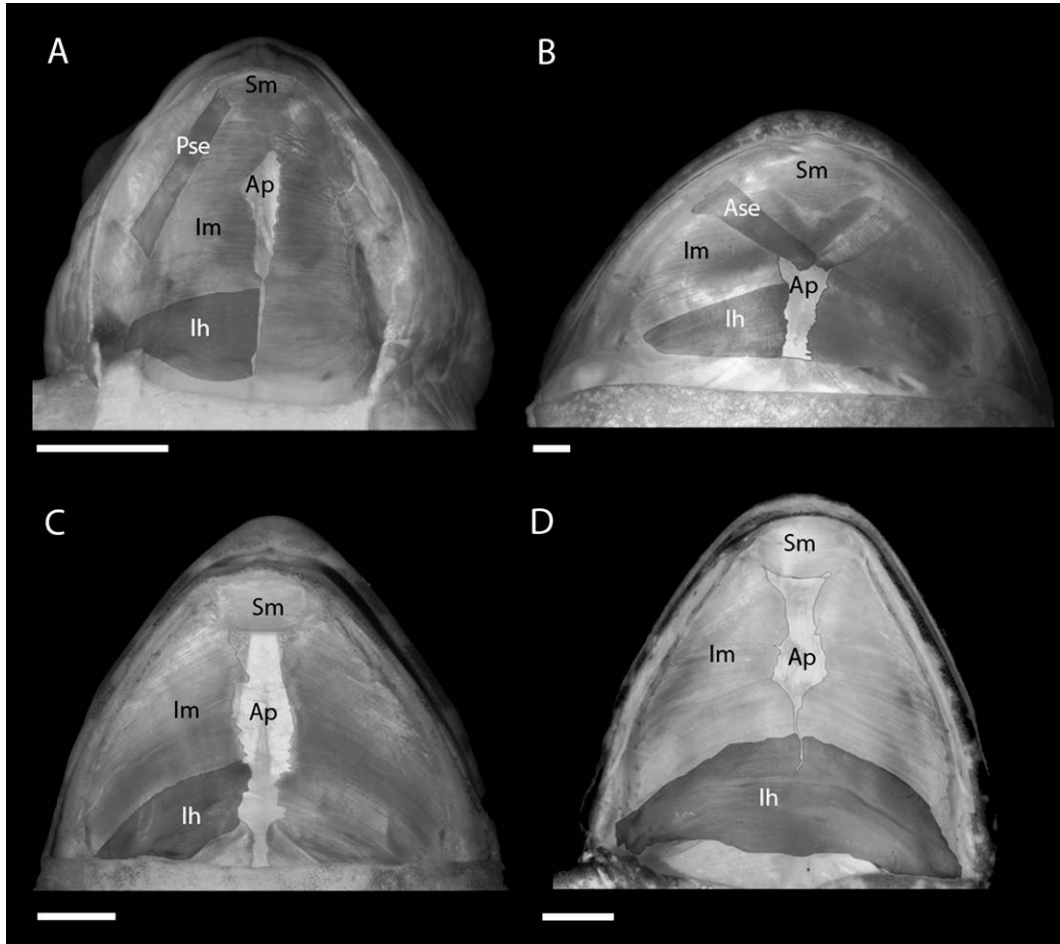
Fig. 5.—Submandibular musculature with selected portions of musculature shaded to enhance contrast. (A) *Brachycephalus ephippium* (CFBH 16836). (B) *Strabomantis biporcatus* (CENAI 2638). (C) *Pristimantis erythropleura* (ICN 18726). (D) *Oreobates barituensis* (MACN 40528). Ase = anterior supplementary element of the m. intermandibularis; Ap = aponeurosis; Ih = m. interhyoideus; Im = m. intermandibularis; Pse = posterolateral supplementary element of the m. intermandibularis; Sm = m. submentalis. Scale = 1 mm.

Character 15. Nature of the fibers of m. interhyoideus: (0) Fibers extending between mandibles without interruption (Fig. 5D). (1) Fibers interrupted medially (Fig. 5A). Our survey of the taxonomic distribution for this character is limited to Terrarana.

Character 16. Nature of the interruption between contralateral portions of m. interhyoideus: (0) Fibers interrupted by median raphe (Fig. 5A). (1) Fibers interrupted by broad median aponeurosis continuous with that of the m. intermandibularis (Fig. 5B,C). Our survey of taxonomic distribution for this character is limited to Terrarana, where the two states occur.

Discussion

Although our sample of 22 species of Terrarana is only a small fraction of the > 970 recognized species of this clade, the examined species were chosen to broadly represent the diversity of the clade. As such, we propose that seven of the character states defined above are synapomorphies of Terrarana (Characters [State]: 1 [1], 2 [3], 5 [1], 6 [0], 8 [0], 9 [1], 10 [1]) while other eight

characters vary within Terrarana and might prove useful to delimit internal clades in future studies. Another character (7) is invariable in Terrarana but is informative for other large clades of Anura.

Urogenital Anatomy

Our results indicate that the fusion of the Wolffian ducts (Character 1, State 1) is a synapomorphy of Terrarana. Beyond Terrarana, only *Heleophryne purcelli*, *Hemisus guineensis*, and *Hemisus marmoratus* have been reported to have an anterior fusion of Wolffian ducts (Character 2 [2]; Bhaduri, 1953; Bhaduri and Basu, 1957). Although the Wolffian ducts coalesce anteriorly to form a common duct in these three species, the terminal portion of the duct extends some distance within the cloacal wall before opening, as seems to occur in most anurans studied so far (Bhaduri, 1953; Bhaduri and Basu, 1957; Bhaduri and Mondal, 1967). In the species of *Eleutherodactylus* studied by Bhaduri (1953) and in all species of Terrarana we examined with serial sections, however, the common Wolffian duct opens into the cloaca as soon as it coalesces with its wall, at least in males. The situation in females is less clear (Figs. 39, 45 of Bhaduri, 1953), but this might provide another synapomorphy of Terrarana. Although the morphology of the fused Wolffian ducts of these groups requires more study, the phylogenetic position of these taxa refutes their homology, with the anterior fusion of the Wolffian ducts arising independently in Terrarana, *Heleophryne*, and *Hemisus*. The posterior fusion of the Wolffian ducts observed in *Pristimantis fenestratus* (Character 2 [2]) is tentatively interpreted as a transformation from a plesiomorphic anterior fusion (Character 2 [3]).

Evidence from ontogeny might provide morphological evidence for the nonhomology of the fusion of the Wolffian ducts in these groups. Urogenital system ontogeny has never been studied in *Heleophryne purcelli*, *Hemisus guineensis*, or *Hemisus marmoratus*, however, and has been examined in only a few species of Terrarana (Sampson, 1904; Lynn, 1942; Lynn and Lutz, 1946; Adamson et al., 1959; Valett and Jameson 1961). Adamson et al. (1959) described the development of the

urogenital system in *Eleutherodactylus martinicensis* and noticed an early posterior fusion of the Wolffian ducts before opening in the dorsolateral aspect of the hindgut. Sampson (1904), working with another species of *Eleutherodactylus* (formerly considered *E. martinicensis* but later claimed to be a different species on biogeographic arguments; Lynn, 1942), stated that both Wolffian ducts meet sagittally by the time the gut differentiates from the archenteron. Lynn (1942) described the Wolffian ducts of *E. nubicola*, including their opening into the cloaca, but did not state whether there is a single, shared opening or two separate ones. Unfortunately, he did not describe the development of Wolffian ducts in embryos after Stage 11 (for stage equivalences with other developmental series see table 3 of Townsend and Stewart, 1985). Although not mentioned in their legends, figures including the opening of the ducts (Figs. 76,77 in Lynn, 1942) suggest a possible fusion before their entrance into the cloaca. If this is correct, then the anterior fusion of the ducts observed in the adults examined here and previously (Bhaduri, 1953) were attained early during ontogeny.

Although the anterior fusion of the Wolffian ducts is conspicuous (based on gross dissection of the abdominal cavity), more effort is required to score the other character states related to the Wolffian ducts. For example, subterminal fusion of the Wolffian ducts just anterior to the cloacal wall (Character 2 [1]) has been reported in several species of *Anaxyrus*, *Duttaphrynus stomaticus*, *Agalychnis dacnicolor*, *Xenophrys parva*, *Scaphiopus couchii*, *Chrysobatrachus cupreonites*, and *Aubria subsigillata* (Bhaduri, 1953; Bhaduri and Basu, 1957). Without serial sections, however, it is not possible to discriminate between that condition, terminal fusion within the cloacal wall (Character 2 [0]), and entirely independent Wolffian ducts with separate openings (Character 1 [0]).

An additional structure that deserves attention when examining the condition of the Wolffian ducts is the urogenital sinus. It has been described as a diverticulum of the dorsal wall of the cloaca that reaches the Wolffian ducts before they open into the cloaca and is presumed to be involved in sperm storage

(Bhaduri, 1953). The Wolffian ducts lie parallel to the sinus within the urogenital capsule and can either fuse (*Ascaphus truei* and *Rhinophynus dorsalis*) or remain separate (*Arthroleptis sylvaticus*, *Kassina senegalensis*, *Microhyla ornata*) before opening into it (Bhaduri and Rudra, 1944; Bhaduri and Basu, 1957). If the urogenital capsule that surrounds both the Wolffian ducts and the urogenital sinus is a continuation of the cloacal wall, then the fusion found of the Wolffian ducts in *A. truei* and *R. dorsalis* are examples of terminal fusion within the cloacal wall (Character 2 [0]).

Additional variation in Wolffian duct morphology might also prove phylogenetically relevant. For example, in many adult anurans the Wolffian ducts dilate anteriorly to form seminal vesicles, and Bhaduri (1953) emphasized the seminal vesicles of the species of *Eleutherodactylus*. The seminal vesicles of all species of Terrarana we examined were similarly conspicuous, but there is extensive variation in size and shape, presenting either large, curved external edges or distinctively straight profiles. However, the seminal vesicles become greatly reduced in reproductively inactive individuals (Spengel, 1876), and larger samples of more species and individuals are required to formulate explicit hypotheses of homology.

Vascular Anatomy

The dorsolumbar veins collect blood from the dorsal trunk musculature and spinal cord and merge with Jacobson's veins. Literature concerning the number and insertion of these veins in the renal portal vein is scarce, but our results extend existing observations to 60 species. In anurans, Millard (1941), Stephenson and Stephenson (1947), and Szarski (1948) described two dorsolumbar veins, one anterior and one medial, in *Xenopus*, *Leiopelma*, *Bombina*, and *Pelobates*. The number of dorsolumbar veins occurring in *Ascaphus*, however, remains unknown (Noble, 1931).

In the three bufonids we studied (representing *Melanophryniscus*, *Nanophryne*, and *Rhinella*), there is a medial dorsolumbar vein and a second anterior vein similar to that described in basal anurans (Appendix 2). The taxonomic distribution of the anterior dorso-lumbar vein in bufonids and close relatives deserves further study, as it could also be a synapomorphy of that family. The remaining hyloids (except Terrarana) and one ranoid (Appendix 2) present one medial dorsolumbar vein.

Within Terrarana, we observed only the posterior dorsolumbar vein. Previously, a posterior dorsolumbar vein has only been reported on one side of a few abnormal specimens of *Hoplobatrachus tigerinus*, for which the usual condition is a single medial dorsolumbar vein (Mathtur and Sharma, 1938; Sharma, 1955). As such, we suggest that the absence of the medial dorsolumbar vein (also absent in *Fritziana goeldi*) and presence of the posterior dorsolumbar vein (also present in *Pleurodema bufoninum*) are putative synapomorphies of Terrarana, pending additional studies of hyloid diversity. It should be noted, however, that while always at the posterior end of the kidney, there is some variation in the exact junction point of the dorsolumbar vein. In most specimens, the posterior dorsolumbar vein joins Jacobson's vein. In some specimens, however, the junction is slightly further posterior, with the dorsolumbar vein joining the common iliac vein immediately prior to its contact with the kidney, where it becomes Jacobson's vein. As few specimens were available for study, we do not know if this represents continuous variation or not. One of the two studies (Shore, 1901; Millard, 1949) that had ever addressed in detail the ontogeny of renal portal veins (in *Rana temporaria*; Shore, 1901) reported that its posterior section (from the posterior end of the kidney to the common iliac) originates from the iliac vein. This observation makes it plausible that the point of junction of the posterior dorsolumbar vein on the iliac or Jacobson's vein might just be continuous variation.

Variation in the number and position of the dorsolumbar veins might provide informative characters at different levels of phylogenetic divergence. Whereas anurans have 1–3 dorsolumbar veins, Francis (1934) reported 4–5 dorsolumbar veins associated with each kidney in *Salamandra salamandra*, and Reese (1906) mentioned the junction of six "vertebral veins" directly with Jacobson's vein in *Cryptobranchus alleganiensis*. Details about

the number and point of junction of the dorsolumbar veins are not available from examinations of the vascular system in other salamanders (e.g., Hochstetter, 1888) or caecilians (e.g., Wiedersheim, 1879; Lawson, 1970; Wilkinson, 1992). Two specimens of *Chthonerpeton indistinctum* (Typhlonectidae) dissected during the course of our study presented > 10 veins carrying blood from dorsal musculature to Jacobson's vein. These are probably dorsolumbar veins, although further research is necessary to confirm their homology and determine if the putative loss of dorsolumbar veins provides additional synapomorphies for Anura or more inclusive clades.

We have described a novel lymphatic septum, the pelvic septum, associated with the discoidal fold in Terrarana. This septum is coincident with the discoidal fold in all species studied. Because some species of Terrarana appear to lack a discoidal fold (e.g., Lynch and Duellman, 1997; Padial et al., 2007; Bustamente and Mendelson, 2008; Duellman and Lehr, 2009; Heinicke et al., 2009; Arteaga-Navarro and Guayasamin, 2011), it is necessary to determine whether or not there is a persistent lymph septum despite this absence. Within Terrarana, the septum is known to be absent only in *Brachycephalus ephippium*, which lacks a discoidal fold. An identical pelvic septum to that seen in Terrarana also occurs in *Leptodactylus*, *Physalaemus*, and *Pleurodema* as does an incomplete pelvic septum in *Pseudopaludicola falcipes*. Insofar as discoidal folds also occur in other neobatrachians (e.g., *Limnomedusa*, *Thoropa*), it is likely that they also have a pelvic septum.

Concerning the pectoral septum (as described by Ecker, 1899; Tyler, 1971a; Carter, 1979), we extended the distribution of its occurrence in several clades. Given its presence in all species of Terrarana that we examined, including its sister group *Ischnocnema*, the absence of the pectoral lymphatic septum in *Brachycephalus* can be interpreted as a putative synapomorphy of the genus. Outside Terrarana this septum is absent only in *Rhinoderma*, where it is likely related to the posterior expansion of the vocal sac to hold the developing larvae (Cei, 1962).

Submandibular Musculature

The diversity of the supplementary elements of m. intermandibularis was first documented by Trewavas (1933), who referred to them as superficial slips (also portions, layers, tracts, or fibers) of her m. intermandibularis posterior (her m. intermandibularis anterior corresponds to our m. submentalis). Although Trewavas noted resemblances of the m. intermandibularis in several taxa, the superficial slips did not play any role in her phylogenetic or taxonomic conclusions. As such, the superficial slips were largely ignored until Tyler's (1971b) study of the phylogenetic significance of vocal sac structure in hylids.

Tyler (1971b: 325) described the supplementary elements as "thin, pinnate muscles of such a variety of forms (fide Trewavas, 1933) that it is difficult to propose a simple classification of their structures." Nevertheless, he proposed three morphologies based on the slip's position and origin on the mandibles: (1) an apical element that arises from the lingual surface of the anterior portion of the mandible adjacent to the m. submentalis and extends posteromediad to insert on a median raphe of the primary element of the m. intermandibularis; (2) an anterolateral element that also arises from the lingual surface of the anterior portion of the mandible but extends anteromediad to insert on the ventral fascia of the m. submentalis; and (3) a posterolateral element that originates on the ventral surface of the posterior portion of the mandible and extends anteromediad to insert on the ventral fascia of the primary element.

Tyler (1971b) treated the three morphologies as nonhomologous binary characters. Other workers homologized them as alternative states of the same character (e.g., Burton, 1998; Mendelson et al., 2000; Faivovich et al., 2005). Grant et al. (2006: 91) observed that "the shared origin of the supplementary element on the lingual surface of the mandible superficial to the deeper primary sheet of the m. intermandibularis and the fact that the different morphologies never co-occur are sufficient evidence to treat the supplementary elements of different anurans as a homologous

structure," with fiber orientation treated as an additional transformation series.

Recently, Faivovich et al. (2011) showed that Tyler's (1971b) posterolateral and apical elements co-occur in phyllomedusines, thereby refuting the homology of these structures by way of the test of conjunction (Patterson, 1982). That test also provides a basis to refute the homology of other supplementary elements. Lateral supplementary elements that insert on the m. submentalis and originate both anteriorly and posteriorly co-occur, as do lateral elements with anterior origins and posterolateral elements (Tyler, 1979; Burton, 1986). For this reason we consider the three morphologies to be independent binary characters. Current understanding of the phylogeny of Terrarana (e.g., Hedges et al., 2008; Pyron and Wiens, 2011) further reinforces the nonhomology of these morphologies, which are distributed among distantly related lineages.

"Orthobatrachia"

Heinicke et al. (2009) recently proposed a sister group relationship for Terrarana+Hemiphractidae, a clade that they called Orthobatrachia. Although knowledge of the sister group of Terrarana would be helpful in testing the synapomorphies we have identified, the evidence that Terrarana and Hemiphractidae are sister taxa remains limited. Orthobatrachia was well supported only in Heinicke et al.'s (2009) Bayesian analysis and was not recovered by Pyron and Wiens (2011). Schmid et al. (2012) constrained their analyses to force both Hemiphractidae and Orthobatrachia to be monophyletic. Furthermore, the exclusion of *Cryptobatrachus* from analyses by numerous authors (Wiens et al., 2007; Heinicke et al., 2009; Duellman et al., 2011; Pyron and Wiens, 2011; Blackburn and Duellman 2013) and the weak support for all hypotheses of intergeneric relationships all hinder our ability to target key taxa for comparison with Terrarana. Among the transformations we identified as putative synapomorphies within Terrarana, our study of species within *Gastrotheca* and *Fritziana* indicates that only the absence of the medial dorsolumbar vein and the presence of the posterior dorsolumbar vein occur in hemi-phractids (viz., *Fritziana goeldi*). In the context of the phylogenetic results of Pyron and Wiens (2011), these states evolved independently in the two clades, an interpretation that should be tested by examining the distribution of the characters presented in this paper within other genera of Hemiphractidae.

Final Remarks

We have presented six putative synapomorphies of Terrarana and nine additional characters that vary within the clade and might, therefore, help delimit subclades in future studies. Although we targeted the studied taxa specifically to provide a representative sample of the diversity of Terrarana, the > 900 species we did not examine provide ample opportunities to refute our hypotheses about the homology and taxonomic distribution of the character states we described. Some of the characters require careful dissection or serial sectioning but others can be observed with greater ease (e.g., the anterior fusion of the Wolffian ducts, the supplementary elements of the m. intermandibularis, and the occurrence of the pelvic lymphatic septum). By including these characters in taxonomic accounts, knowledge of their variation and taxonomic distribution will accumulate quickly, which will greatly improve understanding of this large and complex group.

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Species	Sex	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	Ref
TERRARANA																		
Brachycephalidae																		
Brachycephalus ephippium	M	1	3	1	0	1	0	0	0	1	1	0	0	1	0	0	–	l
Ischnocnema guentheri	M	1	3	1	0	1	0	0	0	1	1	1	0	0	0	0	–	l
I. octavioi	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
I. parva	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
Craugastoridae																		
Barycholos pulcher	M	1	3	1	?	1	0	0	0	1	1	1	0	0	0	0	–	l
Euparkerella brasiliensis	?	?	?	?	?	?	?	?	?	?	?	?	1	0	0	?	?	h
Geobatrachus ruthveni	?	?	?	?	?	?	?	?	?	?	?	?	1	0	0	?	?	h
Craugastor alfredi	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
C. andi	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
C. augusti	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
C. bransfordi	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
C. crassidigitus	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
C. fitzingeri	F	1	3	–	–	1	0	0	0	1	1	1	0	0	0	1	1	l
C. fitzingeri	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
C. fleischmanni	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
C. melanostictus	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
C. mexicanus	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
C. necerus	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
C. raniformis	M	1	3	1	0	1	0	0	0	1	1	1	0	0	0	1	1	l
C. raniformis	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
C. rhodopis	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
C. rostralis	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
C. stejnegerianus	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
C. xucanebi	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
Haddadus binotatus	M	1	3	1	0	1	0	0	0	1	1	1	0	0	0	1	1	l
Holoaden bradei	M	1	3	0	?	1	0	0	0	1	1	1	0	0	0	1	1	l
Lynchius flavomaculatus	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
L. parkeri	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
Noblella peruviana[1]	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
Oreobates barituensis	M	1	3	1	0	1	0	0	0	1	1	1	0	0	0	1	0	l
O. quixensis[2]	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
Pristmantis ridens	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. acerus	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. achatinus	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. actites	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. acuminatus	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h

P. altamazonicus	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. audanti	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. cajamarcensis	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. caryophyllaceus	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. chloronotus	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. cruentus	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. erythropleura	F	1	3	–	–	1	0	0	0	1	1	1	0	0	0	1	1	l
P. fenestratus	M/F	1	2	?	?	1	0	0	?	?	1	1	?	?	?	?	?	l
P. festae[3]	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. leoni	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. leucopus	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. molybrignus	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. myersi	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. nicefori	M	1	3	1	0	1	0	0	0	1	1	1	0	0	0	0	–	l
P. nicefori	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. palmeri	M	1	3	0	1	1	0	0	0	1	1	1	0	0	0	1	1	l
P. parvus	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. patriciae	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. permixtus	M	1	3	1	0	1	0	0	0	1	1	1	0	0	0	0	–	h,l
P. peruvianus	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. phoxocephalus	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. prolatus	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. prolixodiscus	M	1	3	1	0	1	0	0	0	1	1	1	0	0	0	0	–	l
P. pseudoacuminatus	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. sanctaemartae	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. truebae	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. unistrigatus	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. urichi	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. variabilis	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. vertebralis	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. wetmorei	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. w-nigrum	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. incomptus	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. riveti	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. surdus	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
P. toftae	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
Strabomantis anomalus	M	1	3	1	0	1	0	0	0	1	1	1	0	0	1	1	1	l
S. biporcatus	M	?	?	?	?	1	0	0	?	?	1	1	0	0	1	1	1	l
S. sulcatus	F	1	3	–	–	1	0	0	?	?	1	1	0	0	1	?	?	l
S. zygodactylus	M	1	3	1	1	1	0	0	0	1	1	1	0	0	0	1	1	l

Eleutherodactylidae

Diasporus diastema	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
D. gularis	M	1	3	1	0	1	0	0	0	1	1	1	0	0	0	1	0	l

Eleutherodactylus abbotti	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
E. albolabris[4]	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
E. alticola	M/F	1	3	0	0	?	?	?	?	?	?	?	?	?	?	?	?	a
E. atkinsi	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
E. auranti	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
E. auriculatoides	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
E. cavernicola	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
E. cooki	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
E. coqui	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
E. counospeus	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
E. cystignathoides	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
E. flavescens	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
E. fuscus	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
E. glandulifer	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
E. haitianus	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
E. hypostenor	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
E. inoptatus	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
E. jamaicensis	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
E. johnstonei	M	1	3	1	0	1	0	0	0	1	1	1	0	0	0	1	1	l
E. karlschmidti	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
E. leprus	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
E. lymbatus	M/F	1	3	?	?	1	0	0	?	?	?	?	0	0	0	?	?	m
E. martinicensis	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
E. minutus	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
E. monoensis	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
E. montanus	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
E. nubicola	M/F	1	3	0	0	?	?	?	?	?	?	?	?	?	?	?	?	a
E. orcutti	F	1	3	0	–	–	?	?	?	?	?	?	?	?	?	?	?	a
E. patriciae	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
E. pezopetrus	M	1	3	1	?	1	0	0	0	1	1	1	0	0	0	?	?	l
E. pictissimus	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
E. pinarensis	M	1	3	0	?	1	0	0	0	1	1	1	0	0	0	?	?	l
E. pipilans	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
E. planirostris	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
E. portoricencis	M	1	3	0	0	?	?	?	?	?	?	?	?	?	?	?	?	a
E. richmondi	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
E. schmidti	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
E. semipalmatus	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
E. sierramaestrae	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
E. wightmanae	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h
E. alcoae	?	?	?	?	?	?	?	?	?	?	?	?	0	0	0	?	?	h

Alsodidae

Species	Sex																	
Alsodes gargola	M	0	–	1	0	0	1	0	1	0	0	1	?	?	?	?	?	l
Eupsophus roseus[5]	M	0	–	1	0	?	?	?	1	0	0	1	?	?	?	?	?	e,l
Alytidae																		
Alytes obstericans	M/F	0	–	?	1	?	?	?	?	?	?	?	?	?	?	?	?	e
Discoglossus pictus	M/F	0	–	?	1	?	?	?	?	?	?	?	?	?	?	?	?	e
Arthroleptidae																		
Arthroleptis sylvaticus	M/F	US	–	0	0	?	?	?	?	?	0	?	?	?	?	?	?	k
Leptopelis karissimbensis	M/F	0	–	0	0	?	?	?	1	?	0	?	?	?	?	?	?	k
L. viridis	M/F	0	–	–	–	?	?	?	?	?	0	?	?	?	?	?	?	k
Batrachylidae																		
Atelognathus salai	M	0	–	1	0	0	1	0	1	0	0	1	?	?	?	?	?	l
Batrachyla antartandica	M	0	–	1	0	0	1	0	1	0	0	1	?	?	?	?	?	l
B. leptopus	M	0	–	1	0	0	1	0	1	0	0	1	?	?	?	?	?	l
B. taeniata	M	0	–	1	0	0	1	0	1	0	0	1	?	?	?	?	?	l
Bombinatoridae																		
Barbourula busuangensis	M/F	0	–	1	1	?	?	?	?	?	?	?	?	?	?	?	?	d
Bombina bombina	M/F	0	–	?	1	?	?	?	?	?	?	?	?	?	?	?	?	e, f
B. orientalis	M/F	0	–	1	1	0	1	1	1	0	0	1	?	?	?	?	?	l
Bufonidae																		
Anaxyrus boreas[6]	M	0	–	1	1	?	?	?	1	?	0	?	?	?	?	?	?	a
A. compactilis[7]	M	1	1	1	1	?	?	?	1	?	0	?	?	?	?	?	?	a
A. fowleri[8]	M	1	0	1	1	?	?	?	1	?	0	?	?	?	?	?	?	a
A. quercicus[9]	M	1	1	1	0	?	?	?	1	?	0	?	?	?	?	?	?	a
A. woodhousii[10]	M	1	0	1	1	?	?	?	1	?	0	?	?	?	?	?	?	a
Ansonia muelleri	M/F	0	–	1	1	?	?	?	?	?	0	?	?	?	?	?	?	d
Bufo bufo	M/F	?	?	?	?	?	?	?	?	1	0	?	?	?	?	?	?	f
Duttaphrynus stomaticus[11]	M	1	0	1	1	?	?	?	1	?	0	?	?	?	?	?	?	a
Melanophryniscus devincenzii	M	?	?	1	1	0	1	1	0	0	0	1	?	?	?	?	?	l
Nannophryne variegata	M	?	?	1	1	0	1	1	1	0	0	1	?	?	?	?	?	l
Rhinella fernandezae	M	1	1	1	1	0	1	1	1	0	0	1	?	?	?	?	?	l
Ceratobatrachidae																		
Platymantis corrugatus	M/F	0	–	1	0	?	?	?	?	?	0	?	?	?	?	?	?	d
P. dorsalis[12]	M/F	0	–	1	0	?	?	?	1	?	0	?	?	?	?	?	?	d
P. papuensis[13]	M/F	0	–	1	0	?	?	?	?	?	0	?	?	?	?	?	?	d
P. vitianus	M	0	–	?	?	?	?	?	?	?	?	?	?	?	?	?	?	e
Dendrobatidae																		
Dendrobates auratus	F	0	–	?	0	?	?	?	?	?	0	?	?	?	?	?	?	a
D. tinctorius	M/F	0	–	1	0	?	?	?	1	?	0	?	?	?	?	?	?	a
Silverstoneia nubicola[14]	M/F	0	–	0	0	?	?	?	?	?	0	?	?	?	?	?	?	a
Dicroglossidae																		
Occidozyga laevis	M/F	0	–	1	0	?	?	?	?	?	0	?	?	?	?	?	?	d
Heleophrynidae																		

Heleophryne purcelli	F	1	2-3?	–	–	?	?	?	?	?	0	?	0	0	0	?	?	a
Hemiphractidae																		
Fritziana goeldii	M	0	–	1	0	0	1	0	1	0	0	1	?	?	?	?	?	l
Gastrotheca christiani	M	0	–	1	1	0	1	0	1	0	0	?	?	?	?	?	?	l
G. masupiata[15]	M/F	0	–	1	1	?	?	?	1	?	0	?	?	?	?	?	?	a
Hemisotidae																		
Hemisus gineensis	M/F	1	M2F?	0	0	?	?	?	1	?	0	?	?	?	?	?	?	k
H. marmoratus	F	1	?	–	–	?	?	?	?	?	0	?	?	?	?	?	?	a
Hylidae																		
Agalychnis dacnicolor[16]	M/F	1	0	1	1	?	?	?	1	?	0	?	?	?	?	?	?	a
Aplastodiscus perviridis	M	0	–	1	1	0	1	0	1	0	0	1	?	?	?	?	?	l
Argenteohyla siemersii	M	0	–	1	0	0	1	0	1	0	0	1	?	?	?	?	?	l
Corythomantis greeningi	M	0	–	1	0	0	1	0	1	0	0	1	?	?	?	?	?	l
Dendropsophus minutus	M	0	–	1	0	0	1	0	0	0	0	1	?	?	?	?	?	l
Hyla cinerea	M	0	–	1	0	0	1	0	1	0	0	1	?	?	?	?	?	l
H. gratiosa	F	0	–	–	–	?	?	?	?	?	0	?	?	?	?	?	?	a
Hypsiboas crepitans[17]	M	0	–	?	0	?	?	?	?	?	0	?	?	?	?	?	?	e
H. pulchellus	M	0	–	1	0	0	1	0	1	0	0	1	?	?	?	?	?	l
H. punctatus	M	0	–	1	0	0	1	0	1	0	0	1	?	?	?	?	?	l
H. raniceps	M	0	–	1	0	0	1	0	1	0	0	1	?	?	?	?	?	l
H. faber	M	0	–	1	0	0	1	0	1	0	0	1	?	?	?	?	?	l
Itapotihyla langsdorfi	M	0	–	1	0	0	1	0	1	0	0	1	?	?	?	?	?	l
Lysapsus limellum	M	0	–	1	0	0	1	0	0	0	0	1	?	?	?	?	?	l
Phyllodytes luteolus	M	0	–	1	0	0	1	0	1	0	0	1	?	?	?	?	?	l
Phyllomedusa azurea	M	0	–	1	1	0	1	0	1	0	0	1	?	?	?	?	?	l
Pseudacris regilla[18]	M	0	–	1	0	?	?	?	1	?	0	?	?	?	?	?	?	a
Pseudis minutus	M	0	–	1	0	0	1	0	0	0	0	1	?	?	?	?	?	l
P. platensis	M	0	–	1	0	0	1	0	1	0	0	1	?	?	?	?	?	l
Scinax nasicus	M	0	–	1	0	0	1	0	1	0	0	1	?	?	?	?	?	l
Smilisca baudini	M	0	–	1	1	0	1	0	1	0	0	1	?	?	?	?	?	l
Sphaenorhynchus botocudo	M	0	–	1	0	0	1	0	1	0	0	1	?	?	?	?	?	l
Trachycephalus typhonius	M	0	–	1	0	0	1	0	1	0	0	1	?	?	?	?	?	l
Hyperoliidae																		
Afrixalus fulvovittatus	M/F	0	–	0	0	?	?	?	1	?	0	?	?	?	?	?	?	k
Chrysobatrachus cupreonites	M/F	1	0	1	1	?	?	?	1	?	0	?	?	?	?	?	?	k
Hyperolius viridiflavus	M/F	0	–	0	0	?	?	?	1	?	0	?	?	?	?	?	?	k
Kassina senegalensis	M/F	0	–	0	0	?	?	?	1	?	0	?	?	?	?	?	?	k
Leiopelmatidae																		
Ascaphus truei	M/F	US	–	1	1	?	?	?	1	?	0	1	?	?	?	?	?	a
Leiopelma hochstetteri	M/F	?	?	?	?	?	?	?	0	0	0	?	?	?	?	?	?	b, c
Leptodactylidae																		
Engystomops pustulosus	M	0	–	?	0	?	?	?	1	?	?	?	?	?	?	?	?	a

Leptodactylus chaquensis	M	0	–	1	0	0	1	0	1	0	1	1	?	?	?	?	?	l
L. latinasus	M	0	–	1	0	0	1	0	1	0	1	1	?	?	?	?	?	l
L. latrans[19]	M	0	–	1	1	?	?	?	1	0	1	1	?	?	?	?	?	e
L. melanonotus	F	0	–	1	0	?	?	?	?	?	?	?	?	?	?	?	?	a
L. pentadactylus	F	0	–	1	0	?	?	?	?	?	?	?	?	?	?	?	?	a
L. podicipinus	M/F	0	–	1	0	?	?	?	1	?	?	?	?	?	?	?	?	a
Physalaemus biligonigerus	M	0	–	1	0	0	1	0	1	0	1	1	?	?	?	?	?	l
Pleurodema bufoninum	M	0	–	1	0	1	1	0	1	0	1	1	?	?	?	?	?	l
P. cinerea	M/F	0	–	1	0	?	?	?	?	?	?	?	?	?	?	?	?	a
Limnodynastidae																		
Adelotus brevis[20]	M	0	–	?	0	?	?	?	?	?	?	?	?	?	?	?	?	e
Limnodynastes dorsalis[21]	M	0	–	?	0	?	?	?	?	?	?	?	?	?	?	?	?	e
L. peronii	M	0	–	?	0	?	?	?	?	?	?	?	?	?	?	?	?	e
Megophryidae																		
Megophrys montana[22]	M/F	0	–	1	0	?	?	?	?	?	?	?	?	?	?	?	?	d
Xenophrys parva[23]	F	1	1	–	–	?	?	?	?	?	?	?	?	?	?	?	?	a
Microhylidae																		
Hypopachus variolosus[24]	M	0	–	?	0	?	?	?	?	?	?	?	?	?	?	?	?	e
Kalophrynus pleurostigma	M/F	0	–	1	0	?	?	–	0	?	0	?	?	?	?	?	?	d
Kaloula picta	M/F	0	–	1	0	?	?	–	1	?	0	?	?	?	?	?	?	d
Microhyla ornata	M/F	US	–	1	0	?	?	?	?	?	0	?	?	?	?	?	?	i
Oreophryne annulata	F	1	?	–	–	?	?	?	?	?	0	?	?	?	?	?	?	d
Uperodon globulosus	M/F	0	–	1	0	?	?	?	?	?	0	?	?	?	?	?	?	a
Myobatrachidae																		
Pseudophryne bibronii	M/F	0	–	?	0	?	?	?	?	?	?	?	?	?	?	?	?	e
Odontophryinidae																		
Proceratophrys avelinoi	M	0	–	1	0	0	1	0	0	0	0	?	?	?	?	?	?	l
Pelobatidae																		
Pelobates fuscus	M/F	0	–	?	0	?	?	?	?	0	0	?	?	?	?	?	?	e
Pelodytidae																		
Pelodytes punctatus	F	0	–	?	0	?	?	?	?	?	?	?	?	?	?	?	?	e
Petropedetidae																		
Conraua crassipes	F	0	–	–	–	?	?	?	?	?	0	?	?	?	?	?	?	k
Phrynobatrachidae																		
Phrynobatrachus keniensis	F	0	–	–	–	?	?	?	?	?	0	?	?	?	?	?	?	a
P. kinangopensis	M/F	0	–	?	0	?	?	?	?	?	0	?	?	?	?	?	?	a
P. natalensis	M/F	0	–	0	0	?	?	?	?	?	0	?	?	?	?	?	?	k
P. versicolor	M/F	0	–	1	0	?	?	?	0	0	0	?	?	?	?	?	?	k
Pipidae																		
Pipa pipa	M	0	–	?	0	?	?	?	?	?	?	?	?	?	?	?	?	e
Xenopus laevis	M/F	0	–	?	0	?	?	?	1	0	0	1	?	?	?	?	?	e,g
Ptychadenidae																		
Ptychadena	M/F	0	–	0	0	?	?	?	1	?	0	?	?	?	?	?	?	k

mascaraniensis

Pyxicephalidae

Species																		
Aubria subsigillata	M/F	M0F1	–	1	0	?	?	?	1	?	0	?	?	¨?	?	?	?	k
Cacosternum boettgeri	M/F	0	–	1	0	?	?	?	1	?	0	?	?	?	?	?	?	k

Ranidae

Lithobates catesbeianus	M	0	–	1	0	0	1	0	1	0	0	1	?	?	?	?	?	l
Pelophylax esculentus	M/F	0	–	1	–	?	?	?	1	0	0	?	?	?	?	?	?	j
Staurois natator	M/F	0	–	1	0	?	?	?	1	?	0	?	?	?	?	?	?	d

Rhacophoridae

Chiromantis rufescens	M/F	0	–	1	0	?	?	?	1	?	0	?	?	?	?	?	?	k
Philautus longicrus	M	0	–	1	0	?	?	?	?	?	0	?	?	?	?	?	?	d
Polypedates leucomystax[25]	F	0	–			?	?	?	?	?	?	?	?	?	?	?	?	e
Polypedates otilophus[26]	M	0	–	1	0	?	?	?	?	?	0	?	?	?	?	?	?	d
Rhacophorus bimaculatus	M	0	–	1	0	?	?	?	1	?	0	?	?	?	?	?	?	a

Rhinodermatidae

Insuetophrynus acarpicus	M	0	–	1	0	0	1	0	1	0	0	1	?	?	?	?	?	l
Rhinoderma darwini	M/F	0	–	1	1	?	?	?	1	0	0	0	?	?	?	?	?	a,l

Rhinophrynidae

Rhinophrynus dorsalis	M	US	–	1	1	?	?	?	1	?	0	?	?	?	?	?	?	a

Scaphiopodidae

Scaphiopus couchii	M/F	0	–	1	0/1	?	?	?	1	?	0	?	?	?	?	?	?	a
S. holbrookii	M/F	M0F1	F=0	1	0	?	?	?	?	?	0	?	?	?	?	?	?	a

Telmatobiidae

Telmatobius hauthali	M	0	–	1	0	0	1	0	1	0	0	1	?	?	?	?	?	l

Using the names: [1]*Phrynopus peruvianus,* [2]*Ischnocnema quixensis,* [3]*Eleutherodactylus trepidotus,* [4]*Eleutherodactylus dixoni,* [5]*Borborocoetes grayi,* [6]*Bufo boreas,* [7]*Bufo compactalis,* [8]*Bufo fowleri,* [9]*Bufo quercicus,* [10]*Bufo woodhousii,*[11]*Bufo stomaticus,* [12]*Cornufer novae-britannae,* [13]*Cornufer meyeri,* [14]*Phyllobates nubcola flotator,* [15]*Gastrotheca boliviana griswoldi,* [16]*Phyllomedusa dacnicolor,* [17]*Hyla xerophyla,* [18]*Hyla regilla,*[19]*Cystignathus ocellatus,* [20]*Cryptotis brevis,* [21]*Heliorana grayi,* [22]*Megophrys monticola,* [23]*Megophrys parva,* [24]*Hypopachus seebachii,* [25]*Polypedates quadrilineatus,* [26]*Rhacophorus otilophus.*

References: [a] Bhaduri, 1953, [b] Stephenson and Stephenson, 1947; [c] Szarski, 1951; [d] Bhaduri and Mondal, 1965; [e] Spengel, 1876; [f] Szarski, 1948; [g] Millard, 1941; [h] Burton, 1998; [i] Bhaduri and Rudra, 1932; [j] Ecker, 1899; [k] Bhaduri and Basu, 1957; [l] This paper; [m] Griffith, 1959.

(?) Not reported; (–) nonapplicable character; (F) female; (M) male; (US) Urogenital sinus

Specimens Examined

Alsodidae: *Alsodes gargola* MACN 38965, 38975

Batrachylidae: *Atelognathus salai* MACN 41324; *Batrachyla antartandica* MACN 45178; *B. leptopus* MACN 45179, 2246; *B. taeniata* MACN 45177,

Bombinatoridae: *Bombina orientalis* MACN 42016

Brachycephalidae: *Brachycephalus epphipium* CFBH 16836, MZUSP-A 128199, 103842, 103788; *Ischnocnema guentheri* CFBH 25607, MZUSP-A 35086

Bufonidae: *Melanophryniscus devincenzii* MACN 38959, 38961; *Nannophryne variegata* MACN 45175-45176; *Rhinella fernandezae* MACN 39352, 39367.

Craugastoridae: *Barycholos pulcher* CFBH 13451,*Craugastor fitzingeri* MACN 18726; *C. raniformis* ICN 10758; *Haddadus binotatus* MACN 17043; *Holoaden bradei* AMNH 103976; *Oreobates barituensis* MACN 40528; *Pristimantis erythropleura* ICN 18726; *P. fenestratus* MZUSP 96174, 86167, 86196; *P. nicefori* ICN 21027; *P. palmeri* ICN 28537; *P. permixtus* ICN 36132; *P. prolixodiscus* ICN 10105; *Strabomantis anomalus* ICN 32598; *S. biporcatus* CENAI 2638; *S. sulcatus* AMNH 22184; *S. zygodactylus* ICN 27482.

Eleutherodactylidae: *Diasporus gularis* ICN 36835; *Eleutherodactylus johnstonei* ICN 42263; *E. pezopetrus* AMNH 63458; *E. pinarensis* AMNH 63008

Hempiphractidae: *Gastrotheca christiani* CENAI 3209; *Fritziana goeldii* CFBH 14819

Hylidae: *Aplastodiscus perviridis* MACN 35170; *Argenteohyla siemersi* CENAI 13884; *Corythomantis greeningi* CFBH 16129; *Dendropsophus minutus* MACN S/N; *Hyla cinerea* MACN 39136; *Hypsiboas pulchellus* MACN 39321, 39326; *H. punctatus* MACN 40110, 40114, 40116, 40117; *H. raniceps* MACN 45054, 45055, 45056; *H. faber* MACN MACN 45273, 45274; *Itapotihyla langsdorfi* MACN 33059; *Lysapsus limellum* MACN 38403; *Phyllodites luteolus* MNRJ 890; *Phyllomedusa azurea* MACN 38371, 38372; *Pseudis minuta* MACN 38345, 38355, 4041, 40475; *P. platensis* MACN 38587, 38588; *Scinax nasicus* MACN 41127, 41129; *Smilisca baudini* MACN 38863; *Sphaenorhynchus botocudo* MACN S/N; *Trachycephalus typhonius* MACN 40545.

Leptodactylidae: *Leptodactylus chaquensis* MACN S/N, *Leptodactylus latinasus* MACN 40726, 40732; *Physalaemus biligonigerus* 38438;.*Pleurodema bufoninum* MACN 39794, 40030.

Odontophynidae: *Proceratophrys avelinoi* MACN 36854

Ranidae: *Lithobates catesbeianus* MACN S/N

Rhinodermatidae: *Insuetophrynus acarpicus* CENAI 6901; *Rhinoderma darwinii* CENAI 5491

Telmatobiidae: Telmatobius hauthali MACN 45180