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The heart and aortic arches of rhinatrematid caecilians (Amphibia: Gymnophiona)

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Previous studies have shown there to be considerable inter-specific variation in the cardiovascular anatomy of five of the six families of caecilians. Observations on the previously unstudied Rhinatrematidae reveal this family to be characterized by a number of cardiovascular features that are unique within the Gymnophiona. These include a poorly developed sinus venosus sinistra, a short truncus arteriosus, separate carotid and systemic arches and the right atrium larger than the left. Character analysis indicates that these unique features are primitive within the Gymnophiona and they provide considerable additional support for the hypothesis that the Rhinatrematidae are the sister-group to all other caecilians. This hypothesis appears to be among the best supported hypotheses of relationships within the Gymnophiona. Caecilian cardiovascular variation provides a useful source of evidence for phylogeny reconstruction that should be integrated into phylogenetic studies of the group.

ADDITIONAL KEY WORDS: — amphibian – circulation – cardiovascular system – character analysis – phylogeny.

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INTRODUCTION

Rathke's (1852) description of the heart and major vessels of *Siphonops annulatus* (Mikan) marks the beginning of studies of the caecilian circulatory system. Following this work, a number of authors have described the structure of the heart and

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configuration of the aortic arches of a variety of caecilian species in a variety of detail. Most of these studies have focused on just one or a few species, with little attempt to be comparative (Weidersheim, 1879; Boas, 1882; Sarasins, 1890; Fuhrmann, 1914; Schilling, 1935; Chaterjee, 1936; Acolat, 1939; Sawaya, 1941, 1948; Lawson, 1966; Toews & MacIntyre, 1978; Putnam, 1970; Wake, 1986; Wilkinson, 1992a). The most comprehensive study to date is that of Ramaswami (1944) who summarized the previous literature and reported comparative information on a further eight species.

At the time of Ramaswami's (1944) study, only a single family of caecilians was recognized. More recently, the higher classification of caecilians has been the subject of several revisions (Taylor, 1968, 1969; Nussbaum, 1977, 1979; Duellman & Trueb, 1986; Laurent, 1986; Lescure, Renous & Gasc, 1986;) with six families currently recognised (Nussbaum & Wilkinson, 1989; Wilkinson & Nussbaum, 1996). Comparative data on the heart and aortic arches are available for representatives of all the currently recognised caecilian families with the single exception of the Rhinatrematidae. The almost 150 years of research on the caecilian circulatory system have demonstrated a considerable degree of interspecific variation. This variation is especially apparent in the configuration of the aortic arches, involving different degrees of bilateral asymmetry through the reduction and loss of the left-hand components of primitively paired arches. Although Ramaswami (1944) recognized that paired aortic arches are primitive within the Gymnophiona, no phylogenetic significance has been attached explicitly to the derived conditions in which arches are reduced. Indeed, Lawson (1970) claimed that the existence, within both Old-World and New-World genera of caecilians, of forms with reduced and asymmetric aortic arches supported the view that the two geographic groups were monophyletic and that "within the two groups varying degrees of asymmetry have been evolved in parallel." From a phylogenetic perspective, the presence of derived characters of the circulatory system in restricted sets of both New-World and Old-World species can only be regarded as evidence that these two geographic groups are not monophyletic.

Phylogenetic analyses, based mainly upon musculoskeletal characters and characters drawn from external anatomy indicate a probable sister group relationship between the neotropical Rhinatrematidae and all other caecilians (Nussbaum, 1977, 1979; Duellman & Trueb, 1986; Hillis, 1991; Wilkinson, 1992b; Wilkinson & Nussbaum, 1996). This hypothesis can be tested by examining characters derived from other morphological systems such as the circulatory system. Here I describe the gross morphology of the heart and aortic arches of the two rhinatrematid genera *Rhinatrema* Peters and *Epicrionops* Taylor, and consider the phylogenetic significance of the considerable differences between these structures in rhinatrematids and in other caecilians.

MATERIAL AND METHODS

The heart and aortic arches were exposed by midventral incisions in formalin fixed, ethanol preserved specimens and studied *in situ*. The most detailed examinations are based on three adult and one larval *Epicrionops sp.* collected by Prof. R.A. Nussbaum (University of Michigan) from San Francisco de las Pampas, Cotopaxi, Ecuador. These specimens are from a single discrete population but, due to uncertainties in the current species level taxonomy of *Epicrionops*, cannot be unambiguously assigned to a particular species at present. They are catalogued in the collections of the University of Michigan Museum of Zoology (UMMZ) as *Epicrionops sp.*, nos. 185875, 185877, 188840 (larval specimen), and 189697. The hearts were also removed to facilitate observation of their dorsal surfaces and investigation of their internal organisation by dissection. Because the vessels of the examined specimens frequently contained coagulated blood or were collapsed, no effort was made to inject the vessels and examination was therefore limited to the most obvious vessels, especially the aortic arches. All observations were made with the assistance of a binocular dissection microscope. Figures were prepared from camera lucida drawings. A more cursory examination of the heart and aortic arches of a single specimen of *Rhinatrema bivittatum* Cuvier, No. 21 in the collection of Dr. D.G. Senn, University of Basel, enabled the basic configuration of the heart and aortic arches of this form to be documented. Extensive observations were made of the cardiovascular anatomy of other caecilians and of outgroups (frog, salamander, lungfish). Details of the results of this broad survey and the material examined will be presented elsewhere.

THE HEART OF *EPICRIONOPS*

The heart lies within a tough transparent pericardium at a distance of about one fifth of the total body length behind the snout tip. It is oriented longitudinally with the single ventricle posteriormost, and with the atria anteriormost and lying dorsal to the conus arteriosus and the proximal aortic arches. The relative position and the orientation of the heart does not differ in adult and larval specimens. The following descriptions are based mainly on UMMZ 185875 and 185877 in which the heart appeared most relaxed. In UMMZ 189617 the heart appears to be fixed in a state of strong ventricular contraction.

Sinus venosus

The sinus venosus is a thin-walled chamber with weak muscular thickenings irregularly dispersed within its wall. It lies mostly dorsal to the ventricle. It is divided, as in all caecilians, into two parts, the sinus venosa principale and sinistra (Fig. 1) although the latter is comparatively poorly developed and there are no valves or folds of the sinus wall marking the junction of the two chambers.

The sinus venosus principale extends posteriorly and right laterally at about 45° to the midline from its union with the atria, about level with the midline of the ventricle, to approximately half-way down the right edge of the ventricle. It is roughly cylindrical in cross section and tapers slightly anteriorly. Blood drains into the sinus venosus principale through two apertures, from the posterior vena cava at its posterior margin and from the common entrance of the renal and right jugular veins at its anterolateral corner. The points of separation between these veins and the sinus are marked only by a sight thickening around the circumference of the aperture.

The sinus venosus sinistra is poorly developed and represents little more than a medial extension of the left common jugular vein. It is partially obscured by the passage of the pulmonary vein across its dorsal surface and partly embedded in the base of the left atrium. Unlike in other caecilians, its lumen is never as wide as that of the sinus venosus principale. The pulmonary vein maintains its integrity from the sinus venosus and enters into the left atrium through a distinct pulmonary aperture.

The sinuatrial aperture is complex (Fig. 2), with the two chambers of the sinus venosus having associated but partially distinct openings into the right atrium referred to here as the sinistral and principale sinuatrial apertures. At its proximal end, the sinus venosus principale extends over the dorsal surface of the right atrium and fuses with it. The principale sinuatrial aperture lies in the same plane as the dorsal atrial wall and is thus ventrally oriented. Internally, the interatrial septum also fuses with the atrial wall at its junction with the left lateral margin of the sinus venosus principale and thus forms the left lateral margin of the principale sinuatrial aperture. Proximal to the aperture, the interatrial septum is expanded slightly across the lumen of the principale sinuatrial aperture so that the left margin of this aperture has the form of a fleshy flap with a convex free edge.

At its junction with the right lateral margin of the sinus venosus principale, the wall of the right atrium is also extended across the lumen of the sinus forming the concave right lateral, and part of the posterior, margins of the principale sinuatrial aperture. The projection of the right atrial wall and the expanded part of the interatrial septum are slightly offset posteriorly when the atrium is relaxed, with the gap between them bridged by an additional short fleshy ridge. The principale

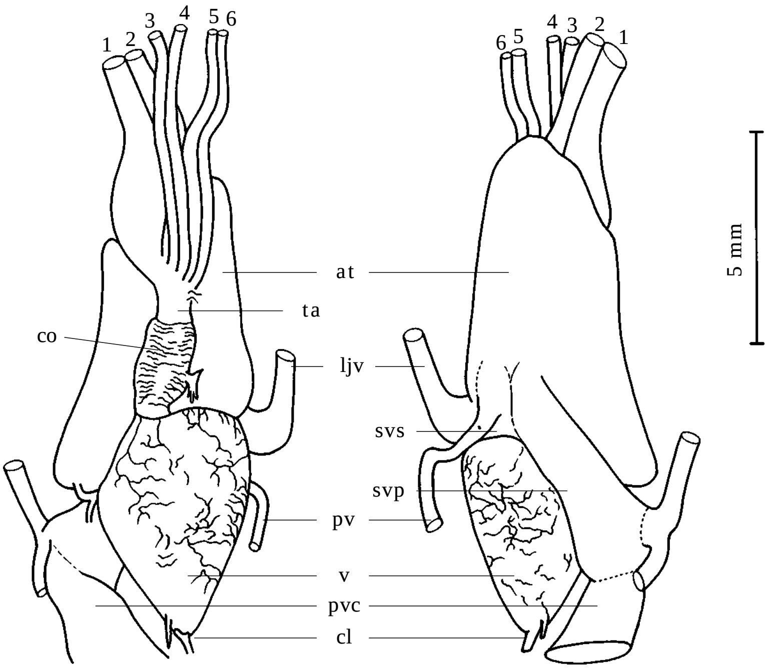

Figure 1. Ventral (A) and dorsal (B) views of the heart of *Epicrionops* sp. Abbreviations: at - atria; cl - cardiac ligaments; co - conus arteriosus; ljv - left jugular vein; pv - pulmonary vein; pvc - posterior vena cava; svp - sinus venosus principale; svs - sinus venosus sinistra; ta - truncus arteriosus; v - ventricle; 1 - right pulmonary; 2 - right systemic; 3 - right carotid; 4 - left carotid; 5 - left systemic; 6 - left pulmonary.

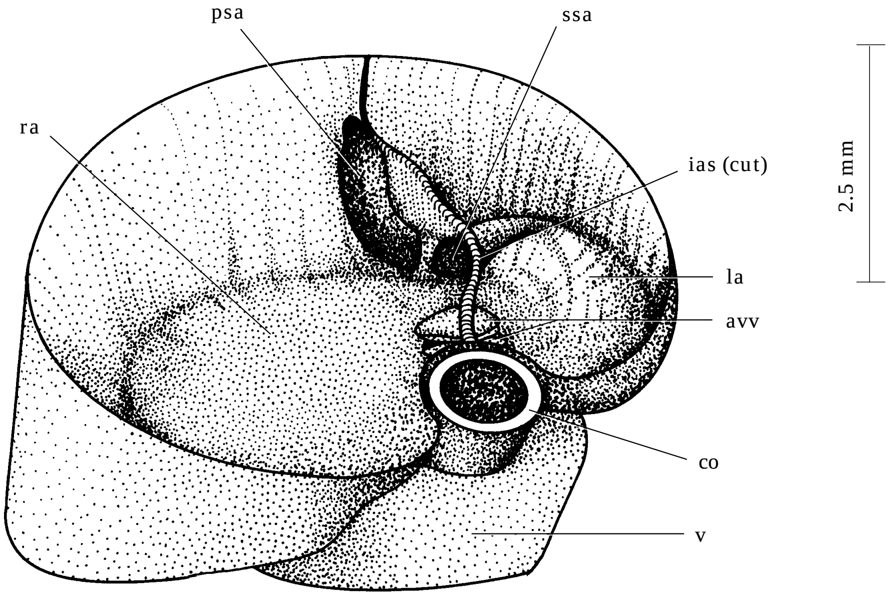

Figure 2. Oblique anteroventral view of the interatrial septum and sinuatrial aperture of the heart of *Epicrionops* sp. Abbreviations: avv - atrioventricular valves; co - conus arteriosus; ias - interatrial septum; la - left atrium; psa - principal sinuatrial aperture; ra - right atrium; ssa - sinistral sinuatrial aperture; v - ventricle.

sinuatrial aperture is thus restricted by mostly complementary, right and left, concave and convex, fleshy projections of the atrial wall and interatrial septum which make the aperture considerably narrower than the proximal lumen of the sinus venosus principale and which may help prevent the flow of blood from the right atrium into the sinus venosus principale during atrial contraction.

The robust fleshy form of the valve-like projection of the right atrial wall differs from most other parts of the atrial wall which are thinner and more highly trabeculate, but resembles in this respect the proximal interatrial septum. The projection extends across the base of the right atrium and, close to the interatrial septum, forms the raised ventral margin of the sinistral sinuatrial aperture. It then extends through the base of the interatrial septum and into the left atrium where it appears to contribute to that part of the atrial base overlying the anterior surface of the embedded sinus venosus sinistra (which is visible, in relief, within the left atrium), but rapidly diminishes in distinctness.

The sinistral sinuatrial aperture is much smaller than the principale (Fig. 2). It is subtriangular with a raised ventral margin formed by the valve-like projection of right atrial wall described above. Its left lateral margin is formed by the interatrial septum. The short robust ridge that extends between the expanded region of the interatrial septum and the projection of the right atrial wall forms a raised oblique right laterodorsal margin to complete the aperture. This ridge thus marks the point of delimitation of the sinus venosus principale and sinus venosus sinistra and is the only common element in the margins of their apertures. The sinistral sinuatrial aperture is oriented anteriorly, in a plane that is roughly perpendicular to the principale aperture. With its raised margins it projects slightly into the lumen of the right atrium and is positioned to the left of the principale sinuatrial aperture.

The sinus venosa sinistra and principale are thus mostly distinct, and certainly do not have a broad area of union bordering a common aperture as has been described for other caecilians. Assessing the functional interrelationships of the apertures and associated structures is not simple. If the expanded region of the interatrial septum and the projection of the atrial wall do serve as a valve which prevents blood flow into the sinus venosus principale during atrial contraction as is suggested by the complementary shape of their edges, it is not apparent whether the juxtaposition of their edges that would occur through atrial contraction would also block the sinistral aperture to any degree.

Atria

Externally there is no indication of the division into left and right atria (Fig. 1). In ventral view, the atrium is divided by the conus arteriosus and proximal aortic arches into left- and right-hand sides which correspond only roughly to the left and right atria. There is considerable variation in the sizes of these sides. In UMMZ 185875, the two sides are of approximately equal width. In the larva the right side is slightly wider than the left, and in the other two adult specimens the right side is much wider than, and dwarfs the left. The right side is bounded anteriorly by the expanded right pulmonary arch and extends posteriorly across the anterolateral surface of the ventricle, partially overlying the right margin of the conus arteriosus. At its posterior margin, the left side lies adjacent to the ventricular base. In UMMZ 185875 it extends further anteriorly than the right, closely approaching the anterior limit of the pericardium, whereas in the larva and other adult specimens the right-hand side is the more extensive anteriorly. In addition the atria of the larval specimen are much smaller relative to the ventricle than in the adult specimens.

Dorsally, the right atrium also extends across the surface of the ventricle so that the ventricle close to its base is enclosed in an atrial envelope. The point of separation of the left and right atria is indicated by the left margin of the anterior limit to the sinus venosus principale which reveals the division to be asymmetrical with the right atrium the larger of the two.

The walls of the atria are mostly composed of a thin membranous connective tissue sheet extending across a network of muscular trabeculae. Internally, numerous trabeculae extend across the lumina of the atria forming a complex network of fibres. An interatrial septum is present lying approximately longitudinal and vertical (Fig. 2). Proximal to the ventricle, it is smooth and fleshy and mostly continuous with the atrial base. It has a free edge that extends across the atrioventricular aperture, dividing it asymmetrically into smaller left and larger right sides, but is continuous dorsal and ventral to the aperture with the atrioventricular valves. Ventrally, the interatrial septum fuses with the atrial wall close to the left margin of the conus arteriosus. Dorsally it is associated with the sinuatrial aperture as described above, and in this area the fleshiness characteristic of the posterior region on the interatrial septum proximal to the base is well developed and more extensively distally. Elsewhere, the septum is formed primarily from trabeculae and becomes progressively more perforate anteriorly. In the adults, the septum curves to the left close to the atrial apex so that the apex clearly belongs to the right atrium despite its being to the left of the midline. The trabeculae of the interatrial septum anastamose with those that are distributed throughout both the atrial walls and lumina so that the septum may appear indistinct from the general network of trabeculae. This is especially true in the larval specimen in which the septum cannot be distinguished from an especially dense network of trabeculae close to the atrial apex.

A particularly dense network of trabeculae is developed on the atrial surface of the right lateral margin of the principale sinuatrial aperture and the adjacent wall of the right atria. This network might contribute resistance to the backflow of blood from the right atrium into the sinus venosus principale.

Ventricle

The ventricle is conal in shape with its pointed apex posterior and its large base making broad contact with the atria anteriorly (Fig. 1). A variable number (two or three) of strong connective tissue cords, the cardiac ligaments, anchor the apical region of the ventricle directly to the pericardium and thus indirectly to the posterior vena cava, liver and trachea, which are all bound to the pericardium by a dense fibrous connective tissue in this region. Additional connective tissue bridges link the ventricle, atria and sinus venosus principale and link the atria to the conus arteriosus and truncus arteriosus.

The external surface of the ventricle is smooth and covered by an extensive plexus of tiny ramifying and anastamosing coronary veins. The walls of the ventricle are thick with a compact outer layer and larger inner spongy layer composed of thick muscular trabeculae, much thicker than the atrial trabeculae. The lumen of the ventricle is enlarged anteriorly and medially into a central cavity which is indented by trabeculae extending mostly anteroposteriorly. A relatively distinct cluster of thick trabeculae lie in this plane along the dorsal wall of the ventricle to the right of the atrioventricular aperture but any resemblance to an incomplete ventricular septum is probably superficial.

In UMMZ 189617 the cone-shape of the ventricle is distorted through contraction producing a more irregular blunt, almost square, apex. In the larval specimen the apex is also less pointed than in the relaxed adult heart, but is gently rounded rather than blunt and irregular.

Conus arteriosus

The conus arteriosus is a cylindrical chamber connecting the ventricle to the arterial system (Fig. 1). It is slightly constricted as its posterior margin adjacent to the right-hand base of the ventricle, and extends anteriorly and left laterally across the ventral surface of the atria. The four specimens differ in the degree to which the conus is bent to the left as it extends anteriorly across the atria. In UMMZ 185875 and the larva bending is least well developed with the conus deviating only slightly from the longitudinal axis of the heart and body. In the other two adult specimens, UMMZ 185877 and 189617, there is an angle of approximately 35° and 45° between the conus and the longitudinal axis of the heart respectively. Variation in the orientation of the conus is correlated with, and at least partially explains, the observed variation in the relative size of the atria lying to the left and right of the conus in ventral view.

The wall of the conus is thick and muscular and its surface is smooth both externally and internally. As with the ventricle, its external surface is covered with numerous tiny coronary veins. Unlike the surface of the ventricle, the coronary veins are mostly oriented transversely around the circumference of the conus.

Within the conus are two rows of large and well formed semilunar valves, one proximal or pygidial and one distal or synangial. The rows lie fairly close to one another rather than being at the extreme proximal and distal ends of the conus. Each row contains three valves: a dorsal, and left and right lateroventral valves. The corresponding valves in the two rows are perfectly aligned and all are of a closely similar size. There is no indication of a spiral fold within the conus arteriosus of *Epicrionops*. The conus of the larva is proportionately shorter and broader than that of the adults.

THE ARTERIAL SYSTEM OF *EPICRIONOPS*

Truncus arteriosus and proximal aortic arches

As blood leaves the conus arteriosus it enters a common chamber from which the vessels comprising the aortic arches arise. This common chamber represents an extremely short ventral aorta or truncus arteriosus less than half of the length of the conus arteriosus and initially slightly narrower than the latter chamber but expanding anteriorly. The walls of the truncus are smooth and considerably narrower the conus and there is no network of coronary veins developed over its surface. There is no indication of any coronary arteries.

The pattern of division of the truncus and origin of the aortic arches (Fig. 3) is as follows. A vertical septum divides the truncus initially into a smaller left and a larger right cavity which rapidly become slightly separated. The left cavity, which represents a short left common systemico-pulmonary trunk, is then almost immediately asymmetrically divided by a further vertical septum (Fig. 3B). The division produces initially a larger lateral pulmonary channel and a smaller, narrower and more medial systemic channel. The two channels remain tightly bound into a systemico-pulmonary trunk until close to the anterior pericardium where they become disassociated and distinct, but the dividing septum becomes more obliquely, dorsolateral-ventromedially, oriented and the relative sizes of the channels change. The systemic expands greatly, becoming much larger than the pulmonary which after some expansion actually decreases in diameter.

The right-hand chamber formed by the initial division of the truncus also expands slightly and, a little anterior to the level of division of the left chamber, develops two grooves in its ventromedial wall. These grooves lead to left and right carotid channels that initially lie within the wall of the right common systemico-pulmonary trunk but which rapidly become separated from it. Initially the two carotids continue as two channels in a single structure (Fig. 3D), but they soon become distinct vessels although they remain adjacent and in contact (Fig. 3E). With the formation of the carotids, the right systemico pulmonary trunk continues to expand so that medially it is adjacent to its opposite with the carotids lying ventral to its ventromedial surface.

In parallel with the left, the right systemico-pulmonary trunk is divided, distal to the separation of the carotids from its wall but posterior to their separation from each other (Fig. 3D), by an oblique (dorsolateral-ventromedial) septum into a slightly larger lateral pulmonary and smaller medial systemic channels. As with the left, the channels do not separate into distinct vessels until close to the pericardium. Before this the pulmonary channel continues to expand dramatically and then suffers an equally substantial reduction in diameter before passing through the pericardium.

Whilst the paired carotids, and the right and left systemics and carotids, form three separate pairs of associated vessels or channels within the pericardium, all the vessels are bound together by a loose mat of connective tissue fibrils with a somewhat compacted superficial layer. This gives the superficial impression of a more extensive and bulbous truncus arteriosus representing a union of all these vessels as is found in other caecilians.

Where the vessels pass through the pericardium there is considerable size asymmetry which is maintained as they run anteriorly with the right pulmonary arch the largest vessel, the right systemic a little smaller, the paired carotids and left systemic approximately equally smaller still and the left pulmonary the smallest. Within the pericardium, the two largest of the proximal aortic arches, the right pulmonary and systemic, expand laterally and dorsally from their origin from the truncus arteriosus so that the much smaller carotid arches run across their ventral surface. The left pulmonary and systemic arches also expand within the pericardium but to a much lesser extent. This proximal expansion interrupts the symmetrical linear arrangement of the arches that is seen at their origin from the truncus so that, if the atria are reflected in dorsal view, the right and left systemics are seen to be

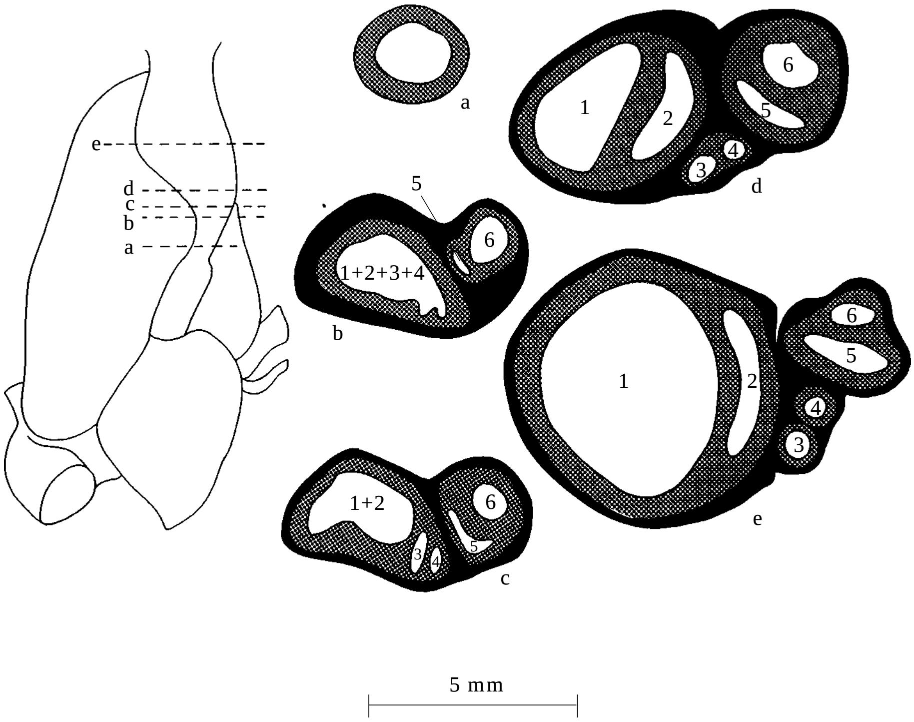

Figure 3. Anterior views of five transverse cross sections (a–e), from most proximal to most distal, of the truncus arteriosus and proximal aortic arches of *Epicrionops* sp. Abbreviations as in Fig. 1. Stippled area is the compact muscular wall of the vessels and septa, dark shading indicates the extent of fibrous connective tissue in which the vessels are embedded. Dashed lines on the outline ventral view of the heart indicate the positions of the corresponding transverse sections (a–e).

adjacent and in contact. The initial linear arrangement is restored as the pulmonary and systemic arches narrow and the vessels pass through the pericardium. Where the left and right systemics are adjacent, the mat of connective tissue fibrils is least well developed so that even superficially the arches are seen to be distinct.

The sizes of the apertures of the arches in the truncus reflect the relative sizes of the arches as they are within the pericardium rather than their relative sizes on leaving the pericardium. Thus the apertures of the carotids are smaller than the apertures of the expanded pulmonary and systemics.

Distal aortic arches

On leaving the pericardium, all six aortic arches, right and left pulmonary, systemic and carotids, are separate vessels although the carotids remain in close contact. In adults, the left systemic and pulmonary arches are thrown into distinctive lateral and then medial bends where they exit the pericardium. In the larva this bending is stronger still and is characteristic of all the arches. Anterior to the pericardium the paired carotid arches are bound to the ventral surface of the trachea. The systemic arches run forward in association with the lateral margins of the trachea. Also associated with these arteries is the insertion of the musculus transversus, which constitutes the innermost layer of the external muscular sheath of caecilians (Naylor & Nussbaum, 1980).

The pulmonary arteries are bound by connective tissue to the lateral margins of the oesophagus. At their anterior limit, a little posterior to the most caudal elements of the glossal skeleton, which are reduced in adult rhinatrematids (Nussbaum, 1977), the carotid arteries separate and, with the systemic arteries, loose their association with the trachea. The pulmonary arteries retain their association with the lateral margin of the oesophagus as it expands laterally to become the pharynx. The carotid and systemic arches also bend laterally so that as the three of each side reach the most laterally expanded point of the pharynx they are oriented transversely and in parallel, with the carotid arteries anterior, the pulmonary arteries posterior and the systemic between them. The anteriormost fibres of the m. transversus also loose their association with the trachea and mirror the lateral curvature of the aortic arches by becoming progressively shorter than the more posterior fibres and shifting their insertion to the lateral margin of the expanding pharynx.

From these most lateral points, the carotid arteries of each side run a short distance anterior along the lateral margin of the pharynx and bifurcate into internal and external carotid arteries. The systemic arches bend back on themselves to form lateral dorsal aortae that are bound by connective tissue to the dorsal surface of the oesophagus. They initially maintain their integrity, but fuse to form a single dorsal aorta about midway between the heart and the pharynx. The single dorsal aorta and right lateral dorsal aorta both give rise to vertebral arteries. The much smaller left lateral dorsal aorta does not appear to give rise to any vessels. The right pulmonary artery bends posteriorly and medially and becomes associated with the right dorsolateral surface of the trachea. As the trachea approaches the level of the heart, it bends to the left to lie along the left dorsolateral margin of the pericardium. The right pulmonary artery follows this deviation in the trachea and then runs into the right lung. The left pulmonary arch initially mirrors the right in forming a posteriorly directed ‘left pulmonary’ artery, but this artery runs along the ventral surface of the oesophagus and eventually disappears. It may be continuous here with a network of tiny vessels that covers the surface of the oesophagus. The right lung, which is well developed, thus receives its own distinct blood supply, whereas the much smaller left lung, which is virtually vestigial, lacks any obvious connection with the ‘left pulmonary’ artery.

The course of the aortic arches of the larva parallels that of the adult with only minor differences apparent in the pharyngeal region. Here the three arches bend laterally and run across the ventral surfaces of the third and fourth ceratobranchials which form at their distal extremes the skeletal support for the sides of the spiracle. The pulmonary arches of each side run across the lateral margin of the pharynx along the anterior edge of the third ceratobranchial, between it and the second ceratobranchial. They then run posteriorly across the dorsal surface of the pharynx dorsal to the spiracle and continue posteriorly as in the adult. The systemic arches run slightly more anterior and are associated laterally with the ventral surface of the second ceratobranchial. Also associated with the second ceratobranchial is a small but distinct second gill. A similar first gill lies anterior to the second in association with the distal tip of the first ceratobranchial. Before curving around the lateral margin of the pharynx each systemic arch gives rise to a small vessel that runs into the base of the second gill. The systemic arches then bend posteriorly to form lateral dorsal aortae which follow the same course as in the adult. The carotid arches of each side run just anterior to the systemics and anterior to the second ceratobranchials. At their lateral most point they also give rise to a small vessel that enters the base of the first gill, before the arch splits into internal and external carotid arteries.

THE HEART AND ARTERIES OF *RHINATREMA*

Only superficial observations on the circulatory system of *Rhinatrema* have been possible. These indicate no differences between *Rhinatrema* and *Epicrionops* in the general form and disposition of the chambers of the heart and the proximal aortic arches. As in *Epicrionops*, the sinus venosus is rather obliquely oriented with a relatively anterior sinuatrial aperture and a poorly developed sinus venosus sinistra, the atria are large and undivided externally, the conus arteriosus extends left laterally across the ventral surface of the atria, and the truncus arteriosus is only poorly developed so that separate paired pulmonary, systemic and carotid arteries arise within the pericardium.

CHARACTER ANALYSIS

The heart and aortic arches of rhinatrematid caecilians display a number of features which differ from conditions present in any other caecilians. In addition, certain features are shared with some but not all those caecilians for which information on the cardiovascular system is available. The overall appearance of the rhinatrematid heart and aortic arches is that of a rather primitive tetrapod lacking several of the distinctive features previously considered to be characteristic of caecilians. Here I present a preliminary formulation of characters to describe and partition caecilian cardiovascular variation and assessments of polarity based on the outgroup criterion. The most informed use of the outgroup criterion depends on the recognition of appropriate outgroup taxa, i.e. on a prior assessment of the phylogenetic position of caecilians within tetrapods. There has never been any clear consensus concerning the affinities of caecilians to the other Lissamphibia, and differing opinions continue to be expressed by competent modern researchers (e.g. Carroll and Currie, 1975; Milner, 1993). This necessitates a conservative approach to the use of the outgroup criterion in which a broad array of potential outgroups are surveyed for comparative information, with the greatest confidence in assessed character state polarities only being possible when the assumed primitive conditions are uniformly distributed across this array of potential outgroups. As is typical of soft anatomical features, substantial information on the cardiovascular system of pertinent fossil taxa such as microsaurs and lysorophiids, which have been considered as possibly closely related to caecilians, is not available and thus the source of comparative outgroup information is restricted to the literature and my own observations on extant taxa including frogs, salamanders, lungfish and amniotes. To this end I have relied drawn heavily upon the works of Goodrich (1930), Francis (1934) and Putnam (1970).

1. Shape of the ventricle. Caecilian ventricles are roughly conical with the apex posterior but there is considerable variation in shape that reflects the width of the base relative to the distance from base to the apex. Rhinatrematids lie at one end of this range of variation having the most relatively broad and short ventricle known for any caecilian. In some caecilians, such as the attenuate caeciliaids *Boulengerula* Tornier and *Indotyphlus* Taylor, the ventricle may be distinctly narrow and highly elongate suggesting a correlation between ventricle shape and body shape. Rhinatrematids fit this pattern: they are stout-bodied forms with stout ventricles. However, relatively slender ventricles are present in some stout bodied forms (e.g. uraeotyphlids) indicating that any correlation between ventricular and body shapes is only partial. Candidate outgroups have broad and short ventricles that are more like those of rhinatrematids than those of any other caecilians suggesting that the rhinatrematid condition is primitive within the Gymnophiona and that the varying degrees of elongation and narrowing seen in other caecilians is derived.

2. Orientation of the sinus venosus principale. In rhinatrematids, ichthyophiids, uraeotyphlids and scolecomorphids, the sinus venosus principale extends obliquely across the dorsal surface of the ventricle. In other caecilians this chamber is oriented transversely in the same plane as the ventricular base. In outgroup taxa the sinus venosus is more longitudinal than transverse suggesting that the oblique orientation of the sinus venosus principale is primitive within the Gymnophiona.

3. Form of the sinus venosus. Rhinatrematids differ from other caecilians in having a poorly developed sinus venosus sinistra that is little more than an extension of the right jugular vein and that is little expanded. In other caecilians, the sinus venosus sinistra is expanded to achieve a broad diameter close to that of the larger sinus venosus principale. In addition, the two chambers are separated by valves or by indentations of the sinus venosus wall at their junction. Comparison with outgroups suggests that the bipartite sinus venosus is a specialization and that the poorly developed condition in rhinatrematids is primitive within the Gymnophiona.

4. Form of the sinuatrial aperture. The unusual form of the sinuatrial aperture of rhinatrematids with partially separate sinistral and principale apertures is not encountered in outgroups or in other caecilians where the sinuatrial aperture is simpler and single. Outgroup comparison therefore suggests that the rhinatrematid condition is derived.

5. Orientation of the interatrial septum. Rhinatrematids and some other caecilians (e.g.

ichthyophiids and uraeotyphlids) have a roughly vertical inter-atrial septum so that the right and left atrial chambers are physically left and right. In contrast, many other caecilians (e.g. typhlonectids and many caeciliaids) have a more oblique, almost horizontal, inter-atrial septum. The vertical inter-atrial septum is more typical of outgroups and may be considered primitive within the Gymnophiona.

6. Relative size of the atria. In rhinatrematids the left atrium is smaller than the right. This is in stark contrast to all other caecilians which have a smaller right atrium. In this respect rhinatrematids resemble frogs and salamanders indicating that the rhinatrematid condition is primitive within the Gymnophiona.

7. Orientation of the conus arteriosus. The conus arteriosus extends anteriorly and slightly right laterally in rhinatrematids. In other caecilians the conus projects either straight anteriorly (e.g. ichthyophiids) or slightly left laterally (e.g typhlonectids, caeciliaids). In outgroups, the conus arteriosus may be a more complex structure involving several regions separated by bends, making its overall orientation difficult to judge. In my judgement the rhinatrematid condition more closely resembles the orientation of the proximal conus arteriosus of frogs, salamanders and lungfish and is probably primitive within the Gymnophiona.

8. Number of valve rows in conus arteriosus. Rhinatrematids have two rows of valves in the conus arteriosus. Other caecilians have either one (e.g. uraeotyphlids) or two (e.g. ichthyophiids) rows of conal valves. Outgroups usually have two or multiple rows of conal valves so that two rows is considered primitive and one row derived within the Gymnophiona. Variation in the size of the caecilian conus arteriosus appears to be correlated with the number of rows of conal valves. Forms with two rows of valves have a relatively elongate conus arteriosus and where there is only one row of valves the conus is relatively short. Thus I have not treated variation in the proportions of the conus as an independent character.

9. Length of the truncus arteriosus. In all caecilians except rhinatrematids, aortic arches are bound together into a single discrete truncus arteriosus that extends between the conus arteriosus and the anterior limit of the pericardium and may extend further anterior than this before the separation of any derivative vessels. The aortic arches form within the truncus arteriosus as septa partition the initially single chamber. The septa are typically thinner than the external walls of the truncus and the pattern of septation shows, like the pattern of aortic arches, much interspecific variation. In rhinatrematids, there is only a very short region of the aortic arches, proximal to the conus arteriosus, where there is only a single undivided chamber. Distally, but within the pericardium, the aortic arches achieve a degree of separation from each other not encountered in other caecilians though the separate units remain loosely bound together by a fibrous connective tissue sheath. Outgroup comparison indicates quite unambiguously that the elongate truncus arteriosus, typical of non-rhinatrematid caecilians, is a derived feature within the Gymnophiona and that the condition in rhinatrematids is primitive.

10. Number of pulmonary arches. Within caecilians pulmonary arches may be paired (e.g. rhinatrematids, ichthyophiids some caeciliaids) or single (e.g. typhlonectids, some caeciliaids). Paired pulmonary arches are primitive.

11. Supply from left pulmonary artery. Rhinatrematids appear unique within the Gymnophiona in having both pulmonary arches present, but in having the left supply the oesophagus rather than the left lung. This appears to be a derived condition as it is not encountered in any outgroup taxa.

12. Path of the pulmonary arches. In rhinatrematids the pulmonary arches extend anteriorly to the level of the posterior pharynx before bending back on themselves and running caudad. Rhinatrematids share this feature with ichthyophiids whereas in many other caecilians (e.g. most caeciliaids) the pulmonary vessels bend posteriorly directly upon or shortly after their exit from the pericardium. The rhinatrematid pattern appears to be primitive within the Gymnophiona, reflecting the circulation of the gills in piscine ancestors, and in gilled larvae and embryos of amphibians.

13. Number of systemic arches. Within caecilians systemic arteries may be paired (e.g. rhinatrematids, ichthyophiids, some caeciliaids) or single (e.g. typhlonectids, some caeciliaids). Paired systemic arteries are primitive.

14. Path of the systemic arteries. As with the pulmonary arteries, the systemic arteries of rhinatrematids and some other caecilians (e.g. ichthyophiids, many caeciliaids) retain the primitive pattern of running anteriorly to the pharynx before doubling back on themselves as lateral dorsal aortae. In typhlonectids and some caeciliaids the systemic arteries do not extend to the pharynx. The rhinatrematid condition is primitive within the Gymnophiona.

15. Separate carotid and systemic arches. The carotid arteries of non-rhinatrematid caecilians arise from common systemicocarotid trunks. Indeed it is generally considered that caecilians lack a discrete carotid arch (Duellman and Trueb, 1986). The condition in rhinatrematids, which lack any extensive systemicocarotid trunk and have separate carotid arches developing within the pericardium, is quite distinctive and clearly primitive within the Gymnophiona.

DISCUSSION

Study of the heart and aortic arches of rhinatrematid caecilians reveals many differences between these structures in this group and in other caecilians. The cardiovascular morphology of rhinatrematids resembles that of frogs and salamanders rather than other caecilians in lacking several distinctive derived features (elongate truncus arteriosus, smaller right atrium and no separate carotid arch) previously considered characteristic of the Gymnophiona. Of the 15 cardiovascular characters outlined above, 6 (1, 3, 6, 7, 9, 15) have primitive conditions restricted to the Rhinatrematidae. This provides considerable additional support for Nussbaum's (1977, 1979) hypotheses that the Rhinatrematidae are the sister-group of all other caecilians which was based largely upon musculoskeletal characteristics. Furthermore, where derived and primitive character states can be identified for other characters, rhinatrematids generally have the primitive condition (2, 5, 8, 10, 12, 13, 14) or a derived condition that is unique to them (4, 11). Thus, in addition to providing additional support for Nussbaum's hypothesis, none of the phylogenetically informative variation detected in the caecilian circulatory system contradicts this hypothesis.

Nussbaum's hypothesis has been controversial because rhinatrematids are zygokrotaphic caecilians, and their hypothesized status as sister-group to all other caecilians might suggests that caecilians were primitively zygokrotaphic. In contrast, others have sought to derive caecilians from stegokrotaphic ancestors (Carroll and Currie, 1975). Walsh (1986) used a reduced set of osteological characters to support the alternative hypothesis that the more stegokrotaphic Ichthyophiidae, rather than the Rhinatrematidae, is the sister-group of all other caecilians, but the slender evidence he found for this hypothesis is strongly contradicted by Nussbaum's (1977,

1979) data and by the evidence from the cardiovascular system presented here. Additional support for Nussbaum's hypothesis has also been derived from a consideration of the larval lateral line system (Wilkinson, 1992b). Phylogenetic analysis of limited caecilian DNA sequence data (Hedges *et al.* 1993) failed to resolve basal relationships within the Gymnophiona. However, although additional tests with more extensive molecular data are desirable, the message from diverse aspects of morphology is clear and is sufficient for Nussbaum's hypothesis to be considered among the best supported hypotheses of phylogenetic relationships within the Gymnophiona.

Trueb & Cloutier (1991) employed 11 cardiovascular characters in their study of phylogenetic relationships of the Lissamphibia. The evidence from the rhinatrematid cardiovascular system suggests that some of their cardiovascular characters may need to be recoded or reformulated. For example, they considered caecilians to have the derived conditions of a right atrium that is smaller than the left, and a sinus venosus that is subdivided by valves or folds. In contrast, rhinatrematids have the primitive conditions of a smaller left atrium and an undivided sinus venosus. Further, the coding of caecilians adopted by Trueb & Cloutier (1991) for several other of their characters is questionable. For example, caecilians are coded as having derived conditions of a subdivided ventricle and a small (reduced) sinus venosus. However, neither of these features is unambiguously characteristic of caecilians. It is beyond the scope of this work to examine what effect revision of these cardiovascular characters would have upon the phylogenetic relationships supported by Trueb and Cloutier's (1991) data.

The character analysis presented here is preliminary as it is focused primarily upon the features which distinguish rhinatrematids from other caecilians but it serves to illustrate the potential utility of caecilian cardiovascular characters for phylogenetic inference. There is also considerable variation within the cardiovascular system of non-rhinatrematid caecilians and this variation should provide a useful source of evidence for reconstructing caecilian phylogeny. Other 'soft' anatomical systems, particularly the urogenital system, may also provide a rich source of characters. Further understanding of caecilian phylogeny will no doubt repay attempts to synthesise the growing body of evidence from diverse anatomical systems.

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REFERENCES

Acolat L. 1939. Variationes de l'appereil respiratoire et de l'appareil circulatoire central chez quelques gymnophiones. *Compte Rendue de l'Association Anatomique du Nancy* **3:** 3–15.

Boas JEV. 1882. Über den Conus arteriosus und die Arterien der Amphibien. *Morphologisches Jarbucher* **7:** 509–551.

Carroll RL, Currie PJ. 1975. Microsaurs as possible apodan ancestors. *Zoological Journal of the Linnean Society* **57:** 229–247.

Chatterjee BK. 1936. The anatomy of *Uraeotyphlus menoni* Annandale. *Anatomische Anzeiger* **81:** 393–414.

Duellman WE, Trueb L. 1986. *Biology of Amphibians.* New York: McGraw-Hill Book Co.

Francis ETB. 1934. *The Anatomy of the Salamander.* Oxford: Oxford University Press.

Fuhrmann O. 1914. Le genre *Thyphlonectes* [sic]. *Memoires de la Societe d'Histoire Naturelle de Neuchatel* **5:** 112–138.

Goodrich ES. 1930. *Studies on the Structure and Development of Vertebrates.* London: Macmillan.

Hedges SB, Nussbaum RA, Maxson LR. 1993. Caecilian phylogeny and biogeography inferred from mitochondrial DNA sequences of the 12S and 16S rRNA genes (Amphibia: Gymnophiona) *Herpetological Monographs* **7:** 64–76.

Hillis DM. 1991. The phylogeny of amphibians: current knowledge and the role of cytogenetics, In: Sessions SK, Green DM. eds. *Amphibian Cytogenetics and Evolution.* San Diego: Academic Press, 7–31.

Laurent RF. 1986. Ordre des gymnophiones. In: Grasse P-P, Delsol M, eds. *Traite de Zoologie, Volume. 14. Amphibiens.* Paris: Masson, 595–608.

Lawson R. 1966. The anatomy of the heart of *Hypogeophis rostratus* (Amphibia: Apoda) and its possible mode of action. *Journal of Zoology* **149:** 320–336.

Lawson R. 1970. The caecilian cardio-vascular system and intra-amphibian relationships. *Journal of Zoology* **160:** 199–229.

Lescure J, Renous S, Gasc J-P. 1986. Proposition d'une nouvelle classificationes amphibiens gymnophiones. *Societe Zoologique de France Mémoire* **43:** 145–177.

Milner AR. 1993. The Paleozoic relatives of lissamphibians. *Herpetological Monographs* **7:** 8–27.

Nussbaum RA. 1977. Rhinatrematidae: a new family of caecilians (Amphibia: Gymnophiona). *Occasional Papers of the Museum of Zoology University of Michigan* **682:** 1–30.

Nussbaum RA. 1979. The taxonomic status of the caecilian genus *Uraeotyphlus* Peters. *Occasional Papers of the Museum of Zoology University of Michigan* **687:** 1–20.

Nussbaum RA, Wilkinson M. 1989. The classification and phylogeny of caecilians (Amphibia: Gymnophiona), a critical review. *Herpetological Monographs* **3:** 1–42.

Naylor BG, Nussbaum RA. 1980. The trunk musculature of caecilians (Amphibia: Gymnophiona). *Journal of Morphology* **166:** 259–273.

Putnam JL. 1970. Heart structure and the phylogeny of the Amphibia. Unpublished PhD. Thesis, Texas A&M University.

Rathke H. 1952. Bemerkungen über mehere Korpelthiere der *Caecilia annulata.* Archiv für Anatomische und Physiologie. **1852:** 335–349.

Ramaswami LS. 1944. An account of the heart and associated vessels in some genera of Apoda (Amphibia). *Proceedings of the Zoological Society of London* **114:** 117–138.

Sarasins P&F. 1890. Zur entwickslungsgeschichte und Anatomie der *Ichthhyophis glutinosus* (Linne). *Ergebnisse naturwissenschaftlicher Forschungen auf Ceylon* **2** parts 1–4.

Sawaya P. 1941. Contribução para a estudio da fisiologia do sistema circulatório do anfibio *Siphonops annulatus* (Mikan). *Boletim do Faculdade di Filosofia, Ciências y Letres Universidade de Sao Paulo serie Zoologico* **5:** 200–233.

Sawaya P. 1948. O sistemo cardiovascular do anfíbio-apodo *Typhlonectes compressicauda compressicauda* (Dum. e Bibr.) *Boletim de Museo Paraense Emilio Goeldi* **10:** 279–289.

Schilling C. 1935. Das Herz von *Hypogeophis* und Seine Entwicklung. *Morphologische Jahrbucher* **76:** 52–91.

Taylor EH. 1968. *The caecilians of the world: a taxonomic review.* Lawrence: University of Kansas Press.

Taylor EH. 1969. A new family of African Gymnophiona. *University of Kansas Science Bulletin* **48:** 297–305.

Toews D, Macintyre D. 1978. Respiration and circulation in an apodan amphibian. *Canadian Journal of Zoology* **56:** 998–1004.

Trueb L, Cloutier R. 1991. A phylogenetic investigation of the inter- and intrarelationships of the Lissamphibia (Amphibia: Temnospondyli). In: Shultze H-P, Trueb L, eds. *Origins of the Higher Groups of Tetrapods: Controversy and Consensus.* New York: Cornell University Press, 223–313.

Wake MH. 1986. The morphology of *Idiocranium russeli* (Amphibia: Gymnophiona), with comments on miniaturization through heterochrony. *Journal of Morphology* **189:** 1–16.

Walsh D. 1986. Systematics of caecilians. Unpublished Ph.D Thesis, McGill University.

Weidersheim R. 1879. *Die Anatomie der Gymnophionen.* Jena.

Wilkinson M. 1992a. Novel modification of the tetrapod cardiovascular system in the West African caecilian *Herpele squalostoma* (Amphibia: Gymnophiona: Caeciliaidae). *Journal of Zoology* **228:** 277–286.

Wilkinson M. 1992b. The phylogenetic position of the Rhinatrematidae (Amphibia: Gymnophiona): evidence from the larval lateral line system. *Amphibia-Reptilia* **13:** 74–79.

Wilkinson M, Nussbaum RA. 1996. On the phylogenetic position of the Uraeotyphlidae (Amphibia: Gymnophiona). *Copeia* **1996:** 550–562.