

A new lungless caecilian (Amphibia: Gymnophiona) from Guyana

Marvalee H. Wake and Maureen A. Donnelly

Proc. R. Soc. B 2010 **277**, 915-922 first published online 18 November 2009
doi: 10.1098/rspb.2009.1662

References

This article cites 32 articles, 1 of which can be accessed free
<http://rspb.royalsocietypublishing.org/content/277/1683/915.full.html#ref-list-1>

Subject collections

Articles on similar topics can be found in the following collections

[taxonomy and systematics](#) (243 articles)
[evolution](#) (1771 articles)

Email alerting service

Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click [here](#)

To subscribe to *Proc. R. Soc. B* go to: <http://rspb.royalsocietypublishing.org/subscriptions>

A new lungless caecilian (Amphibia: Gymnophiona) from Guyana

Marvilee H. Wake^{1,*} and Maureen A. Donnelly²

¹Department of Integrative Biology and Museum of Vertebrate Zoology, University of California, Berkeley, CA 94720-3140, USA

²College of Arts and Sciences and Department of Biological Sciences, Florida International University, Miami, FL 33199, USA

We report the discovery of a single specimen of a small, terrestrial, lungless caecilian, the second known taxon of lungless caecilians. It differs from all other caecilians in lacking open external nares, and from the large aquatic lungless species described by Nussbaum & Wilkinson (Nussbaum, R. A. & Wilkinson, M. 1995 *Proc. R. Soc. Lond. B* **261**, 331–335) in having no significant skull modifications. All modifications are of ‘soft morphology’ (covered external nares and choanae, lung and pulmonary vessel loss, etc.). A new genus and species are described to accommodate this form. Aspects of its skull and visceral morphology are described and considered in terms of the possible life history and evolution of the species, and compared with those of other lungless amphibians.

Keywords: lungless caecilian; terrestrial; Guayana

1. INTRODUCTION

Many new species of amphibians have recently been discovered in museum collections and in the field. Most have the general body features characteristic of their order, but recently a frog (Bickford *et al.* 2008) and a caecilian (Nussbaum & Wilkinson 1995) have been found to lack lungs. Lunglessness in some salamanders, especially the large Family Plethodontidae, has long been known (e.g. Wilder 1896). The lungless caecilian reported is a large aquatic typhlonectid that has a highly modified skull and choanae covered by the oral mucosa, but has large, open nostrils. Nussbaum & Wilkinson (1995) presented a scenario regarding its evolution and function. They placed the species (described as *Typhlonectes eiselti* by Taylor (1968) based on a single specimen without locality (‘South America’) and collection data) in a new genus, *Atretochoana*. Subsequently, an additional preserved specimen was discovered, also without precise locality data (Wilkinson *et al.* 1998). Wilkinson & Nussbaum (1997, 1999) extensively analysed the morphology of *Atretochoana* and of other typhlonectids, and used the 141 characters thus formulated in a phylogenetic analysis of members of the family.

We report the discovery of a single specimen of a small terrestrial caecilian that is also lungless. In contrast to all other caecilians, its nostrils are closed and covered by skin. It also lacks patent choanae (as does *Atretochoana*) but has a rather generalized skull morphology, so that major evolutionary change associated with lunglessness has occurred only in soft tissue morphology (e.g. lungs, cardiovascular system), but not in osteology. This tiny (112 mm total length (TL)) lungless animal provides a marked contrast in its morphology and inferred biology to that of *Atretochoana*. Furthermore, it is by far the most diminutive of New World caecilians (the next smallest species being *Microcaecilia rabei* and *Parvicaecilia*

pricei, with greatest reported total lengths of 185 mm and 191 mm, respectively (Taylor 1968)), and as small as the Old World miniaturized Cameroonian *Idiocranium russelli* (longest reported: 114 mm TL (Wake 1986)) and the equally tiny Seychellean *Grandisonia brevis* (longest reported: 112 mm TL (Taylor 1968)).

We describe the morphology of the little lungless animal, and describe it as a new genus and species in the family Caeciliidae. We also discuss aspects of the possible life history and evolution of the species, and compare them with information on the biology and evolution of other lungless amphibians.

2. MATERIAL AND METHODS

The specimen was fixed in 10 per cent formalin and preserved in 95 per cent ethanol. High-resolution X-ray CT (HRXCT) scans of the skull and anterior vertebrae were prepared in the DigiMorph Facility of the Department of Geology at the University of Texas, Austin, using a FeinFocus microfocal X-ray source with no filter. Measurements were taken with the software IMAGEJ 1.4.1 (Rasband 2008). Contrast and brightness were modified in Adobe PHOTOSHOP 7.0 (San Jose, CA, USA). The scans will be posted on the DigiMorph website (<http://digimorph.org>) for community use. External and internal anatomy were examined and measurements taken by the senior author using a dial callipers under a Wild dissecting microscope; scales were measured using an ocular micrometre on the Wild. The vertebral count was obtained from X-rays. We use a six-family taxonomy of caecilians because issues of paraphyly are not yet fully resolved (see Wilkinson & Nussbaum 2006, pp. 51–52).

3. RESULTS

Genus and species description.

Caecilita iwokramae gen. et sp. nov.

Holotype: CSBD (Center for Biological Diversity, University of Guyana) no. HA 1500, adult female,

* Author for correspondence (mhwake@berkeley.edu).

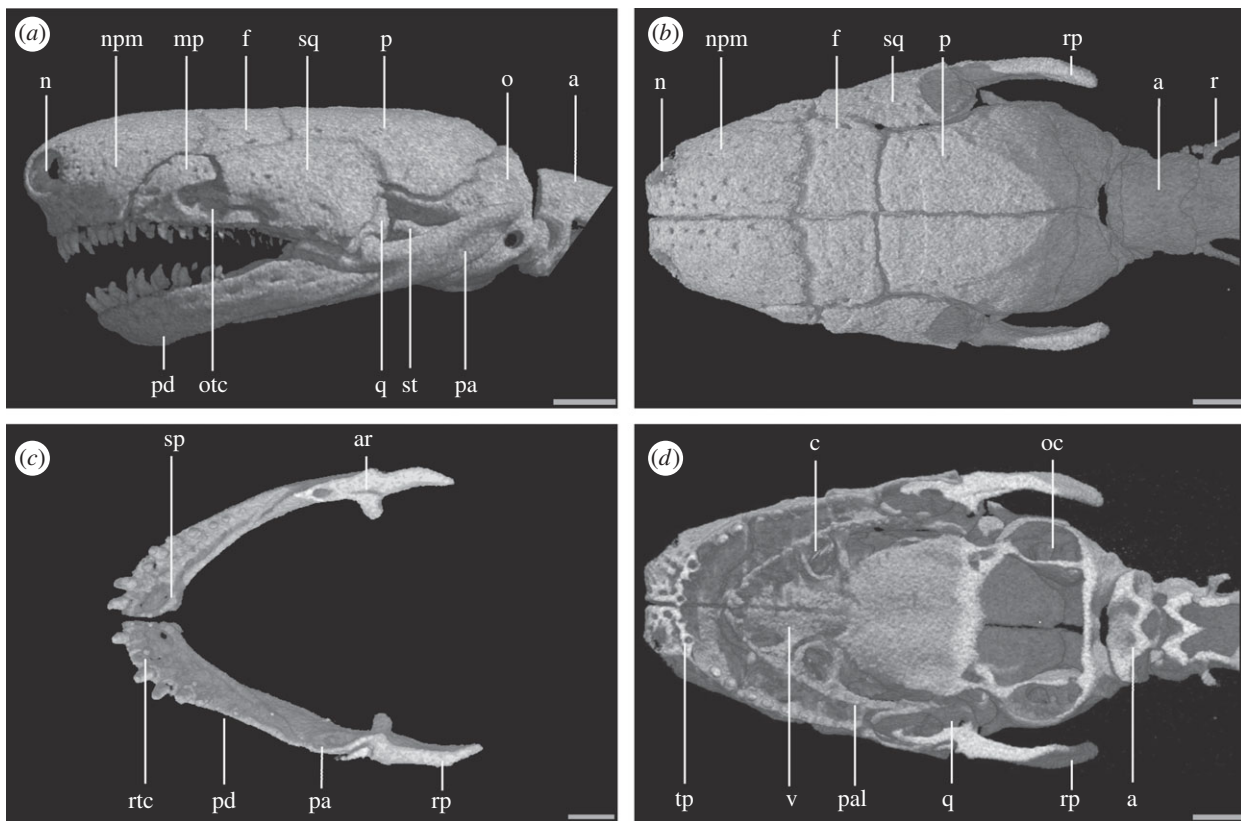


Figure 1. Computerized tomography (CT) scans of skull and anterior vertebrae. (a) Lateral view of skull and atlas. (b) Dorsal view of skull and first two vertebrae. (c) Internal view of lower jaws. (d) Ventral view of skull, plane of section cutting just above the complete ventral surface to illustrate relationships. Abbreviations: a, atlas; ar, articulation; c, choana; f, frontal; mp, maxillopalatine; n, nasal aperture; npm, nasopremaxilla; o, occipital; oc, otic capsule; otc, orbit-tentacular canal; p, parietal; pa, pseudoarticular; pal, palatine; pd, pseudodentary; q, quadrate; r, rib; rp, retroarticular process; rtc, replacement tooth crown; sp, 'splenial' ridge; sq, squamosal; st, stapes; tp, tooth pedicel; v, vomer. Scale bars, (a)–(d) 0.5 mm.

collected opportunistically on 10 March 1997 by members of an Iwokrama Vertebrate Survey Team (see Donnelly *et al.* (2005) for a description of survey activities). Tissues were not taken.

Type locality: Near 'Top Camp' in the Iwokrama Forest of Guyana (4°20' N, 58°48' W, approx. 1000 m altitude). Mixed low-stature scrub forest on high hills.

Diagnosis of genus and species: The specimen is distinct from all other caecilians in its lack of open nostrils. It resembles the typhlonectid *Atretochoana eiselti* in lacking patent choanae and absence of lungs and associated pulmonary vessels, but differs in its small size and terrestrial habit. It is distinguished from Ichthyophiidae and Rhinatrematidae by the lack of a true tail and tertiary grooves, from Uraeotyphlidae by the lack of a true tail and having the atrium undivided externally, from Scolecomorphidae by the presence of a stapes and foramen ovale, and by lacking narial plugs or a relatively large cloacal disk and by having true secondary grooves. It has a combination of features characteristic of members of the family Caeciliidae: fused nasal and premaxilla; septomaxilla, prefrontal, postfrontal and pterygoid lost or fused to adjacent elements; temporal fossae absent, no tail, some primary annuli subdivided by secondary grooves; no tertiary grooves; scales present, aortic arches near heart fused into an elongate truncus arteriosus; atrium undivided externally (see Nussbaum & Wilkinson 1989; Wilkinson & Nussbaum 2006). In addition to the closed nostrils that distinguish the new taxon from all caecilians,

among New World caeciliids, the new taxon is distinguished from members of the genus *Microcaecilia* by the eye not being visible but located in an open orbit (figure 1a), and by the presence of a small vertical keel that curves ventrally from the dorsum on the terminal body annulus (also present in *M. unicolor*, Wilkinson & Nussbaum 2006); from *Caecilia* by the mesethmoid not being exposed dorsally, the absence of inner mandibular teeth, the absence of narial plugs, the tentacle near the nostril and the presence of a terminal keel; from *Oscacaecilia* by the eye, although not visible externally, being in an open socket, the tentacle below the nostril, the mesethmoid not exposed dorsally, narial plugs absent, the vomeropalatine tooth rows not displaced posteriorly and the presence of a terminal annular keel; from *Parvicaecilia* by having long premaxillopalatine tooth rows and a terminal annular keel; from *Brasilotyphlus* by lacking a diastema between vomerine and palatine teeth and by the eye in an open socket; from *Luetkenotyphlus* in having a terminal annular keel; from *Siphonops* by having an unexposed mesethmoid, secondary annular grooves, scales and a terminal annular keel, and from *Mimosiphonops* by the latter three characters; from *Gymnopsis* in having the eye in an open socket and in having a terminal annular keel; and from *Dermophis* by having a terminal annular keel. The new form is distinguished from Old World caeciliids by similar suites of characters: it differs from *Boulengerula* in having the eye in an open socket and by having secondary grooves and

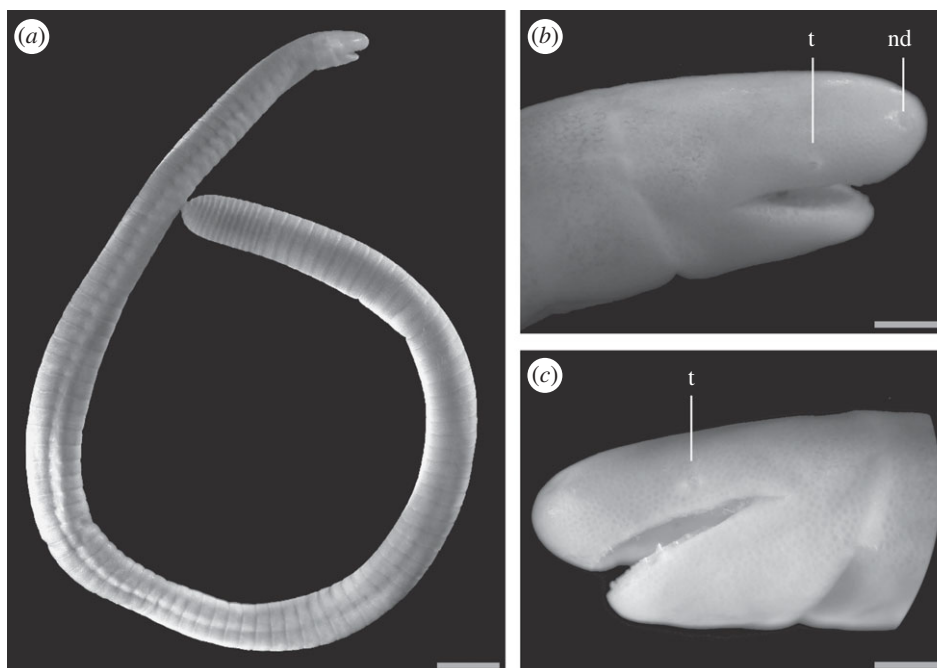


Figure 2. Photographs of *Caecilita iwokrama*. (a) Entire animal. (b) Lateral view of right side of the head. (c) Lateral view of left side of the head. Abbreviations: nd, depression in skin over covered nostril; t, tentacle. Scale bar, (a) 5 mm; (b,c) 1 mm.

scales; from *Gegeneophis* by having the eye in an open socket and by lacking narial plugs on the tongue; from *Geotrypetes* in having the mesethmoid unexposed dorsally and the presence of a terminal annular keel; from *Grandisomia* by having few secondary annular grooves, a terminal annular keel and no narial plugs; from *Herpele* in having the eye in an open socket and lacking narial plugs; from *Hypogeophis* and *Indotyphlus* in having a terminal annular keel; from *Idiocranium* in having the mesethmoid unexposed, frontal in contact with maxillary and a terminal annular keel; from *Praslinia* in having many fewer teeth and a terminal annular keel; from *Schistometopum* in having the mesethmoid unexposed and having a terminal annular keel; and from *Sylvacaecilia* in having a terminal annular keel and lacking narial plugs. See summaries by Nussbaum & Wilkinson (1989), Wake (2003) and Wilkinson & Nussbaum (2006).

Description of the holotype: Type specimen (figure 2a–c) 112 mm total length, 102 primary annuli and six complete secondaries and three incomplete ventrally. Body width 3.5 mm at mid-body and nearly all of the length of the animal posterior to the head. Head length 4.8 mm, head width at jaw articulation 2.9 mm, 1.9 mm at nostrils. Tip of snout 1.1 mm deep from dorsal to ventral surface; lower jaw underslung 0.8 mm from the tip of snout. Eyes not visible, covered by a thick layer of skin, but not bone. Nostrils covered by skin; not patent. Skin indented slightly into rims of nasal capsules (figure 2b,c). Tentacles slightly extruded, visible as small, globose, elevated bulges 0.15 mm diameter; small terminal tip round, slightly shadowed in figure 2b,c. Tentacles 0.3 mm above ventral edge of lip, 2.9 mm posterior to tip of snout, near eyes. Choanae covered by oral mucosa (figure 3a). Vent is small, slightly longitudinal, in a slightly ridged basin in a single annulus, surrounded by four small dorsal lobe-like denticles anteriorly and two larger denticles on each side posteriorly (figure 3b). Terminal annulus keeled (1–2 mm high dorsally);

extends medially from dorsal aspect around the end body, flattening ventrally (figure 3c).

Coloration in preservative: Skin colour nearly uniform light yellow–brown dorsally and ventrally. Melanophore pattern mottled, surrounding light skin glands. Melanophores on dorsum of head more concentrated on snout, fewer over parietal region of head. Throat cream-coloured. Annuli slightly darker posteriorly adjacent to annular groove. Annuli darker and much narrower posteriorly.

Etymology: The generic epithet refers to the small size of the new caeciliid taxon. The species is named for the forest in which the type specimen was discovered.

Conservation status: In the absence of additional specimens and of ecological data, the conservation status of *C. iwokrama* must be considered data deficient in the IUCN classification scheme.

Osteology: *Skull* (figure 1a,b,d)—all data for paired elements are from those of the left side of the skull. Nasopremaxilla 1.6 mm long, one-third of the length of the skull; nostril diameter 0.32 mm and fully perforate. Parietal elongate, 2.1 mm long; frontal short, nearly twice as wide as long (1 mm wide, 0.65 mm long). Paired nasopremaxillae, frontals and parietals each articulate medially; mesethmoid not exposed dorsally. Quadrate block-like, bearing a large articulation facet for the pseudoangular, pterygoid process vertically oriented; overlain at anterior edge by rear part of squamosal; anterior squamosal forms posterior part of orbit. Stapes small, bearing a long anteriorly directed columellar process. Squamosal shelf fully covers the adductor musculature; anterior temporal fossae absent. Maxillary shelf curved dorso-posteriorly, including part of tentacular channel and forming antero-dorsal margin of the open orbit; orbit and tentacular channel complexly continuous. Lower jaw (figure 1c) composed of pseudoangular bearing pronounced articular process and large retroarticular process, and pseudodentary with

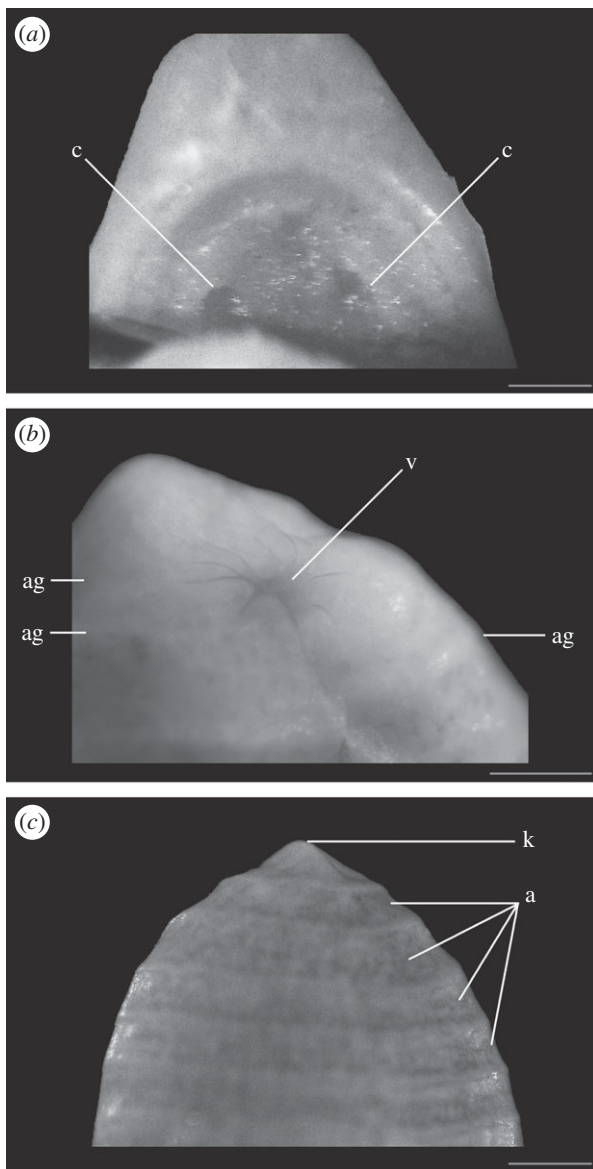


Figure 3. (a) Photograph of roof of mouth showing oral mucosa over choanae, slightly depressed anteriorly into the choanae behind the large anterior choanal rims of palatines (see figure 2d), flattening over choanae posteriorly, providing a continuous, shiny surface. Plane of focus at level of choanae renders teeth poorly resolved. Lower jaw held open with insect pin curved at tip, head supported by paraffin of dissecting dish. (b) Ventral view of body terminus showing vent in a single annulus, surrounded by two larger denticles on each side, and four smaller anterior denticles. (c) Dorsal view of body terminus showing elevated medial keel on terminal segment. Abbreviations: a, annulus; ag, annular groove; c, choana; k, keel; v, vent. Scale bars, (a–c) 0.5 mm.

numerous nerve foramina and an extensive ‘splenial’ ridge lingually. Pseudodentary and pseudoangular loosely bound together. Basisoccipital dominated by large otic components; occipital condyles large, closely articulated with atlantal cotyles. Jugular foramina large. Extensive fusion of sphenethmoid elements to basisoccipital. Short parasphenoid process of os basale separates vomers posteriorly; vomers not contributing to margins of the internal choanae, the latter formed entirely by arms of maxillopalatines. (See Wake (2003) for a general summary of caecilian comparative osteology.)

Dentition (figure 1a,c,d): The tooth formula 8 (left)-7 (right) dentary teeth, 11–10 maxillopalatine, 13–12 pre-vomeropalatine. Teeth pedicellate, crowns held to pedicels by ligaments with slight space between crown and pedicel. Tooth crowns monocuspid, elongate and recurved; longest crowns medial; crowns progressively reduce length posterolaterally in each tooth row.

Anterior vertebrae and ribs: One hundred and seven vertebrae, posteriormost three lacking ribs, the terminal one only a small piece of bone. Atlas short and stout; centrum truncated anteriorly; nuchal crest on neural arch absent; large antero-ventral bicapital cotyles flare laterally. Ventral keel of atlas only slightly developed. Subsequent vertebrae typical of those of most caecilians with well-formed ventral keels and distinct (but not highly elevated) nuchal ridges medially on neural arches. Paired, short bicapital ribs articulate with prominent parapophyseal rib-bearers. Anteriormost vertebrae perforated by spinal nerve foramina. Centrum standard: amphicoelous, slightly spool-shaped, slender centre that likely includes plug of notochordal tissue (not apparent in the CT scans); extended anterior and posterior zygapophyses (figure 1a,b,d).

Viscera: heart superficially typical of that of most caecilians; anterior tip 28 mm posterior to tip of snout. Blood clotted in large atrial cavity; atria undivided externally; invasive internal dissection not performed on holotype. Heart 5 mm long; ventricle 2.5 mm long, 1.1 mm wide at junction with atrium. Aortic arches form long truncus arteriosus; aorta slender, elongate; pulmonary vessels are absent. Clotted blood absent from vena cava. (External morphology of heart and associated blood vessels carefully examined by dissection with fine insect pins to free and expose all vessels; see §4). Lungs not present.

Liver narrow, weakly lobed anteriorly, broader and segmentally deeply lobed posteriorly; pale brown with superficial, contracted melanophores. Gall bladder small but distinct. Spleen spindle-shaped. Gut slender and elongate, not heavily muscular, filled with fragmented, unidentified insect parts. Pancreas small, pale, amorphous, lies below stomach in the mesentery. Kidneys and archinephric ducts elongate, largely retroperitoneal.

Mature female; ovaries (approx. 10 mm long) include four well-yolked ova, 1.2 mm long diameter, in the left ovary and three in the right; several small previtellogenic ova also present in each. Ovaries lie well posterior, commencing 77.4 mm from the tip of head. Fat bodies well developed. Straight oviducts lie lateral to ovaries and medial to fat bodies in ‘mesovaria’.

Skin and scales: Skin has a thin epidermis with keratinized outer layer and a deeper dermis with capillaries near its surface and numerous mucous and mixed granular glands. No evidence of hypervascularization or other differences from skin of terrestrial caecilians based on microscopic examination. Annuli and grooves extend to the end of body; no terminal shield. Small, irregularly shaped (e.g. 0.35×0.11 mm– 0.5×0.2 mm, irregular circumferences) scales present in grooves of 90th primary annulus and those of more posterior annuli. Scales few, scattered, not in rows (four scales recovered from 5 mm opening of the groove of 95th primary; one scale in 7 mm opening of 91st). Scale denticles mineralized, more random than concentric (alizarin staining).

4. DISCUSSION

Lunglessness has evolved independently in members of all three orders of amphibians. Long thought to be restricted to salamanders, lunglessness characterizes all members of the Plethodontidae, the most species-rich family of salamanders (394 species of the 586 in the order Caudata (AmphibiaWeb, 18 August 2009)), and lunglessness has arisen independently in rhyacotritonids, salamandrids and hynobiids as well. The very large, aquatic Brazilian typhlonectid *A. eiselti* was found to be lungless (Nussbaum & Wilkinson 1995) as was the relatively small (26.9–50.5 mm SVL) Bornean aquatic bombinatorid *Barbourula kalimantanensis* (Bickford *et al.* 2008), adding a caecilian and a frog to the lungless amphibian fauna. Our discovery of lunglessness in the small, terrestrial Guyanian caeciliid that we describe herein allows additional evaluation of the evolution of lunglessness. We compare the morphology of the two lungless caecilians, and compare their presumed biology with that of the lungless frog and salamanders. We evaluate the data for *Caecilita* in terms of the scenarios proposed for the origin of lunglessness in other lungless amphibians.

The morphology and presumed habits of the two taxa of lungless caecilians now known contrast markedly in many regards. *Caecilita iwokramae* is a small (112 TL), terrestrial caeciliid. *Atretochoana eiselti* is a large aquatic typhlonectid (represented by two specimens: one 725 (Nussbaum & Wilkinson 1995) or 738 (Wilkinson *et al.* 1998) mm TL and the other 805 mm TL (Wilkinson *et al.* 1998)). Nostrils and choanae in *C. iwokramae* are not patent, the former covered by skin, the latter by a layer of the oral mucosa. Nostrils of *A. eiselti* are large and open; choanae have enlarged, fused valves that bulge into the oral cavity and are covered by oral mucosa. Both taxa are characterized by loss of lungs and pulmonary circulation. Other caecilians, except for typhlonectids, have the left lung well developed, the right usually absent, and a few have a 'tracheal' respiratory component. Typhlonectids, in contrast and often suggested to be correlated with their aquatic lifestyle, have a well-developed left lung that may extend to the cloacal region (*Typhlonectes natans*: M. Wake, personal observations), and also have the right lung well developed and of varying lengths relative to the left among species (although respiratory function has not been determined for most, and the large right lung in *Potamotyphlus* lacks respiratory epithelium and is presumed to be a rheotactic organ (Wilkinson & Nussbaum 1997)). Lung development is more extensive in typhlonectids than that of nearly all other caecilians, rather than less. *Atretochoana eiselti* has extensive modification of its skull such that the squamosal and quadrate are expanded posteriorly and laterally, and there is no postorbital connection between the cheek and the dorsal cranium. The jaw articulation of *A. eiselti* is post-occipital, so that the gape is enlarged, and the cheek is highly mobile because of its lack of connections with the cranium and the stapes. The stapes of *A. eiselti* is enlarged and directed posteriorly, not connected to the quadrate, and a novel muscle extends between the stapes and the cheek and inserts on the stapes. Nussbaum & Wilkinson (1995) interpreted these changes to be the consequences of release of the mouth from its role in lung ventilation. They also

postulate significant physiological changes, so that oxygenation is obtained via the skin circulation, and they suggest that the animal lives in cold-water habitats.

The biology of *C. iwokramae* apparently differs considerably from that of *A. eiselti*. First, *Caecilita* is a member of the most widespread and species-rich family of caecilians, the worldwide (paraphyletic) Caeciliidae. The members of that family are terrestrial, not aquatic. All the modifications concomitant to lunglessness in *C. iwokramae* are those of soft tissue, and some are extensive, such as the loss of lungs and pulmonary circulation. The covering by skin of the nostrils and by oral mucosa of the choanae in *C. iwokramae* intuitively seems to be a logical correlate with the evolution of lunglessness. However, *A. eiselti* retains relatively large, open nostrils but closed choanae, and lungless plethodontid salamanders have both open nares and open choanae, involved in buccal respiration. Given the absence of an open nasal olfactory channel in *C. iwokramae*, we infer that any olfactory chemosensory function is vomeronasal via the tentacle, which is well developed. Typhlonectids, including *A. eiselti*, have small, non-protrusible but presumably functional, tentacles (Wilkinson & Nussbaum 1997). There appear to be no particular modifications of skull structure at all in *Caecilita*, so presumably feeding mechanics are not altered, as they are inferred to be in *Atretochoana* (Nussbaum & Wilkinson 1995; Wilkinson & Nussbaum 1997, 1999). Furthermore, *C. iwokramae* is among the smallest of caecilian species. It is a limbless cylinder (1077.02 mm³ volume: $\pi r^2 h$) with a low surface-to-volume ratio of approximately 1.16 (surface, calculated with body radius and total length, thus not reflecting the slight narrowing of the head and its tip being a 'small' surface component: $2 \cdot \pi r^2 + 2\pi r h$). We presume that it has a low metabolic rate typical of caecilians (Mendes 1941, 1945; Sawaya 1941, 1947; Bennett & Wake 1974), and it lives in a warm tropical environment. The size of *C. iwokramae* is well within the size range of lungless plethodontid salamanders, for which the respiratory physiology is relatively well studied and oxygenation is demonstrably obtained via the skin circulation and the buccal capillaries (Elkan 1955, 1958; Czopek 1962; Whitford & Hutchison 1965, 1967; Hutchison *et al.* 1968; Bennett & Licht 1973). The skin of *C. iwokramae* appears to be typical of that of terrestrial caecilians in terms of epidermis and dermis depth, density of glands and vascularization (see figure 1, Bennett & Wake (1974), for a comparison of the skin of a lungless plethodontid salamander and a terrestrial caecilian with a well-developed lung). It does not have the hypervascularization characteristic of the aquatic *A. eiselti* (Nussbaum & Wilkinson 1995). However, in contrast to the highly mobile tongues of most salamanders and frogs that are used to capture and manipulate prey, the tongues of caecilians are pads of tissue in the floor of the mouth, only slightly free of the jaw if at all, and are composed of the genioglossus muscle fibres and extensive venous sinuses, covered by a thin, glandular mucosa (summarized by Bemis *et al.* 1983). The tongue is involved in prey manipulation once the item is in the mouth (see Bemis *et al.* 1983; O'Reilly 2000). Tiepel (1932) conjectured that the tongue is functional in respiration in caecilians, although he emphasized its role in buccal inflation in lunged caecilians. We suggest that the

vascularity of the tongue might be particularly important in buccal respiration in the lungless caecilians, via narial inspiration in *Atretochoana* and concomitant with feeding in *Caecilita* (because of its imperforate nostrils).

Salthe & Duellman (1973) conjectured that small size might be correlated with evolutionary 'experiments', such as changes in habitat, reproductive mode and physiology and presented a number of examples. It is possible that the evolution of lunglessness might be facilitated by small size as well; the surface-to-volume ratio is relatively high in proportionally smaller animals, so gaseous exchange via skin and the buccal cavity might be more effective in smaller animals. Bickford *et al.* (2008) postulated that 'loss of lungs (in *B. kalimantanensis*, a relatively small frog) may be an adaptation to the combination of higher oxygen content in fast-flowing cold water, the species' presumed low metabolic rate, severe flattening to increase the surface area of the skin and selection for negative buoyancy'. The body size of our small terrestrial caecilian is similar to that of a number of terrestrial lungless plethodontid salamanders. The correlation of small body size and lunglessness in the frog, the salamanders and *Caecilita* may be a common property; lung loss may have been facilitated by effectiveness of cutaneous/buccal respiration in these small-bodied forms, but diverse selection pressures are probably involved. Small size is not concomitant with the evolution of loss of lungs in *Atretochoana*; its large size, hypervascularization of the skin and skeletomuscular modifications appear to be an entirely different response to selection.

Wilder & Dunn (1920), Dunn (1926), Wake (1966) and Beachy & Bruce (1992) all concluded that plethodontid stream dwellers lost their lungs as a rheotropic adaptation for larvae in the cool, fast-flowing mountain brook habitats of Appalachia. Conversely, Ruben & Boucot (1989) stated that plethodontid ancestors probably reduced head size and concomitantly lung development during their evolution in the Mesozoic of Appalachia, then a warm, low-elevation, non-montane region. A consequence of the narrowness of the head was reduced efficiency of the buccopharyngeal force pump to effect lung ventilation, which Ruben and Boucot postulate led to increased dependence on cutaneous gas exchange. Alternatively, Reagan & Verrell (1991) suggested that a shift from aquatic to terrestrial mating was a key event in the evolution of lunglessness in plethodontids. Mating in all caecilians except the secondarily aquatic typhlonectids is terrestrial (and presumably ancestral), insofar as is known, so it is not likely that a shift in mating site is involved in the loss of lungs in *Caecilita* and assuming that *Atretochoana* evolved from an aquatic typhlonectid ancestor, such a shift did not occur in its evolution either. Second, the current habitat of *Caecilita* is certainly warm and at moderate elevation. The head of the animal is indisputably small and narrow, but this is not good evidence of any correlation with lung loss, because many caecilians, of diverse total lengths, have small, narrow heads and have the typical morphology of one elongate lung and some a 'tracheal lung'. The small head-elongate lung morphology characterizes some taxa that have larvae that are free-living in streams for a period as well as direct-developing and viviparous species. Also, our very limited evidence suggests that *Caecilita* is insectivorous,

and might eat invertebrates, on the basis of its gut contents. A diet of invertebrates is typical of many caecilians, which are generalist terrestrial foragers (e.g. Wake 1983; Gaborieau & Measey 2004; Measey *et al.* 2004; Kupfer *et al.* 2005). There is no evidence of either dietary or feeding apparatus modification such as that which occurs in the large aquatic lungless typhlonectid.

Wilkinson & Nussbaum (1997) reviewed the controversy about lung loss cited above in the context of the morphology of *Atretochoana*. Given that 'It is implausible and unparsimonious that lunglessness in *Atretochoana* evolved in a terrestrial environment', Wilkinson & Nussbaum (1997) found that the morphology of typhlonectid heads and lungs provides evidence that the evolution of lung loss in amphibians can occur in aquatic environments, *contra* Reagan & Verrell (1991) and Ruben *et al.* (1993), and as now also seen in the lungless frog (Bickford *et al.* 2008). They found more support for the 'rheotactic' hypothesis than for the 'buccopharyngeal pump' hypothesis for lung loss and the other major morphological changes in *Atretochoana*, and provide alternative explanations for both hypotheses in their tentative scenario for the evolution of lunglessness in *A. eiselti*. We interpret our data to indicate that *Caecilita* evolved from a terrestrial ancestor in a warm, tropical environment. Consequently, we see no evidence at this time that particularly supports either the 'rheotropic' or the 'buccopharyngeal pump efficiency' scenarios in terms of lung loss in *Caecilita*. However, we suggest that small body size and high surface-to-volume ratio, the highly vascular tongue, and the presumably low metabolic rate may have facilitated increased dependence on cutaneous/buccal gas exchange (see Elkan 1955, 1958; Czopek 1962; Whitford & Hutchison 1965, 1967; Hutchison *et al.* 1968; Bennett & Licht 1973), but for reasons not yet determined. The biology of *Caecilita*, including lung loss, may have evolved to be similar to that of plethodontids, particularly the slender, elongate, reduced-limbed, burrowing plethodontids (e.g. *Oedipina*). Furthermore, we emphasize that, in contrast to the scenarios for salamander lunglessness that involve evolution in temperate areas, lunglessness in the frog and the two caecilian species evolved in diverse tropical habitats.

Fieldwork is necessary to obtain crucial ecological and life-history information for these fascinating amphibians. The increasing numbers of discoveries of lunglessness in all three orders of amphibians, with differences in aspects of morphology and presumably physiology, habitats and probable origins, lead us to expect that more species will be found to be lungless, and our understanding and appreciation of the diversity of the biology of amphibians will continue to expand.

We thank the Centre for Biodiversity in Guyana for making the specimen available for study. We appreciate discussion of Iwokrama camps and habitats with Roxroy Bollers, Waldyke Price, Raquel Thomas, Dexter Torres and Graham Watkins. Timothy Rowe, Jessica Maisano and Matthew Colbert produced the invaluable CT scans in the DigiMorph Laboratory at the University of Texas, where the scans will be posted for community use. David Catania and Jonathan Fong took the X-ray of the specimen at the

California Academy of Sciences, and Guinevere Wogan, Museum of Vertebrate Zoology (MVZ), University of California, Berkeley, facilitated its production. We thank David Wake for comments on the manuscript, and David Buckley for producing excellent photographs of the specimen and the preparation of figures. M.H.W. thanks the National Science Foundation for support of her work on the biology of caecilians, most recently through IBN 02-12027 and EB 03-34939. M.A.D.'s fieldwork in Guyana was supported by a GEN/UNDP Assistance Grant to the Iwokrama International Rainforest Programme (project no. GUY92/G31) and a grant from the Florida International University Provost's Office (571244850). This is contribution no. 175 to the programme in Tropical Biology of Florida International University.

REFERENCES

- Beachy, C. K. & Bruce, R. C. 1992 Lunglessness in plethodontid salamanders is consistent with the hypothesis of a mountain stream origin: a response to Ruben and Boucot. *Am. Nat.* **139**, 839–847. (doi:10.1086/285360)
- Bemis, W. E., Schwenk, K. & Wake, M. H. 1983 Morphology and function of the feeding apparatus in *Dermophis mexicanus* (Amphibia: Gymnophiona). *Zool. J. Linn. Soc.* **77**, 75–96. (doi:10.1111/j.1096-3642.1983.tb01722.x)
- Bennett, A. F. & Licht, P. 1973 Relative contributions of anaerobic and aerobic energy production during activity in amphibians. *J. Comp. Physiol.* **87**, 351–360. (doi:10.1007/BF00695269)
- Bennett, A. F. & Wake, M. H. 1974 Metabolic correlates of activity in the caecilian *Geotrypetes seraphini*. *Copeia* **1974**, 764–769. (doi:10.2307/1442690)
- Bickford, D., Iskandar, D. & Barlian, A. 2008 A lungless frog discovered in Borneo. *Curr. Biol.* **18**, R374–R375. (doi:10.1016/j.cub.2008.03.010)
- Czopek, J. 1962 Vascularization of respiratory surfaces in some Caudata. *Copeia* **1962**, 576–587. (doi:10.2307/1441182)
- Donnelly, M. A., Chen, M. H. & Watkins, G. G. 2005 The Iwokrama herpetofauna: exploration of diversity in a Guyanan rainforest. In *Ecology and evolution in the tropics: a herpetological perspective* (eds M. A. Donnelly, B. I. Crother, C. Guyer, M. H. Wake & M. E. White), pp. 428–460. Chicago, IL: University of Chicago Press.
- Dunn, E. R. 1926 *The salamanders of the family Plethodontidae*. Northampton, MA: Smith College.
- Elkan, E. 1955 The buccal and pharyngeal mucous membranes in urodeles. *Proc. Zool. Soc. Lond.* **125**, 685–710.
- Elkan, E. 1958 Further contributions to the buccal and pharyngeal mucous membrane in urodeles. *Proc. Zool. Soc. Lond.* **131**, 335–355.
- Gaborieau, G. & Measey, G. J. 2004 Termitivore or detritivore? A quantitative investigation into the diet of the East African caecilian *Boulengerula taitanus* (Amphibia: Gymnophiona: Caeciliidae). *Anim. Biol.* **54**, 45–56. (doi:10.1163/157075604323010042)
- Hutchison, V. H., Whitford, W. G. & Kohl, M. 1968 Relation of body size and surface area to gas exchange in anurans. *Physiol. Zool.* **41**, 65–85.
- Kupfer, A., Nabhitabhata, J. & Himstedt, W. 2005 From water to soil: trophic ecology of a caecilian amphibian (Genus *Ichthyophis*). *Acta Oecol.* **28**, 95–105. (doi:10.1016/j.actao.2005.03.002)
- Measey, G. J., Gower, D., Oommen, O. V. & Wilkinson, M. 2004 A subtterranean generalist predator: diet of the soil-dwelling caecilian *Gegeneophis ramaswamii* (Amphibia; Gymnophiona; Caeciliidae) in southern India. *C. R. Biol.* **327**, 65–76. (doi:10.1016/j.crvi.2003.11.001)
- Mendes, E. G. 1941 Sobre a respiração (esofágica, traqueal, e cutânea) do *Siphonops annulatus* (Amphibia-Gymnophiona). *Bol. Fac. Fil. Cien. Let. U. Sao Paulo* **1941**, 283–304.
- Mendes, E. G. 1945 Contribuição para a fisiologia dos sistemas respiratório de *Siphonops annulatus* (Amphibia-Gymnophiona). *Bol. Fac. Fil. Cien. Let. U. Sao Paulo* **1945**, 25–67.
- Nussbaum, R. A. & Wilkinson, M. 1989 On the classification and phylogeny of caecilians (Amphibia: Gymnophiona), a critical review. *Herpet. Monogr.* **3**, 1–42. (doi:10.2307/1466984)
- Nussbaum, R. A. & Wilkinson, M. 1995 A new genus of lungless tetrapod: a radically divergent caecilian (Amphibia: Gymnophiona). *Proc. R. Soc. Lond. B* **261**, 331–335. (doi:10.1098/rspb.1995.0155)
- O'Reilly, J. C. 2000 Feeding in caecilians. In *Feeding form, function, and evolution in tetrapod vertebrates* (ed. K. Schwenk), pp. 149–166. San Diego, CA: Academic Press.
- Rasband, W. S. 2008 *IMAGEJ*. Bethesda, MD: U.S. National Institutes of Health. See <http://rsb.info.nih.gov/ij/>.
- Reagan, N. A. & Verrell, P. A. 1991 The evolution of plethodontid salamanders: did terrestrial mating facilitate lunglessness? *Am. Nat.* **138**, 1307–1313. (doi:10.1086/285288)
- Ruben, J. A. & Boucot, A. J. 1989 The origin of the lungless salamanders (Amphibia: Plethodontidae). *Am. Nat.* **134**, 161–169. (doi:10.1086/284973)
- Ruben, J. A., Reagan, N. L., Verrell, P. A. & Boucot, A. J. 1993 Plethodontid salamander origins: a response to Beachy and Bruce. *Am. Nat.* **142**, 1038–1049. (doi:10.1086/285590)
- Salthe, S. N. & Duellman, W. E. 1973 Quantitative constraints associated with reproductive mode in anurans. In *Evolutionary biology of the anurans contemporary research on major problems* (ed. J. Vial), pp. 229–249. Columbia, MO: University of Missouri Press.
- Sawaya, P. 1941 Contribuição para o estudo da Fisiologia do Sistema Circulatório do Anfíbio *Siphonops annulatus* (Mikan). *Bol. Fac. Fil. Cien. Let. U. Sao Paulo* **1941**, 207–270.
- Sawaya, P. 1947 Metabolismo respiratório de Anfíbio Gymnophiona *Typhlonectes compressicauda* (Dum. et Bibron). *Bol. Fac. Fil. Cien. Let. U. Sao Paulo* **1947**, 51–56.
- Taylor, E. H. 1968 *Caecilians of the world: a taxonomic review*. Lawrence, KA: University of Kansas Press.
- Tiepel, H. 1932 Beiträge zur Kenntnis der Gymnophionen. XVI. Die Zunge. *Zeit. f. Anat. u. Entwickl.* **98**, 726–746.
- Wake, D. B. 1966 Comparative osteology and evolution of the lungless salamanders, family Plethodontidae. *Mem. South Calif. Acad. Sci.* **4**, 1–111.
- Wake, M. H. 1983 *Gymnopsis multiplicata* Peters, *Dermophis mexicanus* (Dumeril and Bibron), and *Dermophis parviceps* (Dunn) (Caecilians). In *Costa Rican natural history* (ed. D. H. Janzen), pp. 400–401. Chicago, IL: University of Chicago Press.
- Wake, M. H. 1986 The morphology of *Idiocranium russelli* (Amphibia: Gymnophiona), with comments on miniaturization through heterochrony. *J. Morphol.* **189**, 1–16. (doi:10.1002/jmor.1051890102)
- Wake, M. H. 2003 The osteology of caecilians. In *Amphibian biology*, vol. 5. *Osteology* (eds H. Heatwole & M. Davies), pp. 1811–1878. Chipping Norton, Australia: Surrey Beatty & Sons, Pty. Ltd.
- Whitford, W. G. & Hutchison, V. H. 1965 Gas exchange in salamanders. *Physiol. Zool.* **38**, 228–242.
- Whitford, W. G. & Hutchison, V. H. 1967 Body size and metabolic rate in salamanders. *Physiol. Zool.* **40**, 127–133.

- Wilder, H. H. 1896 Lungless salamanders. *Anat. Anz.* **12**, 182–192.
- Wilder, I. W. & Dunn, E. R. 1920 The correlation of lunglessness in salamanders with a mountain brook habitat. *Copeia* **1920**, 63–68.
- Wilkinson, M. & Nussbaum, R. A. 1997 Comparative morphology and evolution of the lungless caecilian *Atretochoana eiselti* (Taylor) (Amphibia: Gymnophiona: Typhlonectidae). *Biol. J. Linn. Soc.* **62**, 39–109.
- Wilkinson, M. & Nussbaum, R. A. 1999 Evolutionary relationships of the lungless caecilian *Atretochoana eiselti* (Amphibia: Gymnophiona: Typhlonectidae). *Zool. J. Linn. Soc.* **126**, 191–223. (doi:10.1111/j.1096-3642.1999.tb00153.x)
- Wilkinson, M. & Nussbaum, R. A. 2006 Caecilian phylogeny and classification. In *Reproductive biology and phylogeny of Gymnophiona (Caecilians)* (eds J.-M. Exbrayat & B. G. M. Jamieson), pp. 39–78. Enfield, NH: Science Publishers.
- Wilkinson, M., Sebben, A., Schwartz, E. N. F. & Schwartz, C. 1998 The largest lungless tetrapod: report on a second specimen of *Atretochoana eiselti* (Amphibia: Gymnophiona: Typhlonectidae) from Brazil. *J. Nat. Hist.* **32**, 617–627. (doi:10.1080/00222939800770321)