Evolutionary relationships of the lungless caecilian *Atretochoana eiselti* (Amphibia: Gymnophiona: Typhlonectidae)

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Appreciation of the diversity of caecilian amphibians has recently been enhanced by the discovery of a radically divergent aquatic caecilian of the Neotropical Typhlonectidae. *Atretochoana eiselti* is the largest lungless tetrapod and the only lungless caecilian, and it possesses a suite of remarkable cranial modifications that set it apart from all other caecilians. Numerical phylogenetic analyses, using 141 morphological characters, were performed in order to resolve the evolutionary relationships of *Atretochoana* and representatives of all other typhlonectid genera. These analyses yield a single most parsimonious tree, (*Chthonerpeton (Nectocaecilia (Typhlonectes natans, Typhlonectes compressicauda) (Potomotyphlus, Atretochoana))))*, that is both well resolved and, as judged by Bremer support and by bootstrapping, is well supported. This tree is used as a basis for interpreting ecological shifts and associated morphological evolution within the Typhlonectidae. The available data suggest that the rate of morphological evolution in the *Atretochoana* lineage is significantly greater than that in other typhlonectid lineages.

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ADDITIONAL KEY WORDS:—phylogeny – parsimony – morphology – ecology – rates of evolution.

CONTENTS

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INTRODUCTION

The Typhlonectidae is a small group of Neotropical caecilian amphibians that, unlike most other caecilians, are either aquatic or semi-aquatic as adults. The recent discovery of lunglessness and an array of other remarkable morphological features in an aquatic typhlonectid (Nussbaum & Wilkinson, 1995) has highlighted the previously unsuspected diversity of the poorly known caecilian amphibians. An important component of attempts to understand and explain this diversity is the establishment of well-supported hypotheses of phylogenetic relationships for caecilians. Nussbaum & Wilkinson (1995) emphasized the distinctiveness of the lungless caecilian by placing it in its own genus, *Atretochoana*. Subsequently, we presented an extensive comparison of the anatomy of *Atretochoana* with that of other typhlonectid caecilians and discussed in more detail aspects of the evolution of lunglessness and associated features (Wilkinson & Nussbaum, 1997). In the latter work, we considered *Atretochoana* to be a member of a clade including all fully aquatic caecilians, and we identified several derived character states shared by only *Atretochoana* and *Potomotyphlus*, and suggested that they are sister taxa, but we did not test or evaluate the relative support for this hypothesis with any explicit phylogenetic analysis. Here we seek to establish the evolutionary relationships of *Atretochoana* within the Typhlonectidae through numerical phylogenetic analyses of 141 morphological characters drawn from several anatomical systems. We also explore the implications of the phylogenetic framework and associated hypotheses of morphological character evolution for our understanding of typhlonectid evolution.
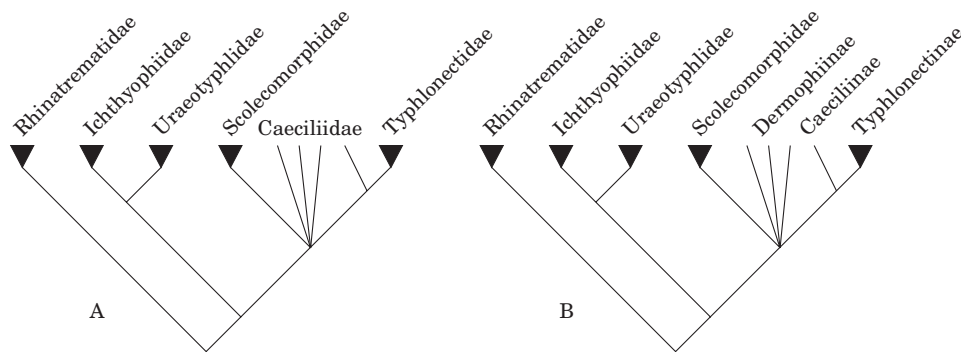
Figure 1. Phylogeny and taxonomy of caecilians. A, conventional view in which recognition of the Typhlonectidae renders the Caeciliidae paraphyletic. B, alternative in which recognition of the Typhlonectinae as a subfamily of the Caeciliidae leaves the monophyletic status of the Caeciliidae uncertain and the monophyletic status and content of the Caeciliinae and Dermophiinae uncertain.

TAXONOMIC BACKGROUND

Monophyly and relationships of the Typhlonectidae

The Typhlonectidae (Taylor, 1968) comprises a small group (5 genera, 13 species) of Neotropical caecilians that are either obligatorily or facultatively aquatic. Current views on the phylogenetic relationships of caecilian families are represented in Figure 1A. Relationships at the base of the caecilian tree are well resolved and well supported, as is the large clade comprising the Typhlonectidae, Scolecomorphidae and Caeciliidae, that Nussbaum (1991) referred to as the 'higher' caecilians (Nussbaum, 1979; Duellman & Trueb, 1986; Hillis, 1991; Hedges, Nussbaum & Maxson, 1993; Wilkinson & Nussbaum, 1996; Wilkinson, 1997a). However, relationships within the higher caecilians are less clear. There is good evidence that both the Scolecomorphidae and the Typhlonectidae are monophyletic (Nussbaum, 1985; Nussbaum & Wilkinson, 1989; Wilkinson, 1997a; and see below), but little evidence, just a single potential synapomorphy, an elongate *m. interhyoideus posterior* (Wilkinson, 1997a), supports monophyly of the large and heterogeneous Caeciliidae (21 genera, 90 species). In contrast, more compelling molecular evidence indicates that some caeciliids are more closely related to the Typhlonectidae than to other caeciliids (Hedges *et al.*, 1993), and it has been suggested that the Typhlonectidae has a caeciliid ancestry (Nussbaum, 1979), and that the Caeciliidae is probably paraphyletic (e.g. Nussbaum & Wilkinson, 1989).

The most distinctive feature of the Typhlonectidae, first reported for *Typhlonectes compressicauda* by Peters (1875), is the unique and derived fusion and expansion of the embryonic gills of each side into sac-like structures, which is now known for representatives of three of the five currently recognised typhlonectid genera. In addition, typhlonectids have a number of characters that appear to be derived within the 'higher' caecilian clade (Table 1). Six of these characters (4, 21–24, 27) appear to be unique to typhlonectids, and, together with the form of their embryonic gills, provide fairly good evidence for typhlonectid monophyly. The other derived characters are shared by some other caecilians, but they are seen in combination in no other caecilian taxa. Typhlonectid monophyly was one of the best supported

TABLE 1. Typhlonectid character states that are considered derived within the 'higher' caecilians. Q = qualifications; ? unknown; * = presumed secondary loss; A = *Atretochoana*; P = *Potomotyphlus*

Character	Q
1. Viviparity	A?, P?
2. Deciduous juvenile dentition	A?, P?
3. Monocusped adult teeth	
4. Small tentacular apertures and tentacles	
5. A single row of valves in the *conus arteriosus*	
6. An almost horizontal interatrial septum	
7. A fused single systemicopulmonary arch	A*
8. A tracheal lung	A*
9. Narial plugs	
10. Relatively superficial and enlarged choanae and choanal valves	
11. No annular scales	
12. No secondary annuli	
13. No postcloacal annuli (or true tail)	
14. Nasal and premaxilla fused	
15. No separate septomaxillae	
16. No separate prefrontals	
17. No separate postfrontals	
18. No separate pterygoids	
19. Secondarily zygokrotaphic skulls	
20. Maxillopalatine widely separated from quadrate	
21. Relatively dorsolaterally oriented occipital condyles	
22. A ventral process of the squamosal bracing against the maxillopalatine	
23. M-shaped ceratohyals	
24. A sliding articulation between the first and second ceratobranchials with no *m. subarcualis II*	
25. Greatly expanded fused third and fourth ceratobranchials	A*
25. A *pars posterosuperficialis* of the *m. cephalodorsosubpharyngeus*	
26. No *m. hyopharyngeus internus*	
27. *M. subvertebralis pars ventralis* with scalloped origin from fascia lateral to the centra	
28. A *pars nuchalis* of the *m. obliquus externus profundus*	

conclusions of a recent phylogenetic analysis using both combined and separate analyses of neuroanatomical and more traditional morphological data (Wilkinson, 1997a), although that study included only two of the five typhlonectid genera. Monophyly is also supported by the lack of any strong conflicting evidence of relationships.

Taxonomic status of the Typhlonectidae

Taylor (1968) established the Typhlonectidae, a familial rank that has been accepted by most workers despite the realization that recognition of the Typhlonectidae renders the Caeciliidae paraphyletic (e.g. Nussbaum & Wilkinson, 1989). Hedges *et al.* (1993) corroborated caeciliid paraphyly in a study based upon mitochondrial ribosomal RNA gene sequences, and, in order to remove the paraphyly, they synonymized the Typhlonectidae with the Caeciliidae, recognizing the Typhlonectinae at subfamilial rank within the Caeciliidae. Hedges *et al.* also noted that the remaining caeciliids included in their study fell into two clades corresponding to Taylor's (1968) Caeciliinae and Dermophiinae, and thereby also implicitly recognized these subfamilies. Unfortunately, the taxonomic sampling of Hedges *et al.* (1993) was limited to 7 of 23 caeciliid genera. In particular, they included none of the taxa that Wake & Campbell (1983) transferred from the Dermophiinae to the Caeciliinae,

so that their phylogeny is equally consistent with both Taylor's and Wake & Campbell's differing conceptions of these caeciliid subfamilies. The limited taxonomic scope also limits any support the molecular data might provide for the monophyly of these subfamilies.

Under both Taylor's and Wake & Campbell's conceptions, the Dermophiinae is simply all those caeciliids that are not caeciliines, and is, therefore, likely to be paraphyletic. The uncertainty of subfamilial classifications of the Caeciliidae is further emphasized by Nussbaum's (1988) demonstration that one of the genera that Wake & Campbell (1983) assigned to the Caeciliinae is a junior synonym of another genus that they included in the Dermophiinae. For these reasons, Nussbaum & Wilkinson (1989) did not recognize any subfamilial taxa within the Caeciliidae. For the same reasons, the proposed recognition by Hedges *et al.* (1993) of the Typhlonectinae as a subfamily of the Caeciliidae, although providing one possible solution to caeciliid paraphyly, introduces additional problems of paraphyly at a lower rank (Fig. 1B), e.g. paraphyly of the Caeciliinae or the Dermophiinae (Wilkinson, 1996a), compounded by uncertainty over the content of these families. More comprehensive molecular studies indicate that that further revision of the classification of the 'higher' caecilians will be required to eliminate paraphyletic familial and subfamilial level taxa (Sheps, Wilkinson, Nussbaum & Cohen, in prep). Here we follow the conservative classification of Nussbaum & Wilkinson (1989) and employ the traditional name Typhlonectidae (*sensu* Taylor, 1968) as our preferred label for the group (based on common usage), while acknowledging that assignment of clades to Linnaean ranks is to a great extent arbitrary and meaningless, and, ignoring the dictates of formal conventions, unnecessary (de Queiroz & Gauthier, 1992).

MATERIAL AND METHODS

Scope

We have examined aspects of the musculature, osteology, the respiratory and cardiovascular systems, and external morphology of the two species of *Typhlonectes* of undoubted validity (Wilkinson, 1996b), *T. natans* and the generotype *T. compressicauda*, the monotypic genera *Atretochoana*, *Potomotyphlus*, and *Nectocaecilia*, and a single species of *Chthonerpeton*, the generotype, *C. indistinctum*. Numbers of postcloacal and nuchal vertebrae were determined using the method of Wilkinson (1989). We provide only brief descriptions of the characters because detailed descriptions of their comparative anatomy have been provided elsewhere (Wilkinson & Nussbaum, 1997). This study also incorporates additional details of the anatomy of *Atretochoana*, from a recently discovered second specimen (Wilkinson *et al.*, 1998). Because of their great rarity in collections, we have been able to make only superficial observations on the other species of *Chthonerpeton*, and we have not included these species in our analyses. Unless otherwise stated '*Chthonerpeton*' refers here to the generotype. Specimens examined are listed in Wilkinson (1989), Wilkinson & Nussbaum (1997), and Wilkinson *et al.* (1998).

For the majority of taxa, sample sizes for osteological and myological observations are small. Thus the possibility that variation has not been adequately assessed and that the delimitation of character states may not correspond to real discontinuities

in the underlying variation is a potentially serious limitation of this study. More adequate samples of *Typhlonectes natans* (>15) have been studied, providing some guide to the extent of variation that may be anticipated in other typhlonectid species (Wilkinson & Nussbaum, 1997). However, inasmuch as it is unsafe to generalize from one taxon to another, some of the characters we employ here may prove to be more variable given larger samples, and thus poorly suited to phylogenetic inference in their present form.

Phylogenetic analyses

Parsimony analyses used the exhaustive or branch and bound search options in PAUP 3.1.1 (Swofford, 1993) that are guaranteed to find all most parsimonious trees. Exhaustive searches also yielded skewness statistics which provide an indicator of data quality (Le Quesne, 1989, Huelsenbeck, 1991). We compared two measures of data quality with their distributions for randomly permuted data, and used permutation tail probabilities (PTPs) as test statistics for null hypotheses that the data are random with respect to phylogeny and thus phylogenetically uninformative. The measures used are: the lengths of most parsimonious trees (Archie, 1989a, Faith & Cranston, 1991), and number of pairwise character (in)compatibilities (Alroy, 1994, Wilkinson, 1992b). For the compatibility-based randomization test, ordered multistate characters were recoded into their binary factors, and correlations within interdependent factors were preserved during the random permutations. The randomization tests also yielded two descriptive statistics: Archie's (1989b) homoplasy excess ratio (HER), and Wilkinson's (1997a) analogous incompatibility excess ratio (IER) ($=IER_1$ of Wilkinson, 1997b).

Hypothesis quality was assessed for all clades recovered in most parsimonious trees by bootstrapping (Felsenstein, 1985), with uninformative characters included or not, and by searching under topological constraints to determine Bremer support (or decay index), the extra steps needed to overturn (or collapse) a clade (Källersjö *et al.*, 1992).

Outgroups and rooting

Appropriate choice of outgroups, either for polarising characters or for rooting trees, requires knowledge of both the diversity of character states in the outgroup and the phylogenetic relations of the various outgroup taxa to the ingroup. Both kinds of information are currently lacking for the non-typhlonectid caecilians, which, as a heterogeneous whole, constitute the outgroup to the Typhlonectidae. In addition, detailed comparative anatomical information, as is now known for typhlonectids, is more sparse for non-typhlonectids. We adopted two strategies for rooting the typhlonectid tree. Firstly, we used *ancestor rooting* in which trees are rooted by a reconstructed hypothetical ancestor (of the Typhlonectidae) comprising the hypothesized primitive states of all characters that could be readily polarized through comparison with non-typhlonectid caecilians. All such polarity decisions are based on original observations of non-typhlonectid caecilians. We have examined representatives of all genera of the families Rhinatrematidae, Ichthyophiidae, Uraeotyphlidae and Scolecomorphidae and 15 genera of the Caeciliidae (*Boulengerula*,

Caecilia, Dermophis, Gegeneophis, Geotrypetes, Grandisonia, Herpele, Hypogeophis, Idiocranium, Indotyphlus, Microcaecilia, Parvicaecilia, Praslinia, Schistometopum, and *Siphonops*). Our observations on these outgroup taxa are less complete than for typhlonectids in most cases, particularly with respect to vertebral morphology, the lower jaws, and deeper cranial and glossal muscles. Outgroup material examined is listed in Nussbaum (1977, 1979, 1985) and Wilkinson & Nussbaum (1997). Where this outgroup is variable, assessments of polarity emphasize the conditions in caeciliids, which are assumed to be the most proximate outgroup (Nussbaum, 1979; Duellman & Trueb, 1986; Hillis, 1991; Hedges *et al.*, 1993; Wilkinson & Nussbaum, 1996); Wilkinson, 1997a,b). About one third of the characters were not polarized because the states in non-typhlonectid caecilians are too heterogeneous, too disparate, or insufficiently known.

Secondly, we included *Caecilia* as a single outgroup and used *outgroup rooting* to root the typhlonectid tree. *Caecilia* was recovered as the sister-group of *Typhlonectes* in Hedges *et al.*'s (1993) molecular phylogenetic study and is thus a good candidate for a proximate outgroup to the typhlonectids. Data for *Caecilia* is based on our original observations, supplemented by the observations of Taylor (1968, 1969). Using *Caecilia*, as opposed to the hypothetical ancestor, allows us to avoid missing entries for nine characters (where the more distant outgroups are variable), but introduces missing entries for seven characters in which the condition in *Caecilia* is unknown or inapplicable.

CHARACTERS

The character data are summarized below and in Table 2. We use two sets of symbols to distinguish between polar and non-polar characters. States of polar characters are denoted by integers, with 0 the assumed primitive condition and successively more derived character states as 1, 2, etc. States of non-polar characters are denoted by capital letters. All multistate characters are linearly ordered with the order represented by the numerical or alphabetic order of the character states (e.g. 0–1–2 etc., A–B–C etc.) and the ordering based on the criterion of intermediacy (Wilkinson, 1992a). We have included phylogenetically uninformative (singlet) characters in order to facilitate comparison of the amount of morphological evolution in different typhlonectid lineages. The data comprise 141 characters, of which 45 are non-polar and 68 are phylogenetically informative under parsimony.

External morphology

1. Body subcylindirical (0), or laterally compressed at least posteriorly (1). Most caecilians, including all non-typhlonectids, *Chthonerpeton* and *Nectocaecilia* have the assumed primitive condition. The derived condition is an obvious adaptation of the aquatic caecilians to their environment.

2. Head, collars, and anterior body disproportionately small (1), or not (0). With the exception of *Dermophis septentrionalis*, which is known only from the holotype and which may represent a teratology (Taylor, 1968), *Potomotyphlus* is unique among caecilians in having the assumed derived condition (Nussbaum & Wilkinson, 1989).

TABLE 2. Character data for typhlonectid taxa, an outgroup (*Caecilia*) and a hypothetical ancestor. Polar characters are represented by numerical codes, non-polar characters by alphabetic codes. Character states of the outgroup that are variable or inapplicable are indicated by 'V' and 'N' respectively and are treated as uncertain in the phylogenetic analyses. Missing entries are otherwise indicated by '?'. See text for additional details.

	Characters
Taxa	1111111111222222222233333333334444444445 1234567890123456789012345678901234567890
Ancestor	00?000?00?0000000000??0??0??0000??00??000000?0?00
Caecilia	00V000V00V00000N0000BA0NN0NNA0000V?00??000000V0?00
Chthonerpeton	00A000B00A0000000000AA0AB0AAA0010BB01AA000000A0A00
Nectocaecilia	00B000A00B1000021010BA0A0BAA0020AA01BA100000A0B00
Typhlonectes natans	10B111A01A1000122011BA0BA0ABB1021AA01BB102100A0B00
T. compressicauda	10B111B01A1000122011BA0AA0ABB1021AA01BB112100A0B00
Potomotyphlus	11B111A11B1000113112BA0AA0ABB2021AA01BB101210B1C10
Atretochoana	10B111B01A1111114212AB1?A1ACB3131BA10BB101321B0B01

	Characters
	1
Taxa	5555555555666666666677777777778888888888999999999 1234567890123456789012345678901234567890
Ancestor	0??0??00?0??00?0000?00??00000?0000???0???00???00
Caecilia	0VVONV?00?0??00?0000A00VA00000?0000??????00??A?00
Chthonerpeton	0AA0AAB01A0AA00B0000A00AB10101A0101BAA0ABB00BAAA00
Nectocaecilia	1AA0AAA01B1AA00A0000B00AA10101A0101BBB0ABA00BBAA01
Typhlonectes natans	0BA0ABA11B2AA00B0000A00AB20101B0101ABB1BAA11ABCB11
T. compressicauda	0AA0ABA11B2AA00B0000A10AB20101A0101ABB1BAA11ABCA11
Potomotyphlus	0AB1BBC00B2BB10C0010A01BB00010A0101ABB1AAA11ABBA11
Atretochoana	0AA0BBC00B1CB01C1111A00BB010?0C1010ABB1AAA11ABBA11

	Characters
Taxa	1111111111111111111111111111111111111111 0000000001111111111222222222233333333344 1234567890123456789012345678901234567801
Ancestor	?000?0??00?0?00000?0000000?0000000000000
Caecilia	V00N?0???00?0A00000A0000000B0?00V0??00000
Chthonerpeton	A000A0BB?1?A0B01000A0010000B0000000000000
Nectocaecilia	D111B0BBB00B0A00100A0000001B0101000000000
Typhlonectes natans	B111B0AAA00A0A00110A0001001B0201101000000
T. compressicauda	B111B0AAA00A00110A0001001B0201200000000
Potomotyphlus	C111B0ABB00A0A00100A0000001B0?01111011100
Atretochoana	C120B1CCB01A1B10101B1100111A1?1???1112111

There is considerable variation in head size within *Potomotyphlus*, some of which is ontogenetic. The smallest, and presumably youngest, specimen we have examined is in the collections of the Carnegie Museum (CM 2906, total length 118 mm, from Rio Negro, Brazil) and has relative head and body proportions similar to those of typhlonectids of comparable size.

3. Total length of largest adults less than (A), or greater than (B) 500 mm. The definition of the states of this character are to some extent arbitrary, but *Chthonerpeton* is unique among typhlonectids for its relatively small size (<400 mm) whereas all other

typhlonectids are known to exceed 590 mm. Two species of *Chthonerpeton*, *C. onorei* and *C. viviparum*, attain much greater lengths than the generotype (Nussbaum, 1986), approaching state B, and a more comprehensive phylogenetic analyses including these species might warrant re-evaluation of this character and recognition of additional character states. Outgroups are too varied to justify polarizing this character.

4. Dorsal 'fin' absent (0), or present (1). Aquatic larval caecilians (e.g. rhinatrematids and ichthyophiids) typically have a small caudal fin, but all adult non-typhlonectids, *Chthonerpeton*, and *Nectocaecilia* lack any trace of a fin and this is considered the primitive condition. Adult *Typhlonectes*, *Potomotyphlus*, and *Atretochoana* have a fleshy dorsal fold of skin that serves as a fin. Taylor (1968:231) noted in his diagnosis of the Typhlonectidae that "A fin remnant may be retained" suggesting homology between the fins of larval rhinatrematids and ichthyophiids and those of adult typhlonectids. However, our current understanding of caecilian phylogeny (Fig. 1) suggests that typhlonectids evolved from ancestors that, like *Chthonerpeton* and *Nectocaecilia*, do not have fins at any stage in their life cycles. Thus we do not consider the fin of adult typhlonectids to be homologous with the caudal fin of aquatic larval caecilians. There may be differences in the extent of the 'fin' among aquatic typhlonectid taxa, but fins are variably expressed in life, and variation in preserved specimens cannot be relied upon to provide meaningful character data for phylogenetic inference, nor for diagnosing species (Wilkinson, 1988, 1996a).

5. Females with broad and rounded (0), or more narrow and pointed (1) body termini. With the exception of caecilians with true tails (rhinatrematids, ichthyophiids, and uraeotyphlids), which are narrow and somewhat pointed in both sexes, female non-typhlonectids, *Chthonerpeton*, and *Nectocaecilia* have bluntly rounded body termini similar in shape to those of males, and this is assumed to be the primitive condition. Females of *Typhlonectes*, *Potomotyphlus*, and *Atretochoana* have distinctive narrow and somewhat pointed body termini, whereas males, as far as is known, have more bluntly rounded or expanded body termini (Gonçalves, 1977; Wilkinson, 1988, 1989).

6. Cloacal disk not bordered (0), or bordered (1) by fleshy folds in females. Non-typhlonectids, *Chthonerpeton*, and *Nectocaecilia* may have the cloacal disc more or less depressed, but it is never surrounded by loose and fleshy folds of skin, and this is considered the primitive condition (Wilkinson, 1989). Large females of *Typhlonectes*, *Potomotyphlus*, and *Atretochoana* have extensive fleshy skin flaps surrounding, and occasionally obscuring, the cloacal disc. Fleshy folds are absent in the Brasilia specimen of *A. eiselti*, but this is thought to be due to its poor physiological condition at the time of preservation (Wilkinson *et al.* 1998).

7. Four (A), or more (B) anterior cloacal denticulations. Potomotyphlus, *Nectocaecilia*, and *Typhlonectes natans* have four anterior cloacal denticulations, other typhlonectids have five or more, with some intraspecific variation evident (Wilkinson, 1996a, b; Wilkinson *et al.*, 1998). *Chthonerpeton* and *Nectocaecilia* tend to have more irregular patterns of cloacal denticulations, though all typhlonectids show some variation, and they are coded for their underlying pattern, ignoring minor variations such as partial asymmetric subdivision or fusion of denticulations. The condition in non-typhlonectids is highly variable both within and between species, and precludes the polarization of this character.

8. Cloacal disk subcircular (0), or with elongate anterior expansion (1). Potomotyphlus is

unique among caecilians in having the assumed derived condition of a 'key-hole-shaped' cloacal disc in which the anteromedial denticulations have an elongate medial border (Taylor, 1968; Nussbaum & Wilkinson, 1989).

9. Annuli demarcated by distinct annular grooves (0), or not (1). Non-typhlonectids, *Chthonerpeton*, and *Nectocaecilia*, have the assumed primitive condition. In contrast, *Typhlonectes*, *Potomotyphlus*, and *Atretochoana* have rather plastic body walls, and their annuli are often poorly indicated, and typically are not demarcated by a distinct groove, although the may be indicated by more or less distinct lines of glands and pigmentation that are probably homologous to the distinct annular grooves of other caecilians.

10. Annuli marked by purplish lines (B), or not (A). *Nectocaecilia* have distinctive purplish lines demarcating their annuli. Similar lines are present in most, but not all, *Potomotyphlus*, which is coded for its modal state. Outgroups, including *Caecilia*, are too varied to justify polarization of this character.

11. External nares ovate (0), or subtriangular (1). With the exception of some larval caecilians (Wilkinson, 1992c), all adult non-typhlonectids, *Chthonerpeton*, and *Nectocaecilia* have sub-circular or ovate external nares (horizontal axes about equal to or longer than their vertical), and this is considered the primitive condition (Wilkinson, 1989). Sub-triangular nares with the vertical axis longer than the horizontal are present in *Typhlonectes*, *Potomotyphlus*, and *Atretochoana*, all of which are believed to be aquatic, and their occurrence only in aquatic larvae of other species with terrestrial adults suggests this morphology is related to breathing at the surface of water.

12. External nares not or barely countersunk (0), or strongly countersunk (1). *Atretochoana* is unique among caecilians in having the assumed derived condition, in which the narial apertures lie at the base of a deep narial depression.

13. External nares small or moderate (0), or much enlarged (1). *Atretochoana* is unique among caecilians in having the assumed derived condition. The enlarged nostrils of this species may be associated with aquatic olfaction (Wilkinson & Nussbaum, 1997). Although this character is taxonomically correlated with character 12, there is no clear logical or biological connection between the size of the nares and their depression, that would suggest that the characters are not independent.

14. Eyes dorsolateral, not within well developed ocular depressions (0), or relatively dorsal and lying within strong ocular depressions (1). *Atretochoana* is unique among caecilians in having the assumed derived condition.

15. Tentacular apertures moderate (0), or small (1). Non-typhlonectids have larger tentacular apertures than typhlonectids. Among typhlonectids, the tentacular apertures of *Chthonerpeton* and *Nectocaecilia* are somewhat larger than those of *Typhlonectes*, *Atretochoana*, and *Potomotyphlus* and this is the assumed primitive condition. *Typhlonectes natans* do not protrude their tentacles (pers. obs.), and it is probable that they are non-protrusible in all aquatic caecilians (Wilkinson & Nussbaum, 1997). Comparable observations are not available for the semi-aquatic *Chthonerpeton* and *Nectocaecilia*, and it is not clear from gross morphology if their tentacles are protrusible.

16. Tentacular aperture approximately equidistant from eye and naris (0), closer but some distance behind naris (1), or immediately behind the naris (2). *Chthonerpeton* has the assumed primitive condition, *Nectocaecilia* and *Typhlonectes* the most derived, with *Potomotyphlus* and *Atretochoana* intermediate (Fig. 2). There is considerable variation in the position of the tentacular aperture among non-typhlonectids, but this does not include the assumed derived states (Wilkinson, 1989). The tentacular apertures of *Caecilia* are
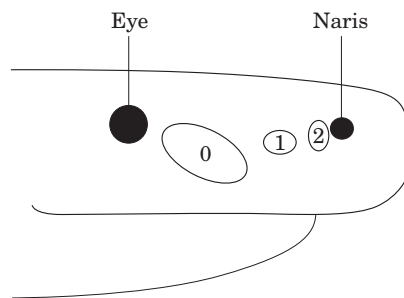
Figure 2. Diagrammatic lateral view of a caecilian head showing positions of the tentacular aperture relative to the eye and naris. Numbers correspond to the states of character 16. Not to scale.
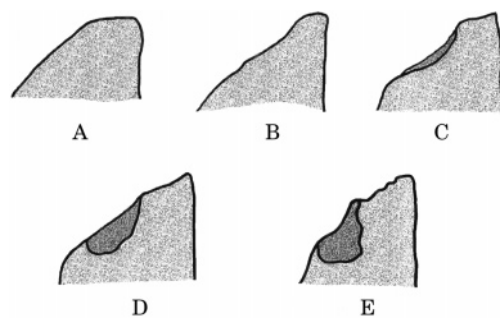

Figure 3. Semi-diagrammatic dorsal view of the left nasopremaxillae of typhlonectid caecilians showing differences in the emargination of the nasal foramen (dark shading). Not to scale. A, *Chthonerpeton*; B, *Nectocaecilia*; C, *Typhlonectes compressicauda*; D, *Potomotyphlus*; E, *Atretochoana*.

far forward, but are also well below, rather than behind the nares. Thus *Caecilia* is coded as inapplicable with respect to this character.

Buccal cavity

17. Choanal valves deep (0), moderately superficial (1), very superficial (2), level with the buccal mucosa (3), or projecting from choanae (4). Non-typhlonectids typically have choanal valves that are deeply recessed within the choanae, and this is considered the primitive condition, with succesively more superficial valves considered sucessively more derived (Wilkinson, 1989). Among typhlonectids, the deepest choanal valves are found in *Chthonerpeton* with increasingly superficial valves typifying *Nectocaecilia*, *Typhlonectes*, *Potomotyphlus*, and *Atretochoana*.

18. Choanae open (0), partially sealed (1), or completely sealed (2). Non-typhlonectids and most typhlonectids have the assumed primitive state of open choanae. *Potomotyphlus* and *Atretochoana* have partially and completely sealed choanae respectively (Nussbaum & Wilkinson, 1995).

Cranium

19. Anterior tips of nasopremaxillae broad and bluntly rounded (0), or narrow and more angulate (1) in dorsal and ventral views. Chthonerpeton and non-typhlonectids share the assumed primitive condition. In all other typhlonectids, the anterior tips of the nasopremaxillae are narrower and more angulate (Fig. 3).

20. Dorsal margins of nasal foramen straight (0), or weakly concave (1), or strongly concave (2). Chthonerpeton, Nectocaecilia, and non-typhlonectids have the assumed primitive condition. In other typhlonectids, the margins of the nasal foramen are emarginated by more extensive cupular cartilages, and this emargination is particularly pronounced in *Atretochoana* and *Potomotyphlus* (Fig. 3).

21. Tentacular groove roofed by bone (B), or not (A). In *Atretochoana* and *Chthonerpeton*, the tentacular groove is not covered with bone, and thus the orbit is open anteriorly. In other typhlonectids, medial and lateral portions of the maxillopalatine grow over the tentacular groove adjacent to the eye and fuse to form a bridge of bone that closes the orbit, with fusion sometimes incomplete in young specimens. In *Caecilia*, the tentacular groove is roofed with bone, but the condition is too variable among other non-typhlonectids to justify an assessment of the polarity of this character.

22. Tentacular groove entirely within maxillopalatine (B), or not (A). Atretochoana is the only typhlonectid in which the tentacular grooves are entirely within the maxillopalatine and do not enter or emarginate the nasopremaxillae. In *Caecilia*, the tentacular grooves emarginate the nasopremaxillae, but the extent of variation within other non-typhlonectids precludes the polarization of this character.

23. Orbit open (1), or closed posteriorly (0). Atretochoana is unique among caecilians in having the assumed derived condition (Nussbaum & Wilkinson, 1995).

24. Postorbital process of squamosal robust (A), or more slender and curved (B). Among typhlonectids, *Typhlonectes natans* generally has a more slender and curved postorbital squamosal process. *Atretochoana* lacks a postorbital squamosal process and is scored as equivocal (i.e. with a missing entry) with respect to the character states recognised here. Variation within the outgroups, and the inaplicability of comparisons to the conditions of stegokrotaphic caecilians (which lack a postorbital squamosal process), preclude the polarization of this character.

25. Maxillopalatine and squamosal contact to form dorsomedial border of the orbit (A), or are separated by orbital process of frontal (B). Among typhlonectids, an orbital frontal process interposing between the maxillopalatine and squamosal is found only in *Chthonerpeton*. *Caecilia* is coded as inapplicable with respect to this character because the orbit is entirely within the maxillopalatine. Other non-typhlonectids are too variable to justify polarizing this character.

26. Squamosal smooth (0), or bearing thickened and elevated anterolateral tuberosity (1). Atretochoana is unique among caecilians in having the assumed derived condition.

27. Posterolateral projection of frontal partially overlying adductor chamber anteriorly (A), or not (B). Nectocaecilia is unique among typhlonectids in having a small but distinct frontal 'shelf' over the anteriormost part of adductor chamber. This character is unpolarised because the conditions in outgroup taxa, many of which are stegokrotaphic and not directly comparable to typhlonectids, are insufficiently known.

28. Parietal sloping strongly (A), weakly (B), or not sloping (C) into adductor chamber. Chthonerpeton and *Nectocaecilia* have strongly sloping parietals. The slope is weaker in mature *Potomotyphlus* and *Typhlonectes* and non-existent in *Atretochoana*. This character is unpolarised because the conditions in outgroup taxa, many of which are stegokrotaphic and not directly comparable to typhlonectids, are insufficiently known.

29. Nasopremaxillary rostral projection less than (A), or greater than (B) 15% total length of skull along dorsal midline. Among typhlonectids, the weakest rostral projection of the snout is seen in *Chthonerpeton* and *Nectocaecilia*. This is comparable to the condition in *Caecilia* but variation within other non-typhlonectids is too great to justify polarizing this character.

30. *Dorsoventral compression of skull/head weak (0), moderate (1), strong (2), or very strong (3)*. The skulls of all caecilians are dorsoventrally compressed to some degree. Among typhlonectids, *Chthonerpeton* and *Nectocaecilia* have the weakest compression, similar to that of non-typhlonectids, and are considered to have the primitive condition. *Typhlonectes*, *Potomotyphlus*, and *Atretochoana* show increasing degrees of compression.

31. *Jaw articulation anterior (0), or posterior (1) to otic capsules*. *Atretochoana* is unique among caecilians in having the presumed derived condition (Nussbaum & Wilkinson, 1995). There are many differences in details of the suspension of *Atretochoana* and other caecilians which are subsumed under this character rather than treated as independent characters.

32. *Ventral process of squamosal absent (0), weakly developed (1), well developed (2), or expanded and plate-like (3)*. Non-typhlonectids lack a ventral process of the squamosal and its presence is considered a synapomorphy of the group. Among typhlonectids, the process is least well-developed in *Chthonerpeton*, and elaborated into a broad plate in *Atretochoana*.

33. *Lateral articulation of squamosal and maxillopalatine relatively broad (0), or short (1)*. Non-typhlonectids, *Chthonerpeton* and *Nectocaecilia* share the presumed primitive condition.

34. *Quadrate exposed dorsally above squamosal (A), or not (B)*. In *Atretochoana* and *Chthonerpeton*, little or none of the quadrate is exposed dorsally because it is covered by the squamosal. The contrasting condition in *Nectocaecilia*, *Typhlonectes*, and *Potomotyphlus* reflects a different orientation of the quadrate which also produces differences in the degree of overlap between the squamosal and quadrate ventrally. The latter variation is not treated as an independent character. Outgroups are too varied to justify polarizing this character.

35. *Articular facet of the quadrate curved (A), or relatively straight (B)*. In *Chthonerpeton*, the articular facet of the quadrate and the corresponding articular surface of the pseudangular is straighter than in other typhlonectids. Outgroups are too poorly known to justify polarizing this character.

36. *Stapes short, anteriorly directed, articulating with quadrate (0), or long, directed posteriorly and free of quadrate (1)*. *Atretochoana* is unique among living amphibians in having the assumed derived condition (Nussbaum & Wilkinson, 1995). There are other details of the shape of the stapes and its articulation with the *foramen ovalis* that also distinguish *Atretochoana* from other caecilians (Fig. 4). These differences are subsumed under this character rather than treated as independent characters.

37. *Quadratoparietal ligament present (1), or absent (0)*. Among typhlonectids, only *Atretochoana* lacks a quadratoparietal ligament. Quadratoparietal ligaments, are generally absent from non-typhlonectids, including other zygokrotaphic forms, suggesting that presence is derived.

38. *Supraotic shelf of os basale relatively weak (A), or strong (B)* Among typhlonectids, *Chthonerpeton* has a relatively weak supraotic shelf above the *foramen ovalis*. Outgroups are too poorly known to justify polarizing this character.

39. *Occipital condyles weakly (A), or more strongly (B) separated ventrally*. The occipital condyles are less widely separated ventrally in *Chthonerpeton* and *Nectocaecilia* than in other typhlonectids. Outgroups are too poorly known to justify polarizing this character.

40. *Long axis of occipital condyles oriented relatively more horizontally (0), or more vertically (1)*. The occipital condyles of typhlonectids are oriented more vertically (dorsolaterally) than in other caecilians. *Chthonerpeton* has less vertical and more horizontal
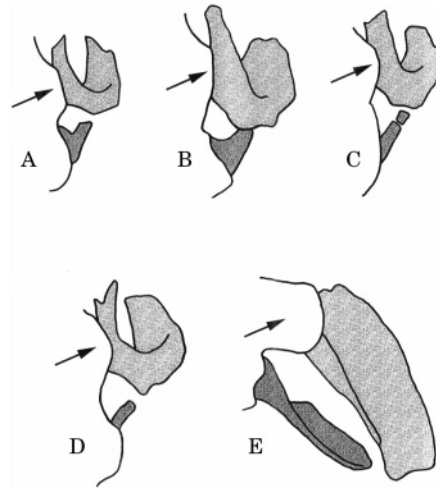
Figure 4. Semi-diagrammatic ventral views of the posterior left margin of the *os basale* (unshaded) of typhlonectid caecilians showing the relations of the basipterygoid process (arrow), quadrate (light shading) and stapes (dark shading). Not to scale. A, *Chthonerpeton*; B, *Nectocaecilia*; C, *Typhlonectes compressicauda*; D, *Potomotyphlus*; E, *Atretochoana*.

(lateral) occipital condyles than other typhlonectids, and this is considered primitive.

41. Ventral margin of foramen magnum visible from above (0), or obscured by dorsal margin (1). Non-typhlonectids typically have the ventral margin of the *foramen magnum* visible from above, and this is considered the primitive condition. The derived condition is found only in *Typhlonectes compressicauda*.

42. Posterior margin of otic capsules transverse (0), transverse proximal to occipital condyles and more oblique laterally (1), or oblique (2). With the exception of the scolecomorphids, which have the otic capsules otherwise modified by the loss of the stapes and *foramen ovalis* (Brand, 1956; Nussbaum, 1985), the otic-capsules of non-typhlonectids, *Chthonerpeton* and *Nectocaecilia* have the assumed primitive condition of transverse posterior margins. The capsules have derived oblique margins in *Typhlonectes* and an intermediate condition in *Potomotyphlus* and *Atretochoana*.

43. Parasphenoid region of palate elongate (0), moderately reduced (1), greatly reduced (2), or exceptionally reduced (3). Non typhlonectids, *Chthonerpeton*, and *Nectocaecilia* have relatively elongate parasphenoid regions similar to those of non-typhlonectids and this is considered the primitive condition. The size of this region is successively reduced in *Typhlonectes*, *Potomotyphlus*, and *Atretochoana*. This character subsumes correlated variation in the sizes of the choanae, narial plugs, and pterygoid processes of the quadrates; the proximity of the maxillopalatines to the basipterygoid processes of the *os basale*; and the position of the mediopalatinal canals and posterior tips of the vomers relative to the choanae; all of which are expected to covary with changes in skull proportions, and which are not treated as independent characters here.

44. Postchoanal process of maxillopalatine long, extending close to vomers (0), short, well separated from vomers (1), or absent (2). With the exception of rhinatrematids and scolecomorphids, non-typhlonectids have well developed postchoanal processes, and
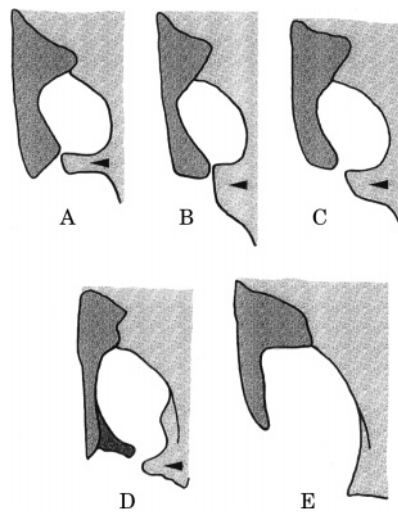
Figure 5. Semi-diagrammatic ventral views of the left choanae of typhlonectid caecilians showing variations in the postchoanal process of the maxillopalatine (arrowhead), the vomer (medium shading), and the pseudovomerine flange of *Potomotyphlus* (heaviest shading). Not to scale. A, *Chthonerpeton*; B, *Nectocaecilia*; C, *Typhlonectes compressicauda*; D, *Potomotyphlus*; E, *Atretochoana*.

this is considered the primitive condition for typhlonectids. The process is reduced in *Potomotyphlus* and absent in *Atretochoana* (Fig. 5).

45. *Basipterygoid processes of os basale short (0), or long (1)*. *Atretochoana* is unique among caecilians in having the assumed derived condition of expanded and relatively broad and elongate basipterygoid processes (Fig. 4).

46. *Vomers at posterior margin of choanae laterally expanded (A), or not (B)*. Among typhlonectids, only *Atretochoana* and *Potomotyphlus* lack posterolaterally expanded vomers (Fig. 5). Outgroups are too varied to justify polarizing this character.

47. *Vomers very narrow between choanae (1), or not (0)*. *Potomotyphlus* is unique among caecilians in having very slender vomers between the choanae (Fig. 5.).

48. *Medial sphenethmoid wall of choanae vertical (A), oblique posteriorly (B), or oblique anteriorly and posteriorly (C)*. *Chthonerpeton* (vertical) and *Potomotyphlus* (slanting throughout) have the extreme states, and all other typhlonectids the intermediate state of this character. Outgroups are too poorly known to justify polarizing this character.

49. *Pseudovomerine flanges of sphenethmoid present (1), or not (0)*. *Potomotyphlus* is unique among caecilians in having the assumed derived condition (Fig. 5).

50. *Choanae with squarish anteromedial corner (A), or not (B)*. Among typhlonectids, *Atretochoana* has a distinctive squarish anterolateral corner where the vomers expand laterally (Fig. 5). Outgroups are too poorly known to justify polarizing this character.

51. *Anterior expansion of vomers partly overlying anteromedial region of choanae (1), or not (0)*. *Nectocaecilia* is unique among caecilians in having the assumed derived state of a small anterolateral flange of the vomer extending across the choanal aperture.

52. *Maxillopalatine vomerine process weakly (A), or well (B) developed*. Among typhlonectids, the maxillopalatines of *Typhlonectes natans* are distinctive in typically having a well developed, elongate vomerine processes at the posterior margins of their articulations with the vomers. Outgroups, including *Caecilia*, are too varied to justify polarizing this character.

53. Maxillopalatine braces against posterior margin of vomer (A), or notches into vomer (B). Among typhlonectids notching of the maxillopalatine into the vomer is seen only in *Potomotyphlus* (Fig. 5). Outgroups are too poorly known to justify polarizing this character.

54. Maxillopalatine does not (0), or does (1) form a relatively large, ventral cheek surface outside mouth. The assumed derived condition is unique among caecilians to *Potomotyphlus*.

55. Suture between nasopremaxilla and maxillopalatine on palate transverse (A), or turning sharply anteriorly (B). *Atretochoana* and *Potomotyphlus* are the only typhlonectids having an angled suture between the nasopremaxillae and maxillopalatine that turns sharply anteriorly. Outgroups are too poorly known to justify polarizing this character.

56. Maxillary margin of mouth curved and concave (A), or relatively straight (B). *Nectocaecilia* and *Chthonerpeton* are the only typhlonectids with the margin of the mouth concave. Outgroups, including *Caecilia*, are too varied to justify polarizing this character.

57. Accessory foramen posterodorsal to antotic foramen, present (A), indicated by a notch in the main antotic foramen (B), or absent (C). *Typhlonectes* and *Nectocaecilia* have a small accessory foramen dorsal and posterior to the main antotic foramen, *Potomotyphlus* and *Atretochoana* have no accessory foramen, and *Chthonerpeton* is intermediate having the accessory foramen fused with the main foramen and indicated by a notch. Outgroups are too poorly known to justify polarizing this character.

58. Sphenethmoid canal lying in a relatively long (0), or short (1) groove. *Typhlonectes* are unique among caecilians in having the assumed derived condition.

59. Narial process of nasopremaxilla absent (0), or present (1). Non-typhlonectids, *Potomotyphlus* and *Atretochoana* have the assumed primitive condition.

Lower jaw

60. Medial wedge of pseudangular relatively elongate (A), or not (B). *Chthonerpeton* has a relatively elongate medial wedge of the pseudangular, and the pseudodentary anterior to it is correspondingly shorter than in other typhlonectids. Outgroups are too poorly known to justify polarizing this character.

61. Dorsal process of medial wedge of pseudangular absent (0), present but not projecting above dorsal margin of mandible (1), or well developed and projecting above dorsal margin of mandible (2). *Chthonerpeton* and non-typhlonectids have the assumed primitive condition, *Potomotyphlus* and *Typhlonectes* the most derived. *Nectocaecilia* and *Atretochoana* are intermediate but differ in details of the form of their dorsal processes (notching into the pseudodentary medially or laterally respectively) such that their presumed homology as represented by the coding of this character is somewhat suspect.

62. Anterior region of splenial ridge relatively short and posterior region long (A), anterior region relatively long and posterior region short (B), or anterior region very long posterior region almost non existent (C). Among typhlonectids, *Potomotyphlus* and *Atretochoana* have increasingly elongate anterior dentigerous regions of the splenial ridge and increasingly shorter adentigerous posterior regions. *Atretochoana* also has more than three times the number of splenial teeth than other typhlonectids (Taylor, 1968), and this variation is subsumed within this character. Outgroups are too poorly known to justify polarizing this character.

63. Subsplenial ridge relatively short (A), or long (B). Among typhlonectids, *Potomotyphlus* and *Atretochoana* have relatively long subsplenial ridges. Outgroups are too poorly known to justify polarizing this character.

64. Splenial fossa well defined anteriorly and splenial ridge elevated (1), or not (0). Potomotyphlus is unique among caecilian in having the assumed derived condition. As a result of the elevated anterior dentigerous region of the splenial ridge, the ridge and teeth are uniquely visible laterally above the dorsal margin of the mandible.

65. Mandibular symphysis highly flexible (1), or not (0). Atretochoana is unique among adult caecilians in having the assumed derived condition.

66. Difference in lengths of ventrolateral and dorsolateral processes of pseudangular small (A), intermediate (B), or great (C). The ventrolateral process of the pseudangular is shorter than the dorsolateral process, but the difference in length varies being weakest in *Nectocaecilia* and greatest in *Potomotyphlus* and *Atretochoana*. Outgroups are too poorly known to justify polarizing this character.

67. Postarticular dorsal tuberosity present on pseudangular laterally (1), or not (0). Atretochoana is unique among caecilians in having the assumed derived state.

68. Retroarticular process of pseudangular moderately (0), or very strongly inflected dorsally and mesially (1). Atretochoana is unique among caecilians in having the assumed derived condition. This is associated with several other features of the elongate lower jaws such as dorsal inflection of the pseudangulars beginning anterior to the articular condyles, and the retroarticular processes being very short (Nussbaum & Wilkinson, 1995). This variation is subsumed within this character rather than treated as independent characters.

69. Anterior tip of pseudodentary narrow and somewhat pointed (0), or blunt and somewhat squarish (1). Potomotyphlus and *Atretochoana* have the assumed derived state. Among non-typhlonectids, caeciliids have the assumed primitive state, but the condition is more variable in other non-typhlonectids.

Dentition

70. Vomeropalatine teeth not or barely visible (0), or clearly visible in lateral view (1). With the exception of *Microcaecilia supernumeraria* (Wilkinson, pers. obs), *Atretochoana* is unique among caecilians in having the assumed derived condition.

71. Palatinal tooth row in line with vomerine teeth anteriorly (A), or forming angle (B). Among typhlonectids, *Nectocaecilia* is distinctive in having the palatine and vomerine teeth series forming an angle where they meet. In *Caecilia*, the tooth rows are also in line, but other outgroups are too varied to justify polarising this character.

72. Adult tooth crowns with pointed (0), or spatulate (1) tips. All adult caecilians, except *Typhlonectes compressicauda*, have the assumed primitive condition of tooth crowns with pointed tips (Wilkinson, 1991).

73. Tooth crowns with conical (0), or more cylindrical shafts (1). All caecilians except *Potomotyphlus* have the assumed primitive condition of tooth crowns with a broad base and conical shaft that narrows distally and ends in a pointed or spatulate distal tip. In contrast, *Potomotyphlus* has a distinctive tooth crown morphology, with more conical shafts, narrow bases, and well developed lateral flanges (Wilkinson, 1991).

74. Less than (A), or more than (B) 25 dentary teeth. Among typhlonectids, *Potomotyphlus* and *Atretochoana* have the greatest number of dentary teeth. Outgroups are too varied to justify polarizing this character.

75. Dentary teeth hypertrophied (A), or not (B). Among typhlonectids, *Nectocaecilia* is exceptional for its large dentary teeth. The dentary teeth of *Caecilia* (and *Oscaecilia*)
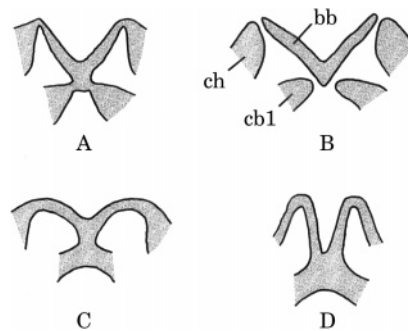
Figure 6. Semi-diagrammatic illustration of variation in the form of the basibranchials (bb) of typhlonectid caecilians and their relation to the ceratohyals (ch) and first ceratobranchials (cb1). Not to scale. A, *Chthonerpeton*; B, *Typhlonectes natans*; C, *Potomotyphlus*; D, *Atretochoana*.

are also relatively large, but other outgroups have small teeth precluding polarization of this character.

Glossal skeleton

76. Cartilage continuous across articulations of anterior glossal skeleton elements (0), weak (1), or interrupted (2). Non-typhlonectids, *Potomotyphlus*, and *Atretochoana* have the assumed primitive condition, *Typhlonectes* the most derived, and *Chthonerpeton* and *Nectocaecilia* are intermediate (Fig. 6). The transformations at each articulation covary taxonomically and are not considered as independent characters because it is likely that a single change in the regulation of chondrification could have produced parallel changes in each of these articulations.

77. Ceratohyals broad (0), or slender (1). *Atretochoana* is unique among caecilians in having the assumed derived condition of extremely long and slender ceratobranchials.

78. Junction of ceratohyal and basibranchial cartilages narrow and pointed (1), or not (0). *Potomotyphlus* and *Atretochoana* are similar to non-typhlonectids in having the assumed primitive condition (Fig. 6).

79. Tips of tracheal cartilages mineralised (1), or not (0). *Potomotyphlus* has the assumed derived state. Mineralization of glossal elements is rare in outgroups and has not been observed in any other typhlonectid. Tracheal cartilages appear to be absent from the vestigial trachea of *Atretochoana* which is coded as equivocal (with a missing entry) with respect to the character states recognized here.

80. Fused third and fourth ceratobranchials with strong medial process (1), or not (0). All typhlonectids except *Potomotyphlus* and *Atretochoana* have the assumed derived condition (Fig. 7).

81. Basibranchial cartilage Y-shaped (A), V-shaped (B), or unconnected (C). Among typhlonectids, the typical pattern is Y-shaped basibranchials, with *Atretochoana* having unconnected and *Typhlonectes natans* intermediate V-shaped basibranchials respectively (Fig. 6). Outgroups are too varied to justify polarizing this character.

82. Laryngeal fascia present (1), or not (0). *Atretochoana* is unique among caecilians in having the assumed derived condition of a tough fascia extending between the medial edges of the fused third and fourth ceratobranchials (Fig. 7).

83. Arytenoids far posterior (0), or close to (1) medial junction of fused third and fourth
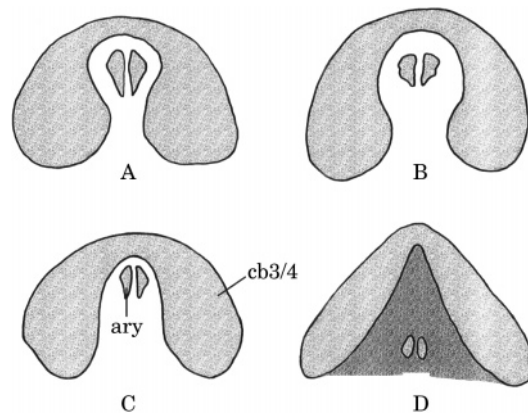
Figure 7. Semi-diagrammatic illustration of the fused third and fourth ceratobranchials (cb3/4) and arytenoids (ary) of typhlonectid caecilians. The heavier shading shows the position of the Laryngeal fascia in *Atretochoana*. Not to scale. A, *Chthonerpeton*; B, *Nectocaecilia*; C, *Potomotyphlus*; D, *Atretochoana*.

ceratobranchials. Non-typhlonectids (Nussbaum, 1979) and *Atretochoana* have the assumed primitive condition (Fig. 7).

84. Dorsal process of arytenoid cartilages present (0), or not (1). Atretochoana is unique among caecilians in having the assumed derived condition of simple rod-like arytenoid cartilages that are devoid of any dorsal processes (Fig. 7).

85. Fused third and fourth ceratobranchials greatly expanded (1), or not (0). Non-typhlonectids and *Atretochoana* share the assumed primitive condition (Fig. 7).

Vertebral column

With the exception of characters that are readily determined from radiographs, knowledge of the vertebral skeleton of non-typhlonectids is mostly insufficient to justify the polarisation of vertebral characters.

86. Neural arches of atlas separated anteroventrally (A), or fused (B). In typhlonectids the ventral margins of the neural arches of the atlas project anteriorly from the body of the centrum. *Chthonerpeton* and *Nectocaecilia* are the only typhlonectids in which these projecting anteroventral margins are fused together.

87. Dorsal anterior margin of neural arches of atlas roughly straight (A), or convex (B). Among typhlonectids, only the neural arches of the atlas of *Chthonerpeton* have a straightish anterior dorsal margin.

88. Posterior zygapophyses of atlas blunt and short (A), or elongate and pointed (B). Chthonerpeton differs from other typhlonectids in having short, blunt posterior zygapophyses on the atlas.

89. Neural arch of atlas without (A), or with (B) a pronounced constriction anterior to the posterior zygapophyses. Non-typhlonectids, *Chthonerpeton* and *Nectocaecilia* have the assumed primitive condition.

90. Posterolateral margins of neural arch of atlas and anterolateral margin of second vertebra oriented anterodorsally (A), or more vertically (B). Among typhlonectids, the more vertical orientation occurs only in *Typhlonectes*.

91. Parasphenes forming anterior projections from centrum of second vertebra (A), or not (B).

Among typhlonectids, only *Chthonerpeton* and *Nectocaecilia* lack parasphenes that project anteriorly from the main body of the centrum on the second vertebra.

92. Strong ventral ridge on parasphenes of trunk vertebrae isolating parapophyses on weak flange (A), or not (B). Chthonerpeton is the only typhlonectid lacking a ventral parasphene ridge on trunk vertebrae.

93. Neural arches bearing strong nuchal crests (0), or not (1). Most non-typhlonectids, *Chthonerpeton*, and *Nectocaecilia* have the assumed primitive condition. Nuchal crests may be present in other typhlonectids but they are weak and generally only found on the anteriormost vertebrae.

94. Vertebrae broad and short (0), or more elongate and narrow (1). Most non-typhlonectids, *Chthonerpeton*, and *Nectocaecilia* have the assumed primitive condition.

95. Diapophyses of second vertebra close to (A), or relatively distant from (B) anterior zygapophyses. Among typhlonectids, only *Chthonerpeton* and *Nectocaecilia* have a strong separation between the diapophyses and anterior zygapophyses.

96. Spinal nerve foramina present (A), or absent (B) from trunk vertebrae. Chthonerpeton is the only typhlonectid with spinal nerve foramina in the neural arches of trunk vertebrae posterior to the fourth vertebra.

97. Ribs broad with broad tips throughout trunk (A), broad proximally but with pointed tips (B), or narrow with pointed tips on all but anteriormost vertebrae (C). Broad ribs with broad tips are characteristic of *Chthonerpeton* and *Nectocaecilia*, narrow ribs and pointed tips are characteristic of *Typhlonectes*, and *Atretochoana* and *Potomotyphlus* are intermediate.

98. Capitulum relatively short (A), or elongate anteriorly (B). Typhlonectes natans is unique among typhlonectids in having the assumed derived condition.

99. Ribs relatively straight (0), or with a strong posterior flexure (1). Non-typhlonectids, *Chthonerpeton* and *Nectocaecilia* have the assumed primitive condition.

100. Less than four (0), or four or more (1) nuchal vertebrae. Most non-typhlonectids and *Chthonerpeton* have the assumed primitive condition (Wilkinson, 1989).

101. Less than 90 (A), 91–105 (B), 106–120 (C), or more than 120 (D) vertebrae. Among the typhlonectids included in the present analysis, *Chthonerpeton* and *Nectocaecilia* have the smallest and greatest numbers of vertebrae respectively, *Typhlonectes* have the intermediate state B, and *Atretochoana* and *Potomotyphlus* have intermediate state C. There is considerable interspecific variation within *Chthonerpeton*, that spans the entire range of character states as defined here (Taylor, 1968; Nussbaum, 1986; Nussbaum and Wilkinson, 1989). If *Chthonerpeton* is monophyletic, this would indicate homoplastic changes in vertebral numbers within the Typhlonectidae and suggest that the character is weak.

102. Three or fewer (0), or more than three (1) postcloacal vertebrae. With the exception of primitively tailed caecilians, non-typhlonectids and *Chthonerpeton* have the assumed primitive condition (Wilkinson, 1989). In fig. 3A of Wilkinson (1989), the presence of several postcloacal vertebrae was wrongly included among characters providing evidence for monophyly of the aquatic caecilians, but, as indicated in the text, supports the monophyly of the aquatic caecilians and *Nectocaecilia*.

Musculature

103. M. depressor mandibulae relatively short (0), moderately long (1), or very long (2). Most non-typhlonectids and *Chthonerpeton* have the assumed primitive condition. Only *Atretochoana* has an extremely elongate depressor (Fig. 8).
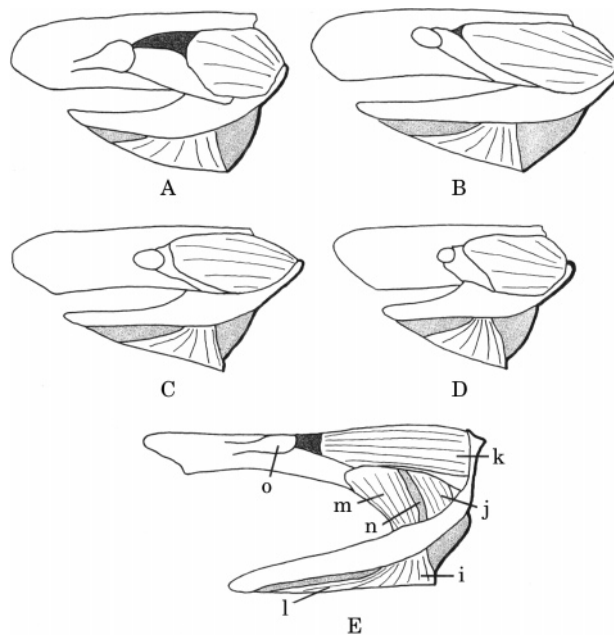
Figure 8. Semi-diagrammatic left lateral view of the superficial cranial muscles of typhlonectid caecilians. Dark shading indicates the temporal fascia. Not to scale. A, *Chthonerpeton*; B, *Nectocaecilia*; C, *Typhlonectes compressicauda*; D, *Potomotyphlus*; E, *Atretochoana*; i = *m. intermandibularis*; j = *m. adductor mandibulae externus minor*; k = *m. depressor mandibulae*; I = *pars symphalis* of the *m. intermandibularis*; n = maxillary branch of the trigemminal nerve; o = orbit.

104. Temporal fascia exposed (0), or mostly concealed by m. depressor mandibulae (1). Zygokrotaphic non-typhlonectids, *Chthonerpeton*, and *Atretochoana* have the assumed primitive condition (Fig. 8). Stegokrotaphic non-typhlonectids lack a temporal fascia but have short medial fibres of the *m. depressor mandibulae* that would not cover the temporal fascia if one were present in a position corresponding to that of typhlonectids.

105. Origin of m. adductor mandibulae comprising widely separated deep and superficial parts (A), or not (B). Among typhlonectids, only *Chthonerpeton* has this origin of this muscle in two widely separated parts. Outgroups are too poorly known to justify polarizing this character.

106. Pars superficialis of m. adductor mandibulae externus major present (1), or not (0). *Atretochoana* is unique among caecilian in having the assumed derived condition.

107. Anterior fibres of m. adductor mandibulae externus relatively vertical (A), more strongly oblique (B), or extremely oblique and nearly horizontal (C). Among typhlonectids, *Atretochoana* has the most horizontal, and *Typhlonectes* and *Potomotyphlus* the most vertical fibre orientation with *Nectocaecilia* and *Chthonerpeton* intermediate. Outgroups are too poorly known to justify polarizing this character.

108. Anterior fibres of m. adductor mandibulae externus minor relatively vertical (A), more strongly oblique (B), or extremely oblique (C). Among typhlonectids, the extremely oblique fibre orientation occurs only in *Atretochoana*, and the most vertical orientation in *Typhlonectes*, with other taxa intermediate. Outgroups are too poorly known to justify polarizing this character.

109. Anteriormost fibres of m. pseudotemporalis originating from surface of m. compressor

glandulae orbitalis (A), or not (B). The association of some fibres of the *m. pseudotemporalis* with the *m. compressor glandulae orbitalis* is found only in *Typhlonectes* among typhlonectids. *Chthonerpeton* lacks an *m. pseudotemporalis* and is coded as equivocal (with a missing entry) for this character. Outgroups are too poorly known to justify polarizing this character.

110. *M. pseudotemporalis present (0), or not (1). Chthonerpeton* and some non-typhlonectids have the assumed derived condition, but most non-typhlonectids have the assumed primitive condition.

111. *M. pseudotemporalis extremely fan-shaped and inserts on pseudangular via long tendon (1), or not extremely fan-shaped and inserting more directly (0). Atretochoana* is unique among caecilian in having the assumed derived condition. *Chthonerpeton* lacks an *m. pseudotemporalis* and is coded as equivocal (with a missing entry) for this character.

112. *M. levator quadrati present (A) or not (B). Nectocaecilia* is the only typhlonectid with an *m. levator quadrati*. Differences in the disposition of the muscle in *Nectocaecilia* caste some doubt on its homology to the muscle of the same name in non-typhlonectids. Outgroups are too poorly known, and those that are better known are too varied, to justify polarizing this character.

113. *Pars stapedialis of m. pterygoideus present (1), or not (0). Atretochoana* is unique among caecilians in having the assumed derived condition (Nussbaum & Wilkinson, 1995).

114. *Dorsal margin of m. interhyoideus posterior free (A), or not (B).* The dorsal margin of the *m. interhyoideus posterior* is not free in *Atretochoana* and *Chthonerpeton* where it forms part of the origin. This character subsumes variation in the visibility of the posterodorsal part of the *m. cephalodorsosubpharyngeus*, which is obscured by the *m. interhyoideus posterior* in *Atretochoana* and *Chthonerpeton*. Outgroups are too varied to justify polarizing this character.

115. *M. interhyoideus posterior relatively thin (0), or thick (1). Atretochoana* is unique among caecilians in having the assumed derived condition.

116. *Main body of thymus covered by m. interhyoideus posterior (1), or not (0). Chthonerpeton* is unique among caecilians in having the assumed derived state.

117. *Pars posterosuperficialis of m. cephalodorsosubpharyngeus broad (0), or narrow (1).* This muscle is narrow in all typhlonectids except *Chthonerpeton*. It is broad in those non typhlonectids where it is present (e.g. *Caecilia*) and this is assumed to be the primitive condition.

118. *M. rectus cervicus with separate set of superficial oblique fibres (1), or not (0). Typhlonectes* are unique among caecilians in having the assumed derived condition.

119. *M. subhyoideus moderately (0), or relatively weakly (1) developed. Atretochoana* is unique among caecilians in having the assumed derived state.

120. *M. subarcualis I moderately (A), or weakly developed (B).* Among typhlonectids, only *Atretochoana* has a weakly developed and narrow *m. subarcualis I* that does not fill the space between the ceratohyals and first ceratobranchials. *Caecilia* has a well developed *m. subarcualis I* but other outgroups are too poorly known to justify polarizing this character.

121. *M. constrictor laryngis present (0), or not (1). Atretochoana* is unique among caecilians in having the assumed derived state.

122. *M. dilator laryngis well (0), or weakly (1) developed. Atretochoana* is unique among caecilians in having the assumed derived state.

123. *M. rectus lateralis present (0), or not (1). Chthonerpeton* is unique among caecilians in having the assumed derived state.

124. M. genioglossus composed of three discrete layers (1), or not (0). Typhlonectes appears to be unique among caecilians in having the assumed derived condition.

125. Left and right halves of m. genioglossus widely separated anteriorly (1), or not (0). Atretochoana is unique among caecilians in having the assumed derived state.

126. Discrete pars symphalis of m. intermandibularis present (1), or not (0). Atretochoana is unique among caecilians in having the assumed derived state (Fig. 8).

127. Origin of m. intermandibularis from mandible broad (0), or relatively narrow (1). Most non-typhlonectids and *Chthonerpeton* have the assumed primitive condition (Fig. 8).

128. Posteriormost fibres of each block of m. obliquus externus superficialis short (A), or as long as more anterior fibres (B). Among typhlonectids, only *Atretochoana* has short posterior fibres. *Caecilia* has long posterior fibres, but other outgroups are too poorly known to justify polarizing this character.

129. Anteriormost unit of hypapophyseal muscle composed of interdigitating pear-shaped bundles of fibres (1), or not (0). Atretochoana is unique among caecilians in having the assumed derived state.

Respiratory and circulatory systems

130. Embryonic gills lateral (0), dorsolateral (1), or dorsal (2). The gills of non-typhlonectids and *Chthonerpeton* are lateral, with those of either side widely separated, and this is assumed to be the primitive condition (Wilkinson, 1989). The gills of *Nectocaecilia* are dorsolateral and narrowly separated and those of *Typhlonectes* are dorsal and fused at their bases. Wake (1992) included *Potomotyphlus* in a list of viviparous caecilians on the basis of uncited reports of foetuses, however, as far as we are aware, foetuses and embryonic gills of *Potomotyphlus* and *Atretochoana* are as yet unknown.

131. Lungs present (0), or absent (1). Atretochoana is unique among caecilians in having the assumed derived state (Nussbaum & Wilkinson, 1995). The trachea of *Atretochoana* is also vestigial but this variation is subsumed here and not included as an independent character.

132. Right lung extending to close to posterior end of coelom (1), or not (0). Most non-typhlonectids and *Chthonerpeton* have the assumed primitive condition (Wilkinson, 1989). *Atretochoana* is coded as equivocal.

133. Left lung vestigial (0), well developed, extending close to or just past posterior tip of the liver (1), or very long, extending to close to posterior end of coelom (2). Most non-typhlonectids, *Chthonerpeton* and *Nectocaecilia* have the assumed primitive condition. The most derived condition is restricted to *Typhlonectes compressicauda* (Wilkinson, 1989). *Atretochoana* is coded as equivocal. Wilkinson's (1989) report of well developed left lungs in *Nectocaecilia* was an error.

134. Left lung sac-like and devoid of cartilages (1), or not (0). Potomotyphlus is unique among caecilians in having an expanded left lung that is believed to serve as a hydrostatic organ (Fuhrmann, 1914). *Atretochoana* is coded as equivocal.

135. Capillaries perforating body epidermis (1), or not (0). The assumed derived condition is seen only in *Atretochoana, Potomotyphlus*; and large *Typhlonectes natans*.

136. Capillaries indenting the epidermis of the head (1), or not (0). Atretochoana is unique among caecilians in having the assumed derived state.

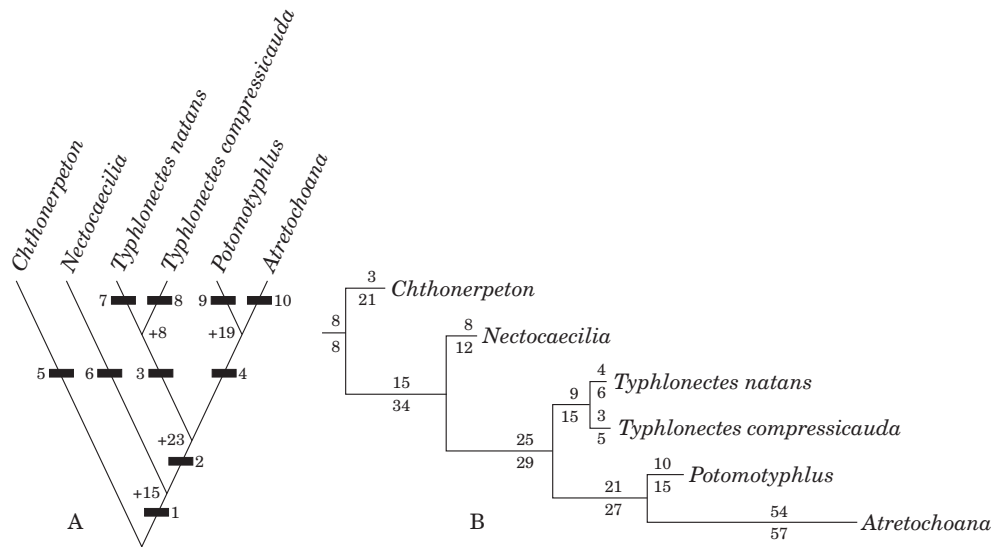*137. Truncus arteriosus septate (0), or not (1).* Most caecilians have septa within the

Figure 9. Most parsimonious trees for the Typhlonectidae based on the character data in Table 2 and ancestor rooting. A, cladogram with numbered edges and Bremer support values for all clades. B, phylogram with edge lengths determined by ACCTRAN character optimization and minimum and maximum possible edge lengths shown above and below the edges respectively.

truncus arteriosus. Among typhlonectids, the assumed derived state is shared by *Atretochoana* and *Potomotyphlus.*

138. Pulmonary and tracheal arteries and veins present and well developed (0), present but common pulmonary artery reduced (1), or all pulmonary and tracheal vessels absent (2). Atretochoana is unique among caecilians in having the assumed most derived state (Nussbaum & Wilkinson, 1995). *Potomotyphlus* has the intermediate state with all the vessels present but with a reduced pulmonary artery. The lack of pulmonary vessels is undoubtably correlated functionally with lunglessness.

139. Interatrial septum degenerate with large perforations (1), or not (0). Atretochoana and *Potomotyphlus* are unique among caecilians in sharing the assumed derived condition.

140. Interatrial septum does (0), or does not (1) closely approach atrioventricular aperture. Atretochoana is unique among caecilians in having the assumed derived state.

141. Sinuatrial aperture proximal to border of atrium and ventricle (0), or displaced anteriorly (1). Atretochoana is unique among caecilians in having the derived state (Wilkinson *et al.*, 1998).

RESULTS

Parsimony analysis using ancestor rooting yields a single most parsimonious tree with a length (L) of 193, a consistency index (CI) of 0.891, and retention index (RI) of 0.817 (L=127; CI=0.835 with uninformative characters excluded). Outgroup rooting yields an identical single most parsimonious tree that differs only slightly in associated tree statistics (L=194, CI=0.887, RI=0.805). This tree (Fig. 9A, B) supports Wilkinson's (1989) hypotheses that *Chthonerpeton* is the sister-group of all

other typhlonectids and that the fully aquatic caecilians (*Typhlonectes*, *Potomotyphlus*, and *Atretochoana*) are a clade, and Wilkinson & Nussbaum's (1997) interpretation of *Atretochoana* and *Potomotyphlus* as sister taxa. It also provides the first support from a numerical phylogenetic analysis for the monophyly of *Typhlonectes*.

With ancestor rooting, lengths of the 945 possible binary trees range from 193 to 285 with a mean of 264.78 and are highly significantly left skewed ($g_1 = -0.925$) suggesting that the data are significantly non-random. More direct randomization tests confirm this. The parsimony and pairwise (in)compatibility PTPs are 0.01 and 0.001 respectively, the lowest possible values for the number of randomly permuted data sets, allowing rejection of the null hypothesis that the data are no more congruent than would be expected of structurally comparable but random (and thus phylogenetically uninformative) data. HER and IER determined from these randomization tests are 0.725 and 0.614 respectively. Results using outgroup rooting (not shown) parallel those described above for ancestor rooting.

Passing matrix randomization tests represents a minimum requirement for data if they are to be used to infer phylogeny, but such randomization tests provide no indication of the relative support for particular clades. Support for clades in the typhlonectid tree appears to be good. All clades are supported by maximal bootstrap proportions (100%) whether uninformative characters are included in the resampling or not, and whether ancestor or outgroup rooting is used. Bremer support (Fig. 9A) is highest (23) for the aquatic caecilian clade (edge 2), and lowest (8) for *Typhlonectes* (edge 3). Thus these can be considered the best and worst supported clades respectively. Bremer support for the *Atretochoana–Potomotyphlus* clade (edge 4) is also impressive (19). Bremer support for the clade comprising the aquatic caecilians and *Nectocaecilia* (edge 1) is a little lower with outgroup rooting (11) than with ancestor rooting (15).

Unambiguous character optimizations for the typhlonectid tree, and maximum and minimum possible edge lengths are listed in Table 3 and shown in Figure 9B respectively. In this example, Bremer support values are strongly correlated with minimum edge lengths. Using ancestor rooting, 37 of the 193 character trans-formations required by this tree are ambiguous, either in their location on the tree or in the nature of one of their end states (with outgroup rooting 38 of 194 changes are ambiguous). Table 4 is a classification of the unambiguous changes according to character types. The partitioning into character types may be somewhat artificial as functional relationships occur between characters from different systems, but it provides a clear indication of differences between the morphological systems most affected by evolutionary changes occurring on different edges (see Discussion).

DISCUSSION

Our analyses provide an hypothesis of typhlonectid phylogenetic relationships that is both fully resolved and well supported. The randomization test results and tree length distribution statistics are consistent with the view that the data are well structured and phylogenetically informative. Judged by their bootstrap proportions, all hypothesized clades are well supported. Judged by their Bremer support, the aquatic caecilian clade (edge 2) is the best supported hypothesis of typhlonectid relationships, followed by the sister grouping of *Atretochoana* and *Potomotyphlus* (edge

TABLE 3. Unambiguous character state transitions on the edges most parsimonious typhlonectid phylogeny (Fig. 9A). An asterisk indicates homoplastic change. Bold and italic fonts indicate change contingent upon ancestor and outgroup rooting respectively

Edge	0→1	1→0	1→2	2→3	3→4	A→B	B→A	B→C	A→C
0	32 37* 59* 76* 78* 80* 83* 85*								
1	11 17 19 40 61 100 102 103 104* 117 127 **130** 132								
2	1 4 5 6 9 15 20 30 33 42 43 89 93 94 99 133		17 32			28 29 39 56 97	86 91 95		
3	58 118 124		42 76			90	108 109	97	
4	18 44 69 137 138 139	59* 76* 78* 80*	20 30 43	17		46 55 62 63 74		66	57
5	110 116 123					*114*	*21*		
6	51					10* 27 71 112	66 **75**		
7	98					24 52 81*			
8	41 72		133						
9	28 47 49 54 64 73					10* 53		48	
10	12 13 14 23 26 31 36 45 50 65 67 68 70 77 82 84 106 111 113 115 119 121 122 125 126 129 131 136 140 141	37* 83* 85* 104*	18 44 103 138	30 32 43	17	22 34* 114* 120	21* 128	28 62 108	81*

TABLE 4. Numbers of unambiguous character changes on the edges of the most parsimonious typhlonectid phylogeny (Fig. 9A) classified according to character type. E = external and buccal; C = cranial; L = lower jaw; D = dental; G = glossal; V = vertebral; M = muscular; R = respiratory and cardiovascular; T = total. Numbers in parentheses indicate differences due to outgroup rooting

Edge	E	C	L	D	G	V	M	R	T
					Character types				
0	–	3	–	–	5	–	–	–	8
1	2	2	1	–	–	2	4(3)	2(1)	13(11)
2	7	10	–	–	–	8	1	–	26
3	–	2	–	–	1	2	4	–	9
4	2	8	4	1	3	–	–	3	21
5	–	–(1)	–	–	–	–	3(4)	–	3(5)
6	1	2	1	2(1)	–	–	1	–	7(6)
7	–	2	–	–	1	1	–	–	4
8	–	1	–	1	–	–	–	1	3
9	3	5	1	1	–	–	–	–	10
10	5	15	4	1	6	–	16	5	52

4), and the basal position of *Chthonerpeton* (edge 1). Least well-supported, and thus least compelling is the conclusion that *Typhlonectes* is monophyletic (edge 3). We suggest that the phylogeny provides a sound basis for examining patterns of character evolution in the Typhlonectidae.

Phylogeny, morphology and ecology

The tropical distribution and secretive habits of caecilians have ensured that we are profoundly ignorant of most aspects of their ecology and natural history. We know that *Chthonerpeton* is semi-aquatic because it has been collected both in water and in terrestrial burrows, and that *Typhlonectes* and *Potomotyphlus* are fully aquatic and have only ever been collected in water. We know also that *Typhlonectes compressicauda* lives in aquatic mud burrows (Moddie, 1978) and that the *Typhlonectes natans* of the Lago de Maracaibo, Venezuela burrow in mud between submerged rocks at the lakeside (Wilkinson, pers. obs.). Our only knowledge of the ecology of *Nectocaecilia* is that it has been collected in a water-filled, artificial pit within a few days of its construction, and that this was not close to any natural water courses or bodies (S. Gorzula, pers. comm.) demonstrating both an affinity for water and ability to survive terrestrial conditions. Thus we consider *Nectocaecilia* to be probably semi-aquatic. The ecology of *Atretochoana* is unknown. Mapping ecological and distribution data onto the phylogeny suggests that (1) the ancestral typhlonectid was a semi-aquatic lowland form; (2) the transition to fully aquatic habits evolved in the line leading to the *Atretochoana–Potomotyphlus–Typhlonectes* clade (edge 2); and (3) the ancestral caecilian habit of burrowing persisted during the invasion of the aquatic environment.

The paucity of available ecological data means that interpretations of the evolution of the Typhlonectidae are frequently contingent upon inferences from morphology. For example, *Atretochoana* has been inferred to be aquatic on the basis of morphology. A fin and lateral body compression are not expected of terrestrial or semi-aquatic caecilians (Nussbaum & Wilkinson, 1995). To the extent that the phylogenetic position of *Atretochoana* is not dependent upon the evidence provided by fins and lateral body compression, our phylogenetic analysis provides additional support for

this inference (see below). Similarly, regarding the evolution of *Atretochoana*, Wilkinson & Nussbaum's (1997) conclusions were based on inferences from morphology, ecology, and phylogeny (the sister group relationship of *Potomotyphlus* and *Atretochoana* and the monophyly of the aquatic caecilians), and were necessarily speculative. The strong support for these phylogenetic relationships provided by the present numerical analyses helps to establish the robustness of one important link in the inferential chain. Much stronger tests would be provided by ecological data for *Atretochoana* and *Potomotyphlus*.

The origin of the Typhlonectidae

Character transformations associated with edge 0 are characters that support typhlonectid monophyly but are reversed within the Typhlonectidae. Most of these characters, (and three of the synapomorphies of the Typhlonectidae identified in Table 1) are glossal and represent elaborations of the size, musculature, and flexibility of the typhlonectid buccal pump. Typhlonectids are the only 'higher' caecilians with tracheal lungs, and, with the exception of *Atretochoana*, they have longer lungs than most caeciliids. Thus increased lung size and ventilatory capacity seems to be an important feature in the origin of typhlonectids and may have allowed ancestral typhlonectids to survive longer periods of submersion. The significance of other changes associated with the origin of the Typhlonectidae (such as embryonic gill fusion, quadratoparietal ligaments), particularly with respect to the hypothesised shift to a semi-aquatic ecology, are not obvious.

The phylogenetic position of Chthonerpeton

The large difference between the minimum and maximum possible lengths of edge 1 (the sister group relationship between *Chthonerpeton* and the *Nectocaecilia*–aquatic caecilian clade) and the line leading to *Chthonerpeton* (edge 5) is due in part to 12 non-polar characters in which *Chthonerpeton* differs from all other typhlonectids. These characters can be equally parsimoniously optimized as either autapomorphies of *Chthonerpeton* or synapomorphies of the other typhlonectids. Thus determination of outgroup conditions and character polarity may be expected to provide additional support for edge 5 or edge 1.

Under ancestor rooting, there are only three unambiguous autapomorphies of *Chthonerpeton* and all are muscular. Outgroup rooting identifies an additional muscular and a cranial synapomorphy. The most interesting unambiguous autapomorphy is the absence of the *m. rectus laterales*, which is expected to have important biomechanical consequences and perhaps other morphological correlates. The *m. rectus laterales* function as the most dorsal component of the external muscular sheath of all other caecilians. The loose association of the sheath and the deeper vertebral musculture and vertebral column facilitates, and may be an adaptation for locomotion and burrowing (Naylor & Nussbaum, 1980; O'Reilly, Ritter & Carrier, 1997). The unknown consequences of the reduction of the sheath in *Chthonerpeton* merit investigation. Five of the non-polar characters that could be autapomorphies of *Chthonerpeton* are vertebral (87, 88, 92, 96, 101), including changes in the atlas, low number of vertebrae, presence of spinal nerve foramina, and ridge development on

parasphenes. However, with the possible exceptions of low number of vertebrae and parasphene ridges, these features are unlikely to be related to the change in the external muscular sheath.

Under ancestor and outgroup rootings respectively, 13 or 11 unambiguous changes unite *Nectocaecilia* with the aquatic caecilians (edge 1). These unambiguous changes and the 12 additional potential synapomorphies come from all categories of characters except glossal and dental. The diversity of characters lends support to the phylogenetic hypothesis, but provides little evidence of any adaptation to a particular environment. Three synapomorphies (long right lung, more postcloacal vertebrae, and subtriangular external nares) may be aquatic adaptations suggesting that the last common ancestor of the *Nectocaecilia*–aquatic caecilian clade may have been more aquatic than the last common ancestor of all typhlonectids. The implication that *Nectocaecilia* is more aquatic than *Chthonerpeton* is a prediction that cannot be evaluated on the basis of the little available ecological information for these taxa. *Nectocaecilia* has either six or seven diverse cranial and dental autapomorphies (depending on the rooting) that provide little guidance on the factors influencing its evolution since it diverged from its last common ancestor with the aquatic caecilians.

The origin of fully aquatic caecilians

Some 26 unambiguous changes in external morphology, the cranium, vertebral column, and respiratory systems support the aquatic caecilian clade (edge 2). This provides strong support for the clade and suggests that becoming fully aquatic was a major transition in typhlonectid evolution. Some of the synapomorphies (e.g. fins, lateral body compression, smaller tentacular apertures, larger choanae, elongate left lung) are clearly aquatic adaptations, and some (e.g. flatter head, indistinct annuli, less robust vertebrae and slender curved ribs) may be associated with aquatic locomotion or burrowing in less challenging substrates. However, not all of the synapomorphies of the aquatic caecilians are obvious aquatic adaptations (e.g. size of cloacal disk, skin folds about the disc in females, size of the nasal, and shape of otic capsules).

The origin and evolution of Typhlonectes

Nine unambiguous cranial, glossal, vertebral, and muscular synapomorphies support the monophyly of *Typhlonectes* (edge 3). Three of these characters (separation of glossal cartilages, and increased complexity of the *m. rectus cervicus* and *m. genioglossus*) represent further elaboration of the buccal pump over that which characterized the origin of typhlonectids and the last common ancestor of the aquatic caecilians. This suggests that additional adaptation to an aquatic existence employing pulmonary gas exchange occurred in the ancestors of *Typhlonectes*. Both *Typhlonectes compressicauda* and *T. natans* have few unambiguous autapomorphies, just three and four respectively, underscoring the morphological similarity of these species and perhaps suggesting a relatively recent speciation. Indeed, (Dunn 1942) considered them to be subspecifically related. Distribution records suggest that these two species are allopatrically isolated, with *T. compressicauda* distributed throughout the Amazon and the Guianas, and *T. natans* restricted to Colombia and northwestern Venezuela.

Atretochoana and *Potomotyphlus*

Several elaborations of the glossal skeleton associated with the origin of the Typhlonectidae and interpreted as ventilatory adaptations for the ancestral semi-aquatic habits, are reversed either in edge 4, leading to the *Atretochoana–Potomotyphlus* clade, or in edge 10, the line leading to *Atretochoana*. Both *Atretochoana* and *Potomotyphlus* appear, on the basis of independent evidence, to be less dependent upon pulmonary respiration and buccal ventilation than other typhlonectids (Wilkinson & Nussbaum, 1997). *Atretochoana* is lungless. *Potomotyphlus* has the left lung modified into a hydrostatic organ, a reduced pulmonary arch, partly sealed choanal valves, and reduction in aortic and atrial septation. The phylogeny suggests that those morphological correlates of decreased reliance upon pulmonary respiration that are common to *Potomotyphlus* and *Atretochoana* are homologous, and that lunglessness in *Atretochoana* was presaged by changes in the ancestry of the *Atretochoana–Potomotyphlus* clade. There is an element of circularity here because these common features also provide evidence for the *Atretochoana–Potomotyphlus* clade and might plausibly be considered as consequences of a common but convergent trend. However, other characters that are not obviously correlated with respiratory changes, particularly changes in the cranium and lower jaw, also provide substantial evidence for evidence for the *Atretochoana–Potomotyphlus* clade. The diversity of this evidence strengthens our confidence that *Atretochoana* and *Potomotyphlus* are each other's closest known relatives, and that reduction in the importance of pulmonary respiration began before the divergence of these taxa (Wilkinson & Nussbaum, 1997).

Ten unambiguous changes, including changes in head size and the cloacal disc that make the external morphology of *Potomotyphlus* quite distinctive, are associated with the line leading to *Potomotyphlus* (edge 9). In the absence of direct ecological information, Wilkinson & Nussbaum (1997) argued that *Potomotyphlus* was unlikely to be a burrower because its relatively small head and development of the left lung into a large hydrostatic organ make it poorly suited to burrowing. We also suggested that abandonment of hypoxic mud burrows may have been the ecological step that favoured a reduction in pulmonary, and an increase in cutaneous, gas exchange in the common ancestry of *Potomotyphlus* and *Atretochoana*. Other than to assert the existence of this common ancestry, the phylogeny is uninformative with respect to this hypothesis because there are no pertinent ecological data for *Potomotyphlus* and *Atretochoana*.

We have discussed previously the evolutionary changes in the line leading to *Atretochoana* (edge 10), and how they may be correlated with the invasion of more lotic environments (Wilkinson & Nussbaum, 1997). The phylogeny has little significance for this scenario, and we will not discuss it in any detail here, but it does imply an unusually high rate of morphological evolution associated with the origin of lunglessness in *Atretochoana*. The phylogenetic analysis implies that some 52 unambiguous changes, of all character types, except vertebral, occurred on edge 10. The sheer number of changes serves to emphasize the degree of evolutionary divergence of *Atretochoana* from other caecilians including its closest relatives. As can be seen in the phylogram (Fig. 9B), this produces a marked asymmetry in edge lengths across the entire typhlonectid tree and suggests marked asymmetries in evolutionary rates. If the hypothesis that *Atretochoana* and *Potomotyphlus* are sister taxa is correct, it implies that these lineages have the same absolute age and that there has been a much higher rate of evolution (and over five times as many unambiguous

evolutionary changes) in the *Atretochoana* line (edge 10) than in the *Potomotyphlus* line (edge 9). Assuming that character sampling is unbiased, a simple binomial test allows us to reject the null hypothesis that each character change is equally likely to be associated with either edge (i.e. that there are equal rates of morphological evolution in each lineage). Similarly, the amount of evolution in the *Atretochoana* line (and thus the rate of evolution) is significantly greater than the total amount of evolution in the edges or combinations of edges leading to any of the more remote typhlonectid relatives of *Atretochoana*. It would clearly be interesting to determine whether the morphological evolution of *Atretochoana* is coupled with enhanced rates of molecular evolution, or whether its extraordinary morphological evolution is essentially de-coupled from the evolution of 'housekeeping' genes. The main limitation to such investigation will be the availability of tissue samples for molecular systematic analyses. When described, *Atretochoana* was known only from a single specimen, with the imprecise type locality of 'South America' raising concerns that this taxon may be extinct. The discovery of a more recently collected second specimen (Wilkinson *et al*, 1998) improves the chances that *Atretochoana* is not extinct and that, with sufficient effort in the field, additional specimens will be found enabling molecular, physiological, and biomechanical studies of this radically divergent taxon.

Ecological shifts and phylogeny

Phylogenetic relationships within the Typhlonectidae now represent one of the best supported parts of the generally poorly resolved caecilian phylogenetic tree. This is due, at least in part, to the more detailed study that this group has received, but it may also reflect the ecological history of the group. Quality of phylogenetic hypotheses is sometimes promoted by distinct ecological shifts within the history of a group (Arnold, 1990), which promote adaptation and distinct synapomorphies. In the present case, the best supported clade is associated with the major transition from semi- to fully aquatic lifestyles, and the longest edge is associated with the complete transition from pulmonary to cutaneous respiration. In contrast, there is no clear evidence of any major ecological shift in the origin of *Typhlonectes*, which is the least well supported clade. The *Potomotyphlus–Atretochoana* clade is also well supported, suggesting a major ecological shift, associated with the decreased import-ance of pulmonary gas exchange, in their common ancestor. This is consistent with Wilkinson & Nussbaum's (1997) suggestion of a shift out of mud burrows, but we await the appropriate ecological observations to be able to test this hypothesis.

formatted disk or can be downloaded from http://www.bio.bris.ac.uk/research/
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