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Biogeography and evolution of body size and life history of African frogs: Phylogeny of squeakers (*Arthroleptis*) and long-fingered frogs (*Cardioglossa*) estimated from mitochondrial data

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ABSTRACT

The evolutionary history of living African amphibians remains poorly understood. This study estimates the phylogeny within the frog genera *Arthroleptis* and *Cardioglossa* using approximately 2400 bases of mtDNA sequence data (12S, tRNA-Valine, and 16S genes) from half of the described species. Analyses are conducted using parsimony, maximum likelihood, and Bayesian methods. The effect of alignment on phylogeny estimation is explored by separately analyzing alignments generated with different gap costs and a consensus alignment. The consensus alignment results in species paraphyly, low nodal support, and incongruence with the results based on other alignments, which produced largely similar results. Most nodes in the phylogeny are highly supported, yet several topologies are inconsistent with previous hypotheses. The monophyly of *Cardioglossa* and of miniature species previously assigned to *Schoutedenella* was further examined using Templeton and Shimodaira–Hasegawa tests. *Cardioglossa* monophyly is rejected and *C. aureoli* is transferred to *Arthroleptis*. These tests do not reject *Schoutedenella* monophyly, but this hypothesis receives no support from non-parametric bootstrapping or Bayesian posterior probabilities. This phylogeny provides a framework for reconstructing historical biogeography and analyzing the evolution of body size and life history. Direct development and miniaturization appear at the base of *Arthroleptis* phylogeny concomitant with a range expansion from Central Africa to throughout most of sub-Saharan Africa.

1. Introduction

Three of the world's most studied animal species have their origins in sub-Saharan Africa (e.g., Strait and Wood, 1999; Evans et al., 2004; Keller, 2006). However, this interest in the fly *Drosophila melanogaster*, the frog *Xenopus laevis*, and ourselves, *Homo sapiens*, has not stimulated sufficient study of the diversity and diversification of African animals on a continent-wide scale. Four of the biodiversity hotspots defined as conservation priorities by Myers et al. (2000) are located in continental sub-Saharan Africa and there are numerous African regions with both many endemic species and threatened species (Burgess et al., 2004). The diverse fauna and flora of many African regions remain poorly documented and most regions have received little research attention from conservation biologists (e.g., Pimm, 2007). To better develop our understanding of African biodiversity, it is necessary to incorporate species from regions across sub-Saharan Africa and to integrate diverse forms of organismal data.

Throughout Africa's history, oscillating climatic regimes have caused the repeated expansion and contraction of different habitat types (e.g., Axelrod and Raven, 1978; Colyn et al., 1991; Stokes et al., 1997; Nichol, 1999). As these changes occur over broad time scales, the phylogenetic relationships of organisms that have diversified and dispersed across the continent can illuminate the historical connections between various regions and thus the history of the African continent. Such work moves a step beyond regional comparisons based on floristic or faunal surveys by explicitly including a historical component; both the order of cladogenic events and branch lengths can inform patterns of spatial and temporal diversification. By combining a phylogenetic approach with information from organismal biology, such as ecology and life history, we can test hypotheses regarding the processes of diversification that generated Africa's endemic biodiversity.

Among African vertebrates, amphibians exhibit remarkable patterns of geographic, reproductive, and anatomical diversity. Nonetheless, most African amphibians remain poorly studied in comparison to the diverse faunas of North and South America or Southeast Asia and the Malay Archipelago. Within sub-Saharan Africa, there are several regions of high anuran diversity (Stuart et al., 2004), a few of which correspond to previously recognized

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biodiversity hotspots, including the Upper Guinean Forests, the Cameroon Volcanic Line, and Eastern Arc Mountains (Brooks et al., 2002). Previous phylogenetic studies of anuran genera spanning regions of sub-Saharan Africa have found differing patterns of relationship among regions (Wieczorek et al., 2000, 2001; Vences et al., 2003a,b, 2004; Evans et al., 2004; Dawood and Uqubay, 2004; Idris, 2004; Measey et al., 2007). Other studies have been restricted to samples from particular geographic regions within Africa (e.g., Richards and Moore, 1996; Dawood et al., 2002) and thus unable to resolve the historical relationships between regions with high anuran diversity. Van der Meijden et al. (2004, 2005, 2007) and Bossuyt et al. (2006) provided the first phylogenetic evidence for two radiations of ranoid frogs that are endemic to sub-Saharan Africa. However, these studies did not focus on relationships between, and especially not within, the Africa genera. In general, there has been limited biogeographic study on the scale necessary to understand the history of African anuran clades across the continent.

Several frog clades found throughout sub-Saharan Africa exhibit a broad range of organismal diversity. Squeakers and long-fingered frogs are one such clade, which currently comprises two genera, *Arthroleptis* and *Cardioglossa* (Fig. 1; Frost et al., 2006). Together, these two genera have an ecological, altitudinal, and geographic range spanning much of the habitat accessible to amphibians across sub-Saharan Africa (Fig. 1). In general, these are terrestrial leaf-litter frogs that feed on a diverse range of terrestrial arthropods, including many ants and termites (Blackburn and Moreau, 2006). *Arthroleptis* and *Cardioglossa* exhibit a range of adult body sizes (13–54 mm snout-vent length; i.e., more than a fourfold difference), are characterized by two contrasting life histories, and exhibit the most reduced karyotype ($2n = 14$) known in anurans (Bogart and Tandy, 1981). All but one species of *Cardioglossa* are believed to have the ancestral anuran life history characterized by a free-living, feeding tadpole (Lamotte, 1961; Blackburn et al., 2008). *Cardioglossa aureoli* and all *Arthroleptis* are believed to have direct development in which metamorphosed froglets hatch directly from terrestrial eggs (e.g., Guibé and Lamotte, 1958; Lamotte and Perret, 1963; IUCN, 2006). Lastly, males of nearly all *Arthroleptis* and *Cardioglossa* share a common suite of secondary sexual characters. Males of most species exhibit spines lining the surface of the third and sometimes second and first fingers. In addition, males of many species have unusually elongated third fingers, which are unique among vertebrates and may be a synapomorphy uniting these genera (e.g., Noble, 1931). However, the function of these long fingers remains enigmatic (Amiet, 1989). The diversity of male secondary sexual structures in these genera are evolutionary labile and may be the product of changing sexual selection forces (Blackburn, in press).

The relationships among the smallest and largest *Arthroleptis* species have been contentious. Historically, most small *Arthroleptis* species were placed in the genus *Schoutedenella*, which was synonymized with *Arthroleptis* by Frost et al. (2006) based on the results of a preliminary phylogenetic analysis of this group. Some authors (Loveridge, 1957; Schmidt and Inger, 1959; Poynton, 1964, 1976, 2003) have argued that the small species are essentially "small versions" of the larger *Arthroleptis* and thus should be included in this genus. In contrast, study of morphological diversity in an evolutionary context suggested to other authors (e.g., Laurent, 1940, 1973, 1981; Laurent and Fabrezi, 1985) that the large (i.e., *Arthroleptis*) and small (i.e., *Schoutedenella*) species are not only each monophyletic but are also not sister taxa. Clearly, these are alternative phylogenetic hypotheses that cannot both be true. The latter hypothesis implies either more than one origin of direct development in this clade, or that larvae re-evolved in *Cardioglossa*. To date, there has been no attempt to resolve these discrepancies by combining a broad taxon sampling with modern phylogenetic methodology.

This study of *Arthroleptis* and *Cardioglossa* aims to establish, as thoroughly as possible, an understanding of the evolution and diversity of an anuran clade that is restricted to continental sub-Saharan Africa. There are three principal goals. The first is to investigate the monophyly, intergeneric, and interspecific relationships of *Arthroleptis* and *Cardioglossa*. As part of this analysis, I analyzed the effect of alignment on the inference of phylogenetic relationships. Second, given an estimate of phylogeny, I evaluated the evolution of body size and life history; these characters have played a central role in previous attempts to organize the taxonomic diversity of these genera. Third, I evaluated the biogeographic history

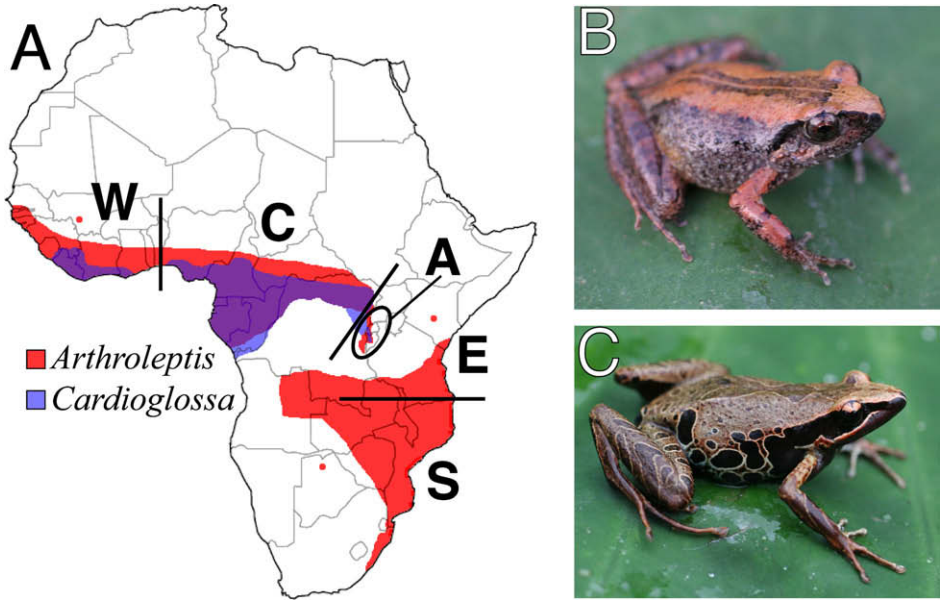

Fig. 1. (A) Geographic ranges of *Arthroleptis* (red) and *Cardioglossa* (blue); areas of overlap are depicted in purple. Geographic zones used in the reconstruction of historical biogeography are indicated (W: Western Africa; C: Central Africa; A: Albertine Rift Mountains; E: Eastern Africa; S: Southern Africa). (B) *Arthroleptis* "*poecilonotus*" in life (MCZ A-137982, subadult; Obang, Northwest Province, Cameroon). (C) *Cardioglossa melanogaster* in life (MCZ A-137905, adult female; Nsoung, Southwest Province, Cameroon).

within *Arthroleptis* and *Cardioglossa* to determine the spatial diversification of these genera across five geographic zones in sub-Saharan Africa. The results of the above analyses facilitate an interpretation of the relationship, if any, that exists between patterns of diversity of body size, life history, and biogeography.

2. Materials and methods

2.1. Taxon sampling

All species of *Arthroleptis* and *Cardioglossa* for which tissues were available in museum collections were included in this analysis (Appendix 1); the assignment of species to genera follows Frost (2007). Unfortunately, many species of *Arthroleptis* and *Cardioglossa* are represented only by type specimens, which cannot be damaged and, in any event, tissues from these are likely unusable in molecular phylogenetic studies. Fieldwork in Malawi (in 2005) and Cameroon (in 2004 and 2006) significantly supplemented the taxon sampling, including the discovery of new species (e.g., Blackburn, 2008; in this study). In recognizing taxa included in this study as new species, I adhere to the general lineage concept outlined by de Queiroz (1998) and use the combination of phylogenetic data and diagnosable morphological traits as criteria to recognize these species. In addition to data collected in this study, DNA sequence data were obtained from GenBank for two ingroup species (*Arthroleptis wahlbergii*: FJ151052; *Cardioglossa gratiosa*: DQ283176). The species included in this analysis represent all species groups of *Cardioglossa* (Amiet, 1981; Blackburn, 2008) and *Arthroleptis* species previously assigned to *Schoutedenella* (e.g., Frost, 2004), as well as the subgenera *Abroscaphus*, *Arthroleptulus*, and *Coracodichus* (Laurent, 1940, 1957, 1961); no tissue samples were available for *Arthroleptis adolfifriederici*, the type species of *Abroscaphus* (Laurent, 1940, 1957). Institutional abbreviations follow Leviton et al. (1985) with two additions: AMCC, Ambrose Monell Cryo Collection (American Museum of Natural History, New York); MM, Museums of Malawi, Blantyre.

I collected mitochondrial DNA sequence data (see below) from 112 specimens representing 26 ingroup species and six outgroup genera. The ingroup species represent nearly half of the described species diversity (26 of 54 species) of *Arthroleptis* and *Cardioglossa*: 15 of 37 species of *Arthroleptis*, 11 and 17 species of *Cardioglossa*, and at least three new species of *Arthroleptis*. Notably, the sampling of species previously assigned to *Schoutedenella* (Frost, 2004) is very low; only four of the 19 species could be included in the analysis (*A. schubotzi*, *A. sylvaticus*, *A. xenodactylus*, and *A. xenodactyloides*). However, this is par for the course when working on poorly known African vertebrates. Much of the diversity of these small species occurs in regions, such as eastern Democratic Republic of Congo, that have been essentially inaccessible to researchers for decades. Six species of *Arthroleptis* (*A. bivittatus*, *A. brevipes*, *A. carquejai*, *A. krokosua*, *A. milletihorsini*, and *A. stridens*) are known only from the holotype specimen, and fully eleven of the species previously assigned to *Schoutedenella* (*A. discodactylus*, *A. hematogaster*, *A. milletihorsini*, *A. mossoensis*, *A. phrynoides*, *A. pyrrhoscelis*, *A. spinalis*, *A. stridens*, *A. troglodytes*, *A. vercammeni*, and *A. zimmeri*) are known only from type specimens. In at least one case, the type and only specimen is lost (*A. milletihorsini*; Ohler, pers. comm.), and most of the diversity of small species was unavailable even for morphological study. However, as the species included span the range of geography, elevation, ecology, life history, and body size found in *Arthroleptis* and *Cardioglossa*, this sampling is adequate for addressing basic questions of phylogenetic relationships, character evolution, and biogeography.

Over the past seventy years, the close relationship of *Arthroleptis* and *Cardioglossa* has not been contested. Historically, most suprageneric taxonomies proposed in the era before molecular phylogenetics (Laurent, 1942, 1949, 1951, 1961, 1973, 1979; Dubois, 1981, 1983, 1984, 1985, 1986, 1992; Frost, 1985) reflected some close relationship of these genera to the Astylosternidae (sensu Dubois, 1992; *Astylosternus*, *Leptodactylodon*, *Nyctibates*, *Scotobleps*, and *Trichobatrachus*) and the Hyperoliidae (sensu Liem, 1970; Drewes, 1984; e.g., *Afrixalus*, *Hyperolius*, *Kassina*, and *Leptopelis*). Nearly all molecular phylogenetic studies have upheld the close relationship of these taxa to a clade comprising *Arthroleptis* (including *Schoutedenella*) and *Cardioglossa* (Vences et al., 2000, 2003a, 2003b; Biju and Bossuyt, 2003; van der Meijden et al., 2004, 2007; Scott, 2005; Bossuyt et al., 2006; Frost et al., 2006). In addition, most molecular phylogenetic studies have found that the above taxa are part of a larger African ranoid clade that includes the Brevicipitidae (sensu Frost et al., 2006; e.g., *Breviceps* and *Callulina*) and the monotypic Hemisotidae (*Hemisus*). As this study focuses on the relationships between and within *Arthroleptis* and *Cardioglossa*, rather than the higher-level relationships of African ranoid frogs, representative species of each of the above genera were included to serve only as outgroups in analyses. One species of each outgroup genus was included (Appendix 1) and, based on previous larger phylogenetic studies (e.g., Frost et al., 2006; Roelants et al., 2007), the outgroup genera *Hemisus*, *Breviceps*, and *Callulina* were used to root the tree.

To confirm or establish identification, voucher specimens for tissue samples were compared to the type specimens of nearly every species of *Arthroleptis* and *Cardioglossa* included in this analysis (see Appendix 1). The only voucher not examined is a specimen (MTSN 9178) of *Arthroleptis nikeae* that could not be obtained on loan. Relevant type material was not examined for only three species: *Arthroleptis stenodactylus*, *A. sylvaticus*, and *A. xenodactyloides*. However, specimens from the type localities were examined for both *A. sylvaticus* and *A. xenodactyloides*. The type material of *A. stenodactylus* is lost and presumably destroyed during the Second World War (Frost, 1985); other *A. stenodactylus* were examined from near the type locality in eastern Tanzania to confirm identification. Because populations often referred to *A. poecilonotus* likely represent multiple cryptic species (Rödel, 2000a; Rödel and Agyei, 2003; Rödel and Bangoura, 2004; Rödel et al., 2005), I refer to this taxon as *A.* "*poecilonotus*". Even when checked against the holotype, specimens of *Arthroleptis reichei* proved difficult to identify. There have been no previous suggestions that this is a species complex, but I cannot consider the identification of *A. reichei* specimens in this analysis definitive.

2.2. Molecular data

Genomic DNA was extracted from tissue samples using the Qiagen DNeasy Tissue Kit (Cat. No. 69506). A continuous stretch of mitochondrial DNA (~2400 bp) comprising genes encoding 12S and 16S ribosomal RNA and the intervening gene encoding transfer RNA for valine, was amplified using either two or four overlapping sets of the primer pairs of Darst and Cannatella (2004) as well as standard PCR conditions similar to those used by those authors. In a few cases, taxon specific primers were developed to amplify regions close to polynucleotide regions that inhibited amplification using the other primers (not shown). The PCR conditions used were standard and the thermal cycle profile was as follows: 94 °C (3 min); 35 cycles of 94 °C (30 s), 48 °C (30 s), 72 °C (1 min); 72 °C (7 min). The annealing temperature was sometimes modified (±3 °C) to improve the quality of the PCR product. PCR products were sequenced using cycle sequencing and the ABI Prism BigDye Terminator kit (v.3.1; Applied Biosystems). The thermal cycle profile for sequencing reactions was as follows: 25 cycles of 96 °C (10 s), 50 °C (5 s), 60 °C (4 min). Sequencing reactions were puri-

fied using the Performa DTR V3 96-Well Plates from Edge BioSystems (Cat. No. 63887).

DNA sequences of unequal length from 118 terminal taxa were aligned in ClustalX v.1.83.1 (Thompson et al., 1997). This dataset included 12 exemplars of related outgroup genera, sequences of six of which were obtained from GenBank (Appendix 1). Because a 3′ fragment of the 12S rRNA gene of *Cardioglossa aureoli* (CAS 230187) could not be amplified, the data for this taxon were included in the multiple alignment analysis in two fragments (475 bp of 12S and 1447 bp of the remaining sequence). Following alignment, these two sequences were merged, using MacClade v.4.06 (Maddison and Maddison, 2003), to form a single sequence in the phylogenetic analyses with intervening bp considered as missing data. To explore the effect of alignment on phylogeny estimation, three different multiple alignments were generated. These alignments differ in the value used for the gap cost parameter in multiple alignment mode in ClustalX. Gap costs of 10, 15, and 20 were used. The gap cost value of 15 is the default in ClustalX v.1.83.1; the default values were used for all other parameters. Each alignment was checked manually for individual sequences in which the alignment was obviously in error (i.e., large stretches of data consistently one nucleotide position off from the position that would minimize the change across sites). The alignment of only one specimen (*A. stenodactylus*, MCZ A-137021) was obviously in error in all three alignments despite having sequence essentially identical (<0.00% uncorrected pairwise divergence) to that of another specimen from the same locality (*A. stenodactylus*, MCZ A-137022); most nucleotide positions for MCZ A-137021 were one base pair off from those of MCZ A-137022. The correction of this alignment error was the only manual correction made. The three alignments were trimmed relative to a single reference sequence (*A.* "*poecilonotus*", MCZ A-136818) such that most taxa were represented for both the most 5′ and 3′ base pairs of the alignment, which correspond, respectively, to positions 2171–4486 of *Xenopus laevis* mitochondrial genome (GenBank NC-001573).

Subsequently, a consensus alignment was generated in which all nucleotide positions differing between the three alignments were excluded. This follows the exclusion alignment method advocated by Gatesy et al. (1993) that has been employed recently in studies using mitochondrial data (e.g., Leaché and McGuire, 2006; Wiens et al., 2007). In this way, I attempted to exclude characters for which the homology statements are uncertain (e.g., Gatesy et al., 1993). The results of phylogenetic analyses using the consensus alignment were then compared to those of the alignments generated using different gap costs. This comparison enabled me to determine what information, if any, is lost through the use of a consensus alignment in phylogeny estimation.

Mean genetic distances within and between *Arthroleptis* and *Carioglossa* were calculated in MEGA v.4.0.1 (Tamura et al., 2007) using the uncorrected pairwise method and the alignment generated using a gap cost of 15. Standard error was estimated using 500 bootstrap replicates.

2.3. Phylogenetic analysis

Estimates of phylogeny were obtained using parsimony and maximum likelihood (ML) criteria as well as within a Bayesian framework. Parsimony searches, using each of the three alignments generated with different gap costs, were performed in PAUP* v.4.0b10 (Swofford, 2003) using TBR branch swapping in a heuristic search with 500 random addition sequence replicates and gaps treated as missing data. A similar analysis was conducted for the consensus alignment. Support for branches in the parsimony topologies was evaluated using nonparametric bootstrapping with 1000 bootstrap replicates, using TBR and 10 random addition sequences per replicate, as implemented in Nona v.2.0 (Goloboff, 1993) through WinClada v.1.00.08 (Nixon, 2002). Branches present in $\geqslant$70% of the bootstrap trees were considered well-supported following Hillis and Bull (1993; but see Wilcox et al., 2002).

The ML and Bayesian analyses were conducted using all four alignments (i.e., the three alignments generated using different gap costs and the consensus alignment). ModelTest v.3.7 (Posada and Crandall, 1998) was used to determine the best-fit model of sequence evolution. A single partition was used for all analyses of these data. A ML search was conducted in GARLI V.0.95 (Zwickl, 2006) using the model selected by ModelTest, with all parameters estimated, and a neighbor-joining starting tree that was generated in PAUP. This search was conducted three times to make sure that GARLI was not stuck at a local optimum. Each GARLI analysis was terminated at least 3 million generations after the last topological improvement. In each analysis, the last improvement occurred within the first 500,000 generations; the topology was identical among replicates and $-\ln L$ was nearly identical among replicates. Three thousand bootstrap replicates of ML searches were performed using PHYML v.2.4.4 (Guindon and Gascuel, 2003) and a neighbor-joining tree as the starting topology. The PHYML bootstrap searches used the same model of evolution as in GARLI in which all parameters were estimated and six rate categories were used; as in the parsimony bootstrap analyses, topologies present in $\geqslant$70% of bootstrap trees were considered well-supported. To determine whether these data evolved in a manner significantly different from a molecular clock (i.e., constant rates), likelihood scores were calculated in PAUP for each of the phylogenies found from three replicate GARLI searches, for each of the four alignments, both with and without the molecular clock enforced and then evaluated using a likelihood ratio test (Felsenstein, 1981). The phylogenies from the three replicate GARLI analyses were each tested separately because while the topology and $-\ln L$ are essentially identical, branch lengths vary slightly.

Bayesian estimates of phylogeny were obtained using MrBayes v.3.1.1 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003) and the model of evolution selected by ModelTest. Bayesian analysis was run for five million generations, sampled every 1000 generations, using four chains, a temperature of 0.2, and default priors. Because MCMC searches within this Bayesian framework may sometimes be trapped at local optima, three additional replicate analyses were performed. Stationarity of the Bayesian searches was determined by plotting $-\ln L$ of each generation against generation time. The phylogeny, posterior probabilities, and parameter values were then estimated from the post burn-in trees from all four replicates. Topologies with posterior probabilities $\geqslant$95% were considered well-supported (Wilcox et al., 2002; but see Leaché and Reeder, 2002).

The effect of alignment on phylogeny estimation was determined in several ways. Topologies resulting from a particular analytical method, but using different alignments, were qualitatively compared to contrast specific differences between trees (i.e., taxon A is sister to taxon B in this tree but not that tree). The distances between parsimony (from PAUP) and ML (from GARLI) topologies resulting from analysis of different alignments were quantitatively compared using the partition metric of Penny and Hendy (1985) as implemented in PAUP ("symmetric difference method"). Lastly, the measures of nonparametric bootstrap support and Bayesian posterior probabilities were compared for analyses of each alignment. One might expect that the characters excluded from analysis in a consensus alignment would be those with the most homoplasy. Thus, excluding these from analysis would produce relatively higher support values. Alternatively, a consensus alignment may result in the exclusion of characters with autapomorphic states, especially if these are indels, which may cause uncertainty in the alignment of neighboring nucleotides.

2.4. Hypothesis testing

Support for alternative hypotheses of phylogenetic relationships was evaluated in two ways. All topological constraints were generated using MacClade 4.06 and, for the sake of simplicity, only the consensus alignment was used to test alternative hypotheses. First, tree lengths were obtained from parsimony analyses using unconstrained and constrained tree searches conducted in PAUP*. These alternative phylogenetic hypotheses were evaluated using a two-tailed Templeton test (a modification of the Wilcoxon signs ranked test; Templeton, 1983). Because both constrained and unconstrained parsimony tree searches produced multiple most parsimonious trees, all trees were evaluated to determine if the constrained trees were significantly different. The *P*-values that summarize these results are the most conservative possible. Second, the Shimodaira–Hasegawa (SH) test was used to test support for alternative phylogenetic hypotheses in a ML framework (Shimodaira and Hasegawa, 1999; Goldman et al., 2000). Using constrained tree searches in GARLI, maximum likelihood topologies for alternative phylogenetic hypotheses were generated, with each analysis terminating three million generations after the last topological improvement. These topologies were then tested against the ML topology obtained from unconstrained searches using the SH test and 10,000 RELL (resampling estimate of log-likelihood) replicates in PAUP. A significant result ($\alpha = 0.05$) from either the Templeton test or the SH test indicates that the alternative hypothesis is rejected in favor of the topology found by parsimony or ML analyses, respectively.

2.5. Biogeographic history

Historical biogeography was investigated using dispersal–vicariance analysis as implemented in DIVA v.1.1 (Ronquist, 1997). The topologies used for this analysis are the ML phylogenies generated by GARLI for each alignment; these trees were simplified such that all intraspecific relationships, except for those within *A. "poecilonotus"*, were excluded. Because the analyses of the consensus alignment failed to resolve the mitochondrial gene lineages of *C. oreas* and *C. manengouba* as monophyletic (see Section 3), these two species were each given a branch length two orders of magnitude less than other branches in this tree (i.e., 0.00001) to approximate the unresolved relationship between these species; branch lengths of 0.0 may interfere with ancestral state reconstructions of continuous characters (Revell, pers. comm.).

The geographic distribution of each species was obtained from the recent Global Amphibian Assessment (IUCN, 2006). These distributions were then coded as corresponding to five geographic zones (Fig. 1) based loosely on biotic regions recognized by Burgess et al. (2004). These zones are (1) Western Africa (west of, but not including, Nigeria; thus the Upper Guinean Forest Zone is included); (2) Central Africa (including the Lower Guinean Forest Zone, Cameroon Volcanic Line, and lowland forests of Congo River Basin); (3) Albertine Rift Mountains (including eastern Democratic Republic of Congo, Burundi, Rwanda, and Uganda); (4) Eastern Africa (including the Eastern Arc Mountains); (5) Southern Africa (countries south of, and including, Malawi and Mozambique). The geographic ranges assigned to outgroup taxa summarize the entire range of that genus. This is predicated on two assumptions. First, each outgroup genus is assumed to be monophyletic, and, second, the geographic range of extant taxa preserves some representation of their historical distribution.

2.6. Ancestral state reconstructions

Ancestral states for life history were reconstructed using unordered parsimony in Mesquite v.2.0 (Maddison and Maddison, 2007). Data on the life history of each ingroup species were gathered from the literature (Appendix 1). There are surprisingly few reliable published accounts of life history of *Arthroleptis* and *Cardioglossa*. In addition, ongoing research using DNA-barcoding identified tadpoles of several *Cardioglossa* species (Blackburn and Sterne, unpublished data). Life history data for outgroup taxa were taken from Lamotte and Zuber-Vogeli (1954), Amiet (1970, 1971, 1989), Schiøtz (1999), Müller et al. (2007) and my own unpublished data. With the exception of *Leptopelis*, the life history of each outgroup genus is believed to be invariable. Because the only possible direct-developing *Leptopelis* (*L. brevirostris*) is nested within the rest of *Leptopelis* diversity (Idris, 2004), I consider the ancestral life history of *Leptopelis* to have been likely characterized by a tadpole (e.g., Schiøtz, 1999).

The evolution of body size, a continuous character, was reconstructed using weighted squared-change parsimony in Mesquite. Snout-vent length was measured (±0.1 mm) from museum specimens using digital calipers. Maximum body size for each ingroup species was estimated by using the largest snout-vent length (SVL) measured for a species (Appendix 2), regardless of whether that specimen is male or female. This approach has two advantages: (1) it approximates the upper bounds of SVL for each species, and (2) it enables the inclusion of both male and female specimens. Because of error associated with different sampling intensity for each species, these values serve simply as a heuristic measure of maximum body size. Two species in this analysis, *Arthroleptis* sp. nov. 2 and *Arthroleptis* sp. nov. 3 are known only from juvenile specimens, and thus body size data are coded as missing for these species. Ancestral states at nodes immediately ancestral to these two species are not reconstructed. The topologies used for ancestral state reconstruction are the same ML phylogenies used in the above biogeographic analyses.

Body size of outgroup taxa was estimated as follows. For all genera except *Nyctibates*, the maximum snout-vent length was obtained for each species treated in key taxonomic literature (*Afrixalus*: Schiøtz, 1999; *Astylosternus*: Amiet, 1977; *Breviceps*: Channing, 2001, *B. fichus*: Channing and Minter, 2004; *Callulina*: de Sá et al., 2004; *Hemisus*: Laurent, 1972b, *H. barotseensis*: Channing, 2001; *Hyperolius*: Schiøtz, 1999; *Kassina*: Schiøtz, 1999; *Leptodactylodon*: Amiet, 1980, *L. blanci*: Ohler, 1999, *L. stevarti*: Rödel and Pauwels, 2003; *Leptopelis*: Schiøtz, 1999; *Scotobleps*: Perret, 1966; *Trichobatrachus*: Perret, 1966). Then using these maximum snout-vent length values, the median value was calculated for each genus (Appendix 1); results are very similar if mean values of maximum snout-vent lengths are used instead (data not shown). Because the phylogenetic relationships within each of these genera remain largely unclear and were not estimated in this analysis, using the median snout-vent length values is a conservative approach that estimates body size in these genera without skewing ancestral state reconstructions in outgroup taxa to extreme values. Maximum snout-vent length for *Nyctibates corrugatus* was measured directly from three MCZ specimens (MCZ A-136788–89, A-136939).

3. Results

3.1. Parsimony analyses: different gap costs vs. consensus alignment

Because of differences in the numbers of insertions and deletions inferred through multiple alignment using different gap costs, the final data matrices for the three alignments generated vary slightly in length (gap cost 10 [gc10]: 2548 bp; gap cost 15 [gc15]: 2543 bp; gap cost 20 [gc20]: 2533 bp). Homology statements differ for more than 700 characters between the three alignments; when these characters are removed, the resulting consensus alignment is 1800 bp in length. The results of heuristic

Table 1
Summary of parsimony (Pars.; PAUP), ML (GARLI), and Bayesian analyses

Alignment	Total Char.	# Constant	# Pars. inform.	Tree length	# Pars. trees	ML −lnL	Mean bayes −lnL ± standard deviation
Gap cost = 10	2548	936	1366	9601	22445	43229.73[a]	43387.17 ± 13.05
Gap cost = 15	2543	906	1371	9731	7219	43677.72	43835.84 ± 13.11
Gap cost = 20	2533	900	1382	9875	2900	44150.50	44307.33 ± 12.98
Consensus	1800	812	799	4743	624	23532.96	23709.79 ± 14.25

[a] Mean −lnL of three GARLI runs: 43229.44, 43229.88, 43229.86.

parsimony searches using these four alignments are summarized in Table 1.

MP analysis of different alignments produced largely similar patterns of phylogenetic relationships among ingroup taxa (Fig. 2). The primary differences in topologies between analyses pertain to the relative placement of *Arthroleptis wahlbergii* and *Cardioglossa aureoli*. In all parsimony analyses, there is strong support for *A. wahlbergii* being more closely related to large *Arthroleptis* than to *C. aureoli*, *A. sylvaticus*, or *A. xenodactyloides*. However, in the parsimony analyses of the gc10 and gc15 alignments, *A. wahlbergii* is the sister taxon to the remaining large *Arthroleptis*, whereas in the analyses of the gc20 and consensus alignments, it is the sister species of *A. francei*. Similarly, in analyses of gc10, gc15, or consensus alignments, *C. aureoli* is either the sister taxon to or nested within *Arthroleptis*, whereas analysis of the gc20 alignment places it as the sister taxon to *Arthroleptis* and other *Cardioglossa*. The topology found in analysis of the consensus alignment also differs within *Cardioglossa*. In contrast to the analyses of gc10, gc15, gc20 alignments in which the most parsimonious topology is (*C. elegans* (*C. leucomytax, C. occidentalis*) (*C. gratiosa* ((*C. manengouba*, *C. oreas*) *C. pulchra*))), the most parsimonious topology resulting from the consensus alignment is (*C. gratiosa* (*C. elegans* (*C. leucomystax*, *C. occidentalis*)), *C. pulchra* (*C. manengoba*, *C. oreas*)). The gc10, gc15, and gc20 parsimony analyses all produced reciprocal monophyly of *C. manengouba* and *C. oreas*, but the analysis of the consensus alignment produced paraphyly of *C. oreas* with respect to *C. manengouba*. Likewise, analysis of the gc10, gc15, and gc20 alignments resolve *C. gracilis* as the sister taxon to (*C. melanogaster*, *C. schioetzi*), whereas analysis of the consensus alignment located *C. gracilis* as the sister taxon to all other *Cardioglossa* (except *C. aureoli*). Lastly, parsimony analysis of the consensus alignment found *Arthroleptis* sp. nov. 3 to be the sister species of *C. aureoli*, whereas analysis of the other alignments all produce a topology in which *Arthroleptis* sp. nov. 3 is the sister taxon to (*A. xenodactylus* (*A. schubotzi*, *A. xenodactyloides*)). Several nodes found in the parsimony analyses of the gc10, gc15, gc20 alignments did not have $\geqslant$50% bootstrap support in the parsimony analysis of the consensus alignment (Fig. 2).

Nonparametric bootstrapping provides similar levels of high or low support among analyses of the three alignments generated using different gap costs (Fig. 2). There are only two ingroup nodes that have <70% bootstrap support in all three of these analyses (Fig. 2). More interestingly, there are two other ingroup nodes for which the support is <70% in one or two analyses but >70% in the remaining analyses (gray ovals in Fig. 2). In one of these cases, the support for the node uniting *Arthroleptis* sp. nov. 1, *Arthroleptis* sp. nov. 2, and *A. variabilis* ranges from 67% to 90%. In almost every case, bootstrap support from analysis of the consensus alignment is either the same or lower, sometimes much lower, than the support for the same topologies found in analysis of the other three alignments. There are only three cases in which the support for the consensus alignment exceeds that for the other alignments; in two cases, it is only marginally higher (consensus vs. gc10/gc15/gc20: 90 vs. 89/88/81; 82 vs. 75/78/77), whereas it is substantially higher for one node (90 vs. 72/75/78; see Fig. 2 for more details).

In four instances, parsimony analysis of gc10, gc15, and gc20 alignments found a particular interspecific topology that differed from that of the consensus alignment. In two cases, there is high bootstrap support from the gc10, gc15, and gc20 analyses. These topologies and their respective support from these latter analyses are as follows: (*C. gracilis* (*C. melanogaster*, *C. schioetzi*)), 94/83/93; ((*C. leucomystax*, *C. occidentalis*), (*C. gratiosa* (*C. pulchra* (*C. manengouba*, *C. oreas*)))), 84/84/89; (*C. gratiosa* (*C. pulchra* (*C. manengouba*, *C. oreas*))), 61/63/65; (*Arthroleptis* sp. nov. 3 (*A. xenodactylus* (*A. schubotzi*, *A. xenodactyloides*))), 63/64/73.

MP analysis of all four alignments resolved *Scotobleps* as the sister taxon to *Arthroleptis* and *Cardioglossa*. However, the support for this topology is low. Indeed, only the parsimony analysis of the consensus alignment produced bootstrap support >50%, but it was still low in this case (64%). All parsimony analyses found strong support (>70% bootstrap support) for three outgroup clades: (1) *Hemisus* (*Breviceps*, *Callulina*); (2) *Kassina* (*Afrixalus*, *Hyperolius*); and (3) ((*Astylosternus*, *Trichobatrachus*) (*Leptodactylodon*, *Nyctibates*)).

3.2. ML and Bayesian analyses

Both hierarchical likelihood ratio tests and the Akaike information criterion, as implemented in ModelTest, selected the GTR+I+Γ model as the best-fitting model for all four alignments. This model of sequence evolution was used in ML analyses using GARLI and PHYML as well as in Bayesian analyses. Estimated base frequencies were similar among all four alignments, but the estimated transition and transversion rates were consistently higher for the consensus alignment; parameter values estimated by Model Test are reported in Table 2. For each alignment, the hypothesis that the data are ultrametric is strongly rejected ($P < 0.0001$).

Each Bayesian analysis reached stability by 500,000 generations. Thus, to be conservative, the first one million generations (i.e., 1000 trees) from each analysis were excluded from computation of posterior probabilities. The mean −lnL and standard deviation for post burn-in trees are reported in Table 1.

The species-level topologies produced from Bayesian majority-rule consensus and ML analyses from both GARLI and PHYML are all similar. In the ML topology from the gc10, gc15, and gc20 GARLI analyses, every species is monophyletic (Fig. 3). This is not true for the ML topology produced from the analysis of the consensus alignment in which one species, *C. oreas*, is paraphyletic with respect to *C. manengouba* (see Section 4); this is similar to the results from the parsimony analyses. In general, the Bayesian posterior probabilities and ML bootstrap values are similar in that many of the same nodes have either low or high support in both analyses.

There are three major points of difference between the ML species-level topology and the consensus summary of post burn-in Bayesian trees produced from analyses of the four alignments. First, while there is strong support for a clade comprising *A. sylvaticus*, *A. xenodactylus*, and *A. xenodactyloides*, the relationship among these three species are not resolved definitively (Fig. 3). Second, a sister species relationship between *A. wahlbergii* and *A. francei* exhibits high support from Bayesian and ML analysis of the gc20

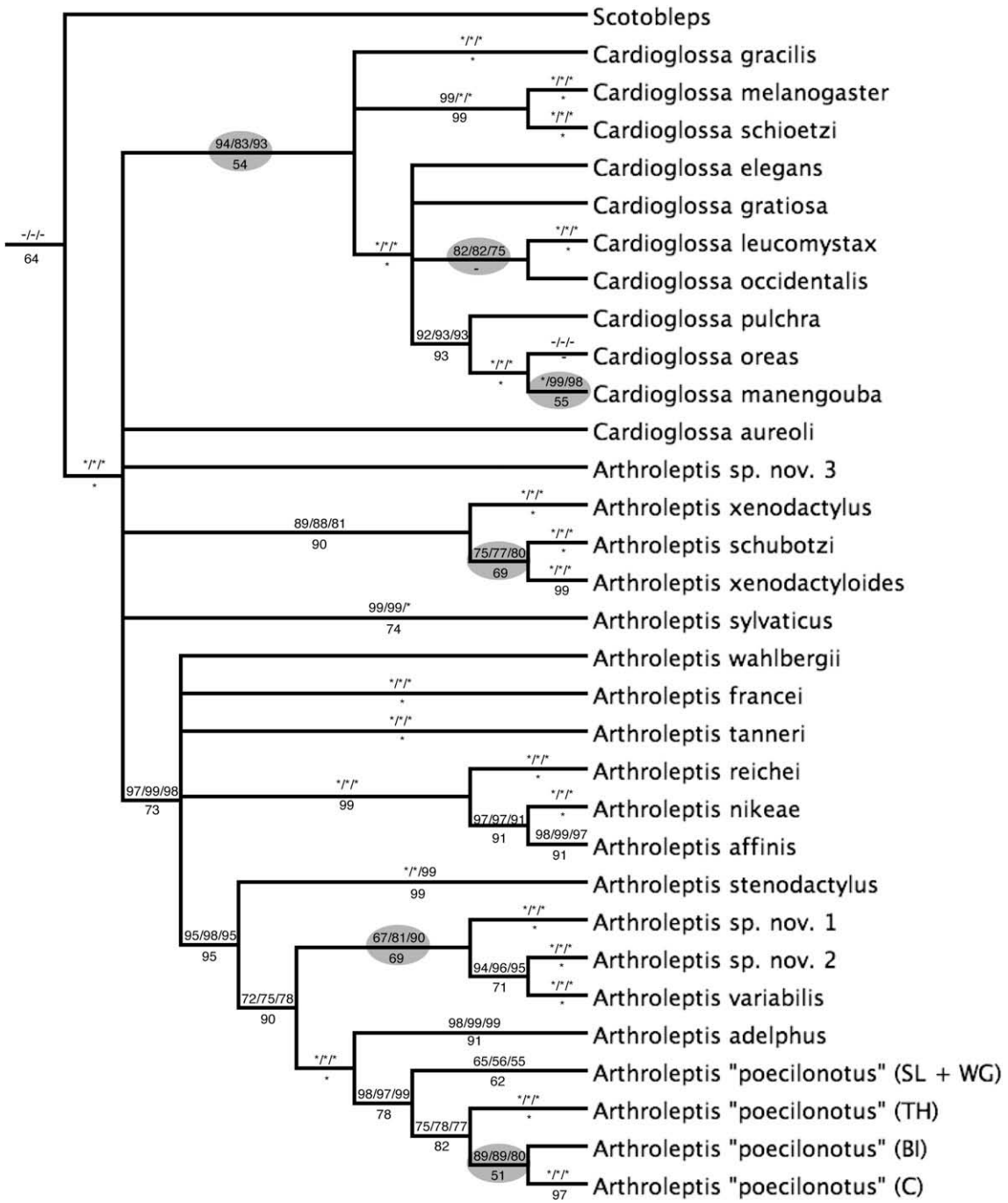

Fig. 2. Strict consensus topology of interspecific relationships based on parsimony analysis. Intraspecific relationships have been collapsed and only the nearest outgroup taxon is shown. Numbers above branches are nonparametric bootstrap proportions for parsimony analyses in which gap costs equal 10, 15, and 20, respectively; numbers below branches are nonparametric bootstrap proportions for parsimony analysis of the consensus alignment. Gray ovals indicate nodes for which nonparametric bootstrap proportions from different analyses span 70% support. Species previously assigned to *Schoutedenella* are indicated. 100% bootstrap support is indicated by " *"; less than 50% bootstrap support is indicated by "-".

Table 2
−Ln likelihood, AIC, and parameter values for the GTR+I+ Γ model selected by ModelTest v.3.7

Alignment	−Ln L	AIC	f(A)	f(C)	f(G)	f(T)	R[A-C]	R[A-G]	R[A-T]	R[C-G]	R[C-T]	I	α
Gap cost = 10	43276.54	86573.08	0.408	0.211	0.140	0.241	4.426	9.296	4.669	1.216	32.786	0.192	0.684
Gap cost = 15	43711.96	87443.92	0.412	0.211	0.136	0.241	4.076	8.968	4.382	1.345	30.500	0.177	0.664
Gap cost = 20	44193.21	88406.41	0.417	0.208	0.134	0.241	3.821	8.195	4.031	1.378	28.146	0.184	0.676
Consensus	23560.77	47141.54	0.376	0.218	0.167	0.240	5.354	15.418	6.622	1.672	47.905	0.214	0.557

and consensus alignments but not the gc10 and gc15 alignments. The relationship of these two species to *A. tanneri* and other large Eastern Arc *Arthroleptis* is also not well resolved. Several analyses (ML: gc10, gc15, gc20; Bayes: gc10, gc15) find that all of these spe-

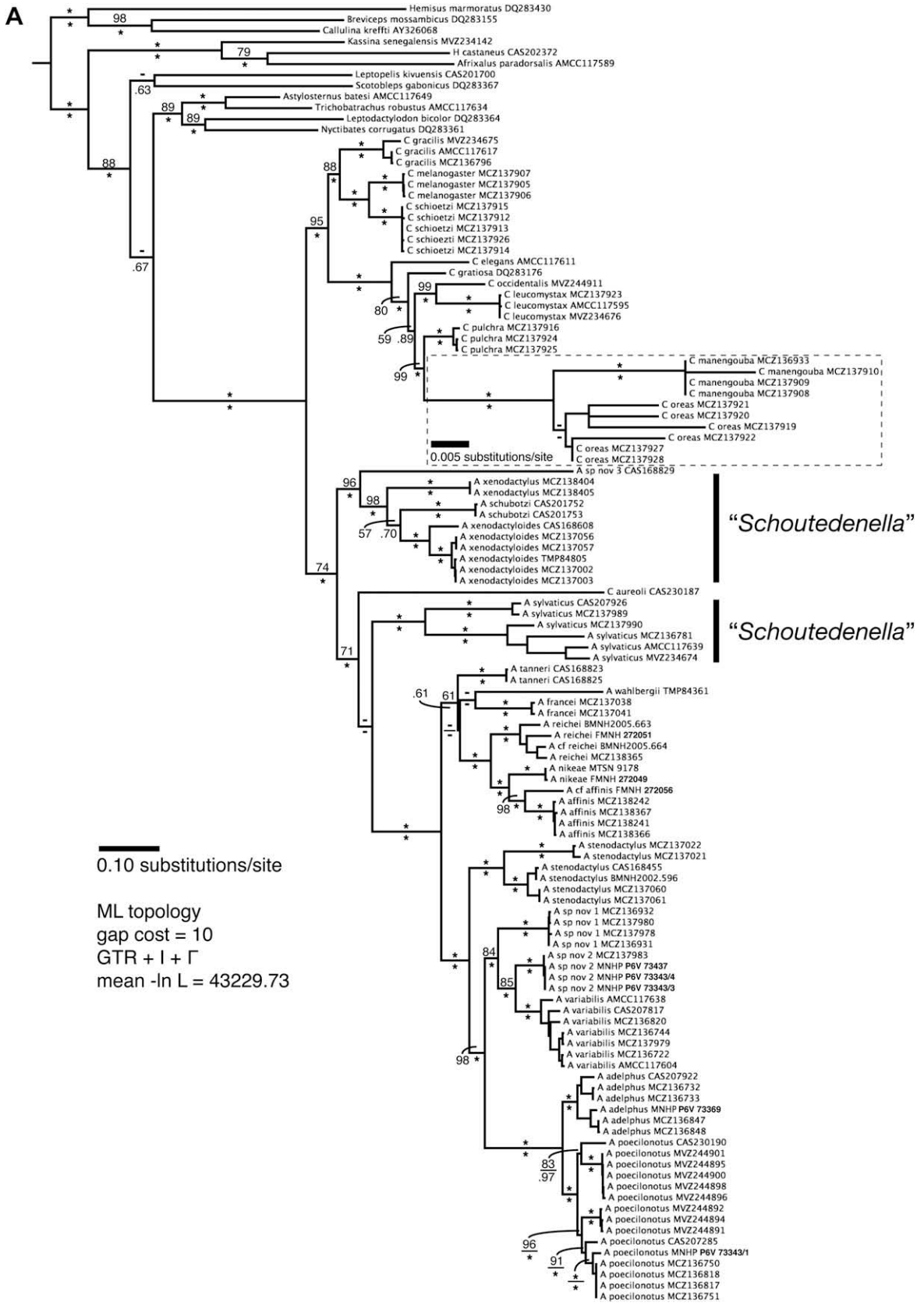

Fig. 3. Maximum likelihood phylograms from analysis of gap cost 10 (gc10; A), gc15 (B), gc20 (C), and consensus (D) alignments. Branches within "dotted line" box are magnified by 100× relative to other branches. Numbers above branches are nonparametric bootstrap proportions from PHYML analysis; numbers below branches are Bayesian posterior probabilities. 100% bootstrap support and 1.00 posterior probability are indicated by "∗"; less than 50% bootstrap support or 0.50 posterior probability are indicated by "−". Species previously assigned to *Schoutedenella* are indicated.

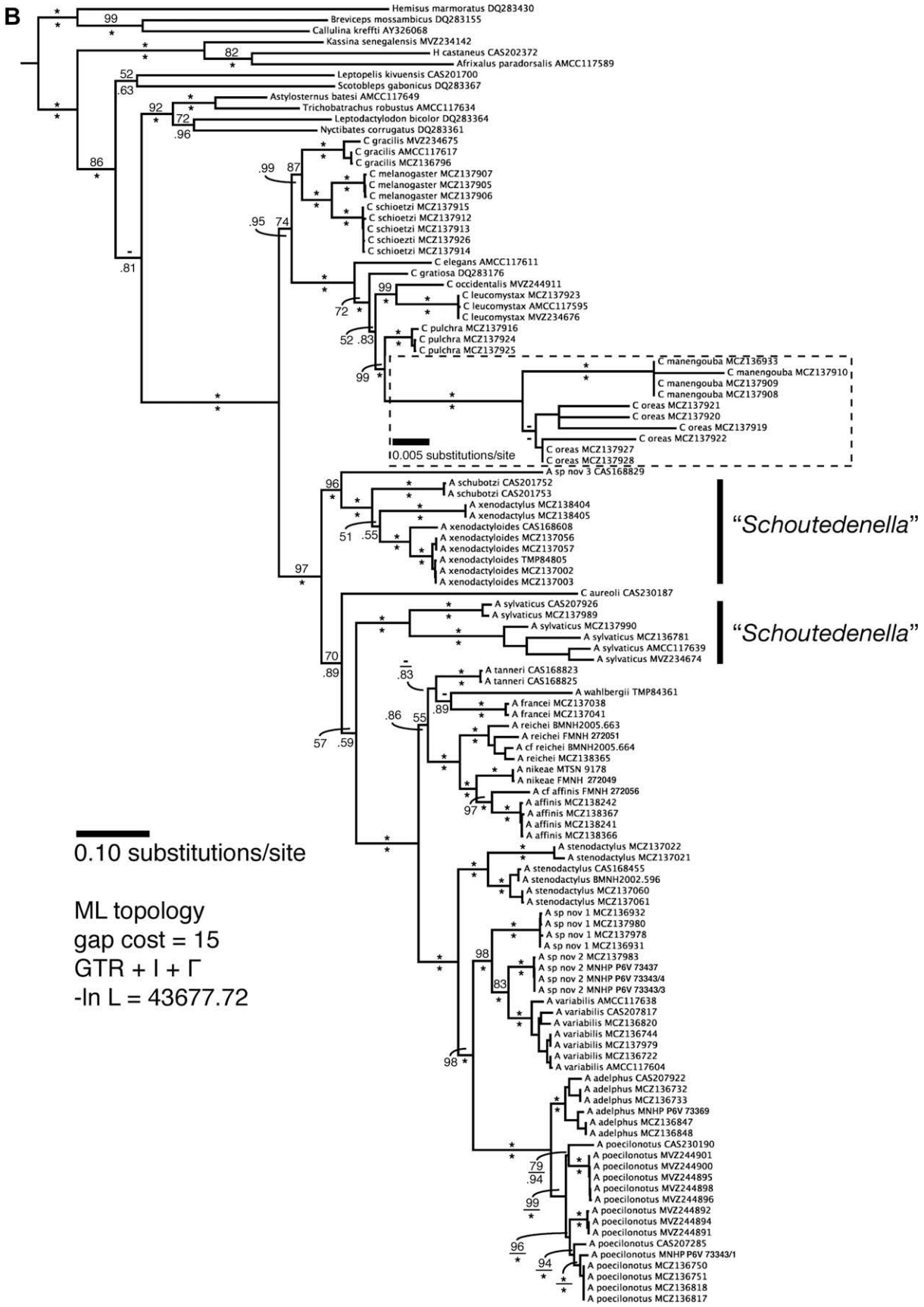

Fig. 3 (*continued*)

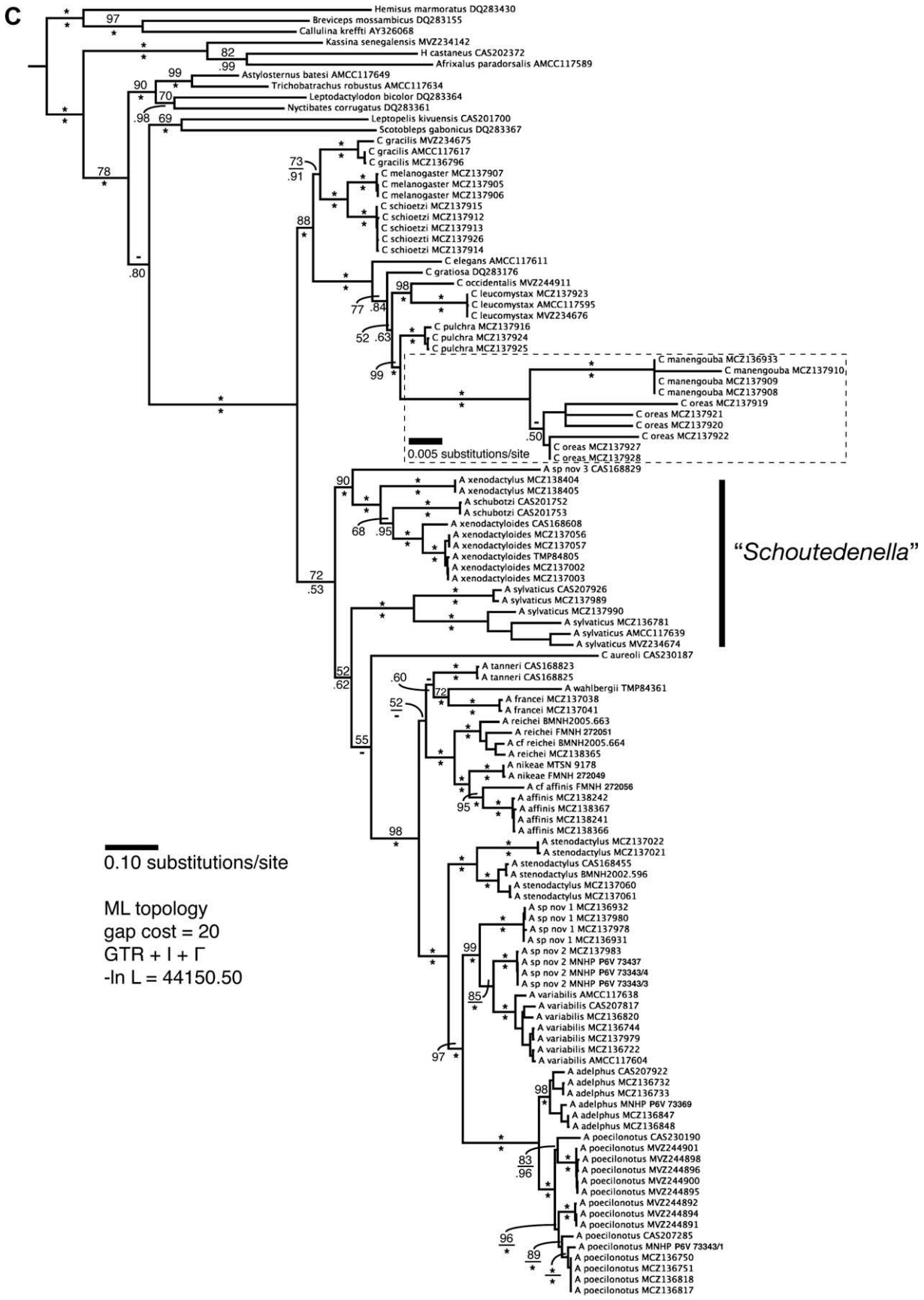

Fig. 3 (*continued*)

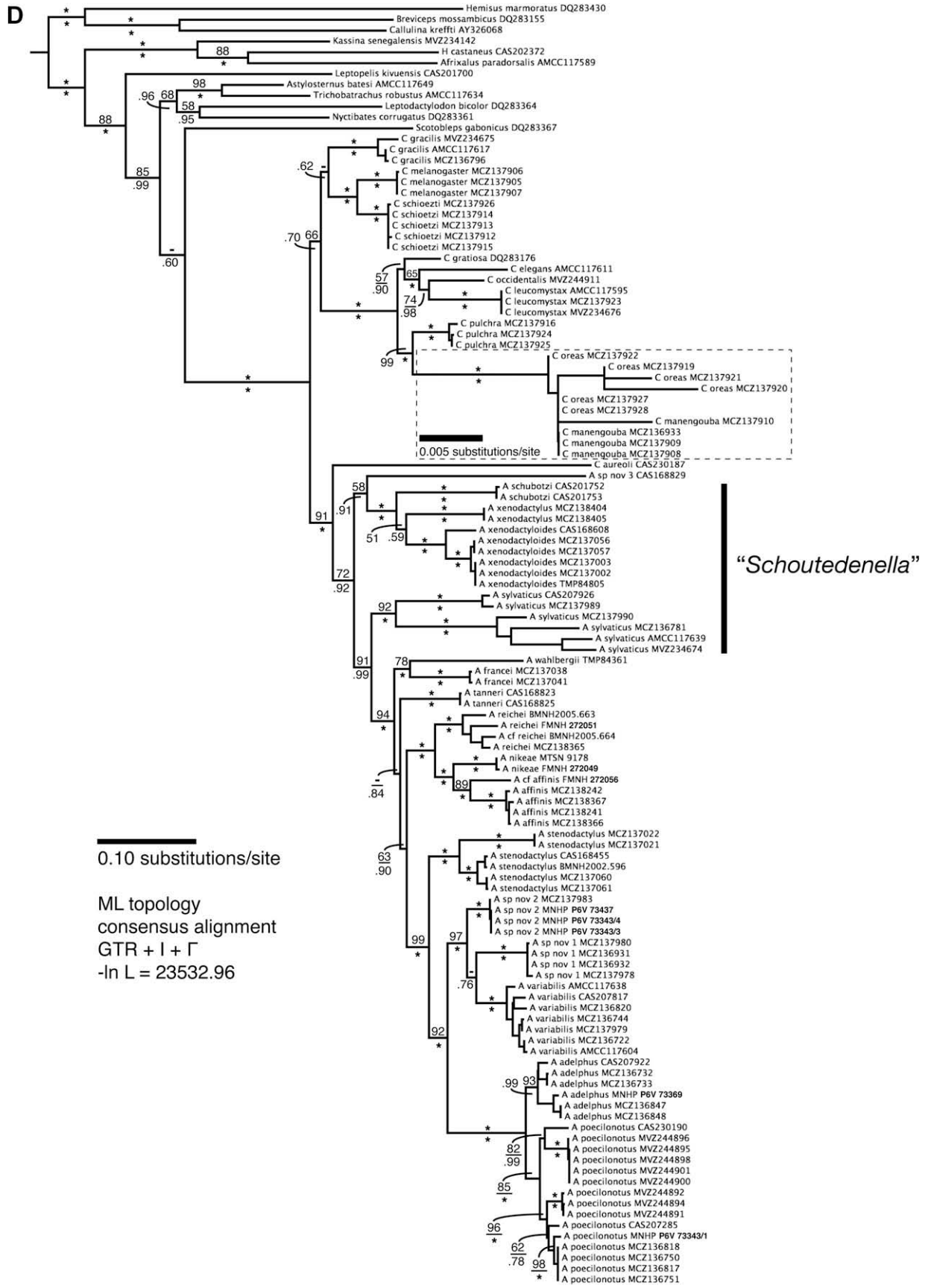

Fig. 3 (*continued*)

Table 3
Posterior probabilities from post burn-in Bayes trees, from analysis of each alignment, for alternative hypotheses of *Cardioglossa aureoli* relationship

Hypothesis	Cons.	gc10	gc15	gc20
Cardioglossa monophyly (including *C. aureoli*)	0.0001	0.0000	0.0000	0.0000
C. aureoli + *Arthroleptis*	0.9995	0.9997	1.0000	0.5300
C. aureoli sister to *Arthroleptis* + other *Cardioglossa*	0.0003	0.0003	0.0000	0.4700
C. aureoli sister to *Arthroleptis*	0.9183	0.0024	0.1082	0.1504
C. aureoli sister to *A. sylvaticus* + "large" *Arthroleptis*	0.0416	0.4349	0.4851	0.0186
C. aureoli sister to "large" *Arthroleptis*	0.0003	0.2344	0.3866	0.3592

cies form a clade, although with low support. The contrasting topology (ML: consensus; Bayesian: consensus, gc20) breaks *A. wahlbergii*, *A. francei*, and *A. tanneri* into one or two lineages that are successively more distant outgroups to the large Eastern Arc lineage. Lastly, while there is strong support from both ML and Bayesian analyses that *Cardioglossa aureoli* is more closely related to *Arthroleptis* (Fig. 3; Table 3), the exact placement of this species varies among analyses. *Cardioglossa aureoli* is resolved as either the sister taxon of *Arthroleptis* (ML: consensus; Bayes: consensus, gc20), the sister taxon of *A. sylvaticus* and larger *Arthroleptis* (ML: gc10, gc15; Bayes: gc15), the sister taxon of large *Arthroleptis* (ML: gc20), or *C. aureoli* has an equivocal relationship to *A. sylvaticus* and large *Arthroleptis* (Bayes: gc10). Bayesian analyses of all four alignments provide unambiguous evidence that *Cardioglossa* (i.e., including *C. aureoli*) is not monophyletic (Table 3).

Nonparametric bootstrap values from PHYML analysis provide largely similar levels of support among analyses of the three alignments generated using different gap costs (Fig. 3). GARLI analyses of gc10, gc15, and gc20 resolved a sister species relationship between *A. walhbergii* and *A. francei*, but bootstrap support varied across analyses and was >70% only in the gc20 analysis. As with the parsimony analysis, bootstrap support from analysis of the consensus alignment is generally the same or lower than the support for the same topologies found in the other analyses. There are two cases in which the support for an ingroup topology found in all analyses is the highest in the analysis of the consensus alignment (consensus vs. gc10/gc15/gc20: 91 vs. 74/97/72; 78 vs. −/−/72; − signifies values <50).

The ML and Bayesian analysis of the gc10, gc15, and gc20 alignments resolved *Scotobleps* as the sister taxon of *Leptopelis*, although this relationship receives high support only in the gc20 Bayesian analysis (posterior probability: 1.00). Analysis of the consensus alignment results in a topology in which *Scotobleps* is the sister taxon to *Arthroleptis* and *Cardioglossa*, but support for this topology is low.

3.3. Testing alternative phylogenetic hypotheses

Three alternative phylogenetic hypotheses were tested against the topologies obtained through parsimony and ML analyses (Table 4). There is strong support from ML and Bayesian analyses that *Cardioglossa aureoli* is more closely related to *Arthroleptis* than to other *Cardioglossa*, thus rendering *Cardioglossa* di-phyletic. The Templeton test further rejected the hypothesis of *Cardioglossa* monophyly ($P \leqslant 0.0343$), but this hypothesis could not be rejected with the SH test ($P = 0.446$). The non-monophyly of species formerly assigned to *Schoutedenella* was strongly supported by Bayesian, ML, and parsimony analyses. However, neither the Templeton test ($P > 0.1$) nor the SH test ($P = 0.414$) could reject the hypothesis of "*Schoutedenella*" monophyly. The hypothesis proposed by Laurent (1973) that *Arthroleptis*, *Cardioglossa*, and *Schoutedenella* are each monophyletic and that *Cardioglossa* and *Schoutedenella* are sister taxa was rejected by both the Templeton ($P \leqslant 0.0002$) and SH ($P = 0.001$) tests.

Table 4
Statistical support for alternative phylogenetic hypotheses described in text, based on analyses using the consensus alignment

Alternative hypothesis	Templeton test Δ steps	SH test Δ ln L
Cardioglossa monophyly	11 ($P \leqslant 0.0343$)*	6.117 ($P = 0.446$)
"*Schoutedenella*" monophyly	11 ($P > 0.1$)	6.822 ($P = 0.414$)
Arthroleptis (*Cardioglossa* + "*Schoutedenella*")	39 ($P \leqslant 0.0002$)*	51.155 ($P = 0.001$)*

* $P < 0.05$.

4. Discussion

4.1. Phylogeny of long-fingered and squeaker frogs

This analysis includes half of the described species diversity of *Arthroleptis* and *Cardioglossa*. As it also includes species from all previously proposed species groups and subgenera of *Arthroleptis* and *Cardioglossa*, and species formerly assigned to *Schoutedenella*, it is among the most comprehensive phylogenetic analyses of any clade of African frogs. There is robust support for the monophyly of a group comprising *Arthroleptis* (squeakers) and *Cardioglossa* (long-fingered frogs). This agrees with recent taxonomies proposed by Vences and Glaw (1991), Dubois (2005), and Frost et al. (2006). In addition, there is robust support for the vast majority of nodes within the phylogeny of both genera. These results are novel as no previous hypotheses for phylogenetic relationships within either *Arthroleptis* or *Cardioglossa* have been explicitly proposed.

These results agree with those from previous phylogenetic analyses in that *Arthroleptis* and *Cardioglossa* are more closely related to each other than to other African ranoid genera in the Hyperoliidae (sensu Liem, 1970) or Astylosternidae (sensu Dubois, 1992; Vences et al., 2003a, 2003b; Scott, 2005; Frost et al., 2006). Frost et al. (2006) found a sister relationship between *Scotobleps* and *Arthroleptis* + *Cardioglossa*, but only analyses of the consensus alignment support this topology and always with low support (MP bootstrap: 64%; ML bootstrap: 51%; Bayesian posterior probability: 0.60). Interestingly, the Bayesian analysis of the gc10, gc15, and gc20 alignments found extremely low posterior probabilities for this sister relationship (Table 5); instead, ML and Bayesian analyses favor a sister relationship between *Scotobleps* and *Leptopelis*. The phylogenetic position of *Scotobleps* differs based on both the method and alignment used for phylogenetic analysis; ML analyses of non-consensus alignments did not resolve *Scotobleps* as the sister of *Arthroleptis* + *Cardioglossa*. Thus, while not designed to explicitly test this hypothesis, this study finds no support for the Arthroleptini proposed by Frost et al. (2006). The larger African clade to which *Arthroleptis* and *Cardioglossa* belong, the Brevicipitidae sensu Dubois (2005), probably originated in the Early Cretaceous (Bossuyt et al., 2006; van Bocxlaer et al., 2006; van der Meijden et al.,

Table 5
Posterior probabilities from post burn-in Bayes trees, from analysis of each alignment, for alternative hypotheses of *Scotobleps* relationship

Hypothesis	Cons.	gc10	gc15	gc20
Scotobleps sister to *Arthroleptis* + *Cardioglossa*	0.6006	0.1016	0.0258	0.0003
Monophyletic "*Astylosternidae*"[a]	0.3819	0.0713	0.0979	0.0000
Scotobleps sister to *Leptopelis*	0.0004	0.6302	0.6323	0.9979

[a] "Astylosternidae" = (*Astylosternus*, *Leptodactylodon*, *Nyctibates*, *Scotobleps*, *Trichobatrachus*).

2007; Roelants et al., 2007). The mean divergence calculated in MEGA between *Arthroleptis* (including *C. aureoli*) and *Cardioglossa* is 17.0% (±1.5%) whereas that within *Arthroleptis* is 12.8% (±1.0%) and within *Cardioglossa* is 8.8% (±0.8%). If one cautiously assumes a constant rate of sequence divergence for *Arthroleptis* and *Cardioglossa* that is similar to that for mitochondrial DNA of other vertebrates (i.e, between 0.6% and 2.0% per million years; Shields and Wilson, 1987; Avise et al., 1992; Mulcahy and Mendelson, 2000), this suggests that the last common ancestor *Arthroleptis* and *Cardioglossa* occurred well after the Mesozoic in either the Oligocene or Miocene.

4.2. The effect of alignment on phylogeny estimation

The effect of sequence alignment on phylogeny estimation is well known (Wheeler, 1995; Morrison and Ellis, 1997; Lee, 2001; Ogden and Rosenberg, 2006). Many investigators are frustrated by the difficulty of producing "meaningful" alignments of certain difficult sequences, such as the stems of ribosomal DNA or untranscribed regions between genes. One solution is to remove those regions in which alignment seems ambiguous (for short review and references, see Lindgren and Daly, 2007). In an effort to be objective about this, there is a growing use of consensus (or "exclusion" sensu Gatesy et al., 1993) alignments that are generated by the removal of characters with uncertain homology statements; this is based on comparison of two or more alignments generated using different parameters or possibly even different methods. I assessed the ways in which results of the analyses of a consensus alignment differ from those obtained by analyses of the alignments used to generate this consensus. This differs from previous work focused on the effect of alignment on phylogeny estimation (e.g., Wheeler, 1995; Morrison and Ellis, 1997; Lindgren and Daly, 2007) by focusing explicitly on the issue of what is different between the results of a "normal" analysis and one in which characters have been removed.

Analyses of the alignments generated using different gap costs (i.e., gc10, gc15, gc20) produced similar estimations of phylogenetic relationships with similar measures of support. However, both the topologies and measures of support from these analyses differed substantially from analyses of the consensus alignment. While specific details are provided in either the Results or relevant sections below, it is beneficial to provide a short summary. In general, the consensus alignment discards potentially informative data that is important both for resolving phylogenetic relationships and for estimating levels of support in parsimony, maximum likelihood, and Bayesian frameworks. The consensus alignment has more than 500 fewer parsimony informative characters than any of the other three alignments (Table 1). Other studies have found a similar relationship between parsimony informative characters and variable regions in which alignment is sensitive to parameter values (e.g., Mugridge et al., 2000; Lindgren and Daly, 2007; see also Källersjö et al., 1999). While estimated base frequencies, proportion of invariant sites, and the α shape parameter are similar for all alignments, the estimated transition and transversion rates are substantially higher for the consensus alignment (Table 2); this is especially true for R[A–T] and R[A–G]. Support for topologies found in analyses of the consensus alignment is either similar to or lower than that for the same topology in the gc10, gc15, and gc20 alignments (see Section 3). There are only a few cases for which support for a given node is higher in an analysis of the consensus alignment, and exceedingly few in which it was substantially higher. In 10 cases, bootstrap values from the four alignments vary across the 70% threshold value proposed by Hillis and Bull (1993). Interestingly, this is also true in three cases when comparing the results of only the gc10, gc15, and gc20 alignments. One might expect that a consensus alignment reduces the number of homoplastic characters and thus produces higher measures of support. At least in this data set, the consensus alignment appears to eliminate informative data and thus gives lower measures of support.

Table 6
Distance (symmetric difference) between parsimony (P) and maximum likelihood (ML) topologies

Alignment	gc10 P	gc15 P	gc20 P	Cons. P
gc10 P	0			
gc15 P	11	0		
gc20 P	10	11	0	
cons. P	29	28	25	0
Alignment	gc10 ML	gc15 ML	gc20 ML	Cons. ML
gc10 ML	0			
gc15 ML	12	0		
gc20 ML	16	12	0	
cons. ML	56	54	54	0

Many nodes are the same between all analyses, but there are more similarities among the results of the gc10, gc15, and gc20 analyses than between these and those from analysis of the consensus alignment. A quantitative measure of the distance between the ML and parsimony topologies demonstrates that the topologies produced from the gc10, gc15, and gc20 analyses are more similar to each other than any of these are to the topologies produced from analysis of the consensus alignment (Table 6).

Importantly, the consensus alignment discards characters that are informative for resolving phylogenetic relationships between closely related species. Both the ML and parsimony topologies from gc10, gc15, and gc20 analyses resolve *Cardioglossa manengouba* and *C. oreas* as reciprocally monophyletic sister species. In contrast, ML and parsimony analyses of the consensus alignment find *C. oreas* to be paraphyletic with respect to *C. manengouba*. This suggests that for species between which there is little genetic divergence, a consensus alignment can discard data that are informative for resolving species boundaries. Given that the characters excluded from the consensus alignment seem likely to be quickly evolving sites, this result is not entirely surprising. In sum, I interpret these results as an empirical example of a consensus alignment discarding informative data, producing in relatively lower measures of support, and possibly overestimating transition/transversion rates for a gene.

For ancestral state reconstructions and biogeographic analysis, I compared the topologies produced from analysis of all four alignments. As an example, the gc10, gc15, and gc20 ML (GARLI) analyses each resolved a clade of larger *Arthroleptis* from Eastern and Southern Africa, whereas these same taxa form a paraphyletic grade in analyses of the consensus alignment. This difference in topology alters the reconstruction of the historical biogeography of this clade.

4.3. Phylogenetic relationships within Cardioglossa

The relationships among *Cardioglossa* species were mostly well resolved in all analyses. In addition, these relationships are in concordance with previously proposed *Cardioglossa* species groups (Amiet, 1972a, 1981; Blackburn, 2008). In an analysis of morphological diversity of all *Cardioglossa*, except *C. alsco* Herrmann et al. (2004) believe to be closely related to *C. pulchra*, Blackburn (2008) found five morphologically recognizable groups: (1) *C. aureoli*; (2) *C. escalerae*, *C. gratiosa*, and *C. nigromaculata*; (3) *C. gracilis*, *C. melanogaster*, and *C. schioetzi*; (4) *C. cyaneospila*, *C. pulchra* (and thus *C. alsco*), *C. venusta*, and *C. trifasciata*; (5) *C. manengouba* and *C. oreas*; and three species (*C. elegans*, *C. leucomystax*, and *C. occidentalis*) for which relationships were unclear based on morpho-

metric data. At least one species from each of these groups was included in this phylogenetic analysis. No species group was found to be paraphyletic, although the taxon sampling does not permit thorough tests of monophyly. There is strong support for all previously suggested pairs of sister species included in the analysis (*C. manengouba* + *C. oreas*, Blackburn, 2008; *C. leucomystax* + *C. occidentalis*, Blackburn et al., 2008; *C. melanogaster* + *C. schioetzi*, Amiet, 1981). A sister species relationship was proposed for *C. manengouba* and *C. oreas* by Blackburn (2008) based on morphological similarities, including the absence of sexual dimorphism in the length of the third finger. A clade comprising the long-snouted species group found by Blackburn (2008), *C. gracilis*, *C. melanogaster*, and *C. schioetzi*, appears in nearly all analyses, although with low support in a few cases. There is sufficient support to indicate at least two well-supported clades of montane species: (1) (*C. melanogaster*, *C. schioetzi*), and (2) *C. pulchra* (*C. oreas*, *C. manengouba*). This indicates multiple colonizations of either the mountains or lowlands; either scenario implies at least two major changes in elevational range during the history of *Cardioglossa*. The relationship of *C. pulchra* to *C. manengouba* and *C. oreas* is further supported by other evidence as these species groups are montane, lack an infratympanal line, and generally exhibit a substantial reduction or complete absence of the dorsal markings found in other *Cardioglossa*.

These results provide strong support that *Cardioglossa aureoli* is neither the sister taxon to nor nested within *Cardioglossa*. Nearly all analyses, of all four alignments, provide strong support that *C. aureoli* is more closely related to *Arthroleptis* than to other *Cardioglossa*. The most parsimonious topology produced from analysis of the consensus alignment resolved *C. aureoli* as the sister taxon of *Arthroleptis* + *Cardioglossa*, but this topology has <50% bootstrap support. ML analysis of the consensus alignment resolved *C. aureoli* as the sister taxon of *Arthroleptis*, but ML analysis of the gc10, gc15, and gc20 alignments placed *C. aureoli* within *Arthroleptis*. The posterior probabilities from Bayesian analysis provide neither unequivocal nor strong support for the relationships uncovered through parsimony and ML analyses (Table 3). Bayesian analyses do clearly indicate that *C. aureoli* is not part of *Cardioglossa*, and nearly all analyses agree that it is most closely related to *Arthroleptis*. Interestingly, Bayesian analysis of different alignments sometimes produced drastically different posterior probabilities for particular hypotheses (Table 3). The results of ML and parsimony analyses are concordant with the morphological study by Blackburn (2008) that found *C. aureoli* distinct from other *Cardioglossa* by its much smaller body size. If indeed *C. aureoli* has direct development (e.g., IUCN, 2006), then this is another point of similarity with *Arthroleptis*. Overall, there is strong support that *C. aureoli* is more closely related to, and probably nested within, *Arthroleptis*. Thus, *C. aureoli* should be included in *Arthroleptis*, at least until additional taxon sampling of *Arthroleptis* species is available to test its phylogenetic position.

4.4. Phylogenetic relationships within Arthroleptis

The relationships among *Arthroleptis* species were well resolved in each analysis, but some nodes differ between analyses. These results agree with morphological studies suggesting that *Arthroleptis* species with larger body sizes, including *A. wahlbergii*, are more closely related to one another than to the small-bodied species formerly placed in *Schoutedenella* (Laurent, 1940, 1941, 1957, 1961, 1973, 1981). In contrast to morphological studies, but in agreement with the recent molecular phylogenetic analysis by Frost et al. (2006), the miniature "*Schoutedenella*" species do not form a clade. While the alternative phylogenetic hypothesis of "*Schoutedenella*" monophyly could not be rejected (Table 4), there is strong support from all analyses that *A. sylvaticus* is more closely related to larger *Arthroleptis* than to other small species (*A. xenodactylus*, *A. xenodactyloides*, and *A. schubotzi*). The posterior probabilities for "*Schoutedenella*" monophyly are extremely low (consensus: 0.0002; gc10: 0.0000; gc15: 0.0000; gc20: 0.0008). The results of the analysis by Frost et al. (2006) are similar except these authors found *A. schubotzi* + *A. xenodactyloides* to be more closely related to larger *Arthroleptis* species. These species with small body sizes exhibit morphological similarities (Laurent, 1940, 1973; unpublished data), but the phylogenetic results presented here and by Frost et al. (2006) indicate that those characters may be symplesiomorphies of *Arthroleptis* and that the morphological similarities of larger species could be autapomorphic states within *Arthroleptis*.

Historically, the smallest "*Arthroleptis*-like" species, which exhibit paedomorphic skeletal anatomy (Laurent, 1940; Maas, 1945), were placed in *Schoutedenella*. This genus was erected by de Witte (1921) to accommodate *S. globosa*, a small species that lacks maxillary and premaxillary teeth that was later synonymized with *A. xenochirus* by Poynton and Broadley (1985). Laurent (1954, 1957) abandoned the lack of teeth as the defining character of *Schoutedenella* because of variation in the presence of teeth among, and possibly within, the *Schoutedenella* species described by de Witte (1921, 1933, 1941). *Schoutedenella* thus came to include many species exhibiting similar skeletal anatomy as well as small species for which the anatomy was unknown but was presumed similar based on having similarly small body size (i.e., Laurent, 1954). Many authors have disagreed with this taxonomy and included all, or nearly all, *Schoutedenella* species within *Arthroleptis* (e.g., Loveridge, 1957; Schmidt and Inger, 1959; Poynton, 1964, 1976, 2003ab). Frost et al. (2006) found *Schoutedenella* paraphyletic with respect to *Arthroleptis* and thus made the former a junior synonym of the latter. In this study, the clade comprising *A. schubotzi*, *A. sylvaticus*, *A. xenodactylus*, and *A. xenodactyloides* could not be rejected based on Templeton and SH tests, but nonparametric bootstrapping and Bayesian posterior probabilities generally provide strong support for the paraphyly of these miniature species with respect to larger ones. Increased taxon sampling will undoubtedly aid in further resolving the phylogeny within *Arthroleptis*, and thus the current generic taxonomy suggested by Frost et al. (2006) should be used.

The analysis of Frost et al. (2006) includes a specimen (CAS 207926) identified as "*Schoutedenella taeniata*" from Bioko Island (Equatorial Guinea); I independently obtained sequence data from this same specimen. The specimen is clearly a juvenile, and while exhibiting a pair of light dorsolateral lines, this character is insufficient to identify it as *A. taeniatus*. These lines are variably present in *A. taeniatus* (Perret, 1966) and can also be present to varying degrees in juveniles and adults of other *Arthroleptis* (pers. obs.). Indeed, in comparing this specimen to adult specimens of *A. taeniatus* (BMNH 1947.2.30.53 [holotype]; MHNG 1040.40–41), there are no additional similarities that suggest these specimens are conspecific. In contrast, the phylogeny presented here indicates a close relationship between CAS 207926 and specimens identified as *A. sylvaticus* from Mt. Manengouba (Cameroon). While *A. sylvaticus* may comprise several cryptic species (see below), the specimens from Nsoung, and thus, by inference, CAS 207926, are not *A. taeniatus*.

Previous discussions of the taxonomy of *Arthroleptis affinis* imply that this species is closely related to *A. adolfifriederici*, *A. francei*, *A. nikeae*, and *A. tanneri* (Skelton-Bourgeois, 1961; Grandison, 1983; Poynton and Broadley, 1985; Poynton, 2003b; Poynton and Loader, 2008). There is generally strong support for a clade comprising *A. affinis*, *A. nikeae*, and *A. reichei*, all of which are endemic to the Eastern Arc Mountains of Tanzania. However, this clade does not contain *Arthroleptis tanneri*, which is also endemic to these mountains. In ML analyses of gc10, gc15, and gc20 alignments, *A. tanneri* is part of a larger clade of Eastern and Southern *Arthroleptis*,

but the support is invariably low. Thus the unusually large body size of *A. tanneri* and *A. nikeae* is not indicative of close phylogenetic relationship. In most analyses, *A. wahlbergii*, from eastern South Africa, was resolved as the sister species to *A. francei*, which is endemic to the Mulanje Massif of southern Malawi. Some results also point towards a sister taxon relationship between (*A. wahlbergii*, *A. francei*) and *A. tanneri*.

There is strong support for a clade comprising *A. stenodactylus* from Eastern and Southern Africa and *A. variabilis*, *A. adelphus*, *A.* "*poecilonotus*", and two undescribed new species, all of which are from Central and Western Africa. *Arthroleptis adelphus* is resolved as the sister taxon of the *A.* "*poecilonotus*" species complex. Each analysis provides support to several distinct clades within the *A.* "*poecilonotus*" species complex (see below).

Laurent (1940, 1941) proposed four subgenera of *Arthroleptis* based on skeletal morphology: the nominal *Arthroleptis*, and *Abroscaphus*, *Arthroleptulus*, and *Coracodichus*. Of these, *Coracodichus* was later raised to the genus level (Laurent, 1957, 1972; Laurent and Fabrezi, 1985) whereas *Abroscaphus* was synonymized with *Arthroleptis* and *Arthroleptulus* with *Schoutedenella* (e.g., Laurent, 1954, 1957). Laurent (1940, 1941) explicitly placed *A. schubotzi* and *A. xenodactylus* in *Arthroleptulus*, a subgenus of *Arthroleptis*, and recognized a close relationship between *Arthroleptulus* and *Schoutedenella*; Laurent (1954) later placed *A. schubotzi* and *A. xenodactylus* in *Schoutedenella*. A close relationship between these two species is supported by the results of these analyses. *Abroscaphus* was only explicitly defined by Laurent (1940) to include *A. adolfifriederici*, which was not included in this analysis. *Coracodichus* was proposed for three East African species, *Arthroleptis stenodactylus*, *A. lonnbergi*, and *A. whytii,* based on the observations that these taxa exhibit coracoids that are strongly bifurcated medially (Laurent, 1940, 1954, 1957); *A. lonnbergi* and *A. whytii* were synonymized with *A. stenodactylus* by Loveridge (1942, 1957). In Boulenger's (1897) description of *A. whytii*, he lists localities in northern Malawi, including the Misuku Mountains from which two *A. stenodactylus* specimens (MCZ A-137060–61) in this analysis were collected. While perhaps a trivial taxonomic point, only a few authors (e.g., Skelton-Bourgeois, 1961) other than Laurent have ever recognized *Coracodichus*. Laurent and Fabrezi (1985) recognized *Arthroleptis* as probably paraphyletic to *Coracodichus* (as alluded to by Laurent, 1972a), and thus *Coracodichus* was abandoned and finally considered a junior synonym of *Arthroleptis*. Yet, Frost et al. (2006) implied that *Coracodichus* could be valid. The results of this analysis provide unequivocal evidence that *Arthroleptis stenodactylus* is embedded firmly within *Arthroleptis*, and thus so are the junior synonyms of this species including *A. whytii* (the designated generotype of *Coracodichus*).

4.5. Cryptic species diversity

There are several unnamed and cryptic species of *Arthroleptis* in the mountains of both Cameroon and Tanzania. While analysis of sequence data alone, especially of only mitochondrial DNA, may be insufficient to warrant the description of new species, morphological data also suggest that these are new species (unpublished data). Specimens collected from Mount Manengouba in Cameroon are both genetically (this study) and morphologically (unpublished data) distinct from those collected in the nearby lowlands. The Cameroonian specimens assigned to *A. adolfifriederici* by Perret (1966) and Amiet (e.g., 1975) correspond to *Arthroleptis* sp. nov. 1 from Mt. Manengouba in this study. Similar to the specimens assigned by Perret (1966) to *A. adolfifriederici*, *Arthroleptis* sp. nov. 1 lacks the light stripe found in *A. variabilis* that extends along the gular midline from the mandibular symphysis to just anterior to the pectoral girdle. *Arthroleptis* sp. nov. 1 is morphologically distinct from *A. adolfifriederici*, which is probably restricted to the Albertine Rift Mountains of Central Africa (unpublished data). A second new species, *Arthroleptis* sp. nov. 2, at least superficially resembles *A.* "*poecilonotus*", but is more closely related to *A. variabilis*; this new species is presently known only from juvenile specimens from the mountains of the Northwest Province in Cameroon. Lastly, there is generally high support that a small *Arthroleptis* from the West Usambara Mountains in Tanzania, *Arthroleptis* sp. nov. 3, is the sister taxon of (*A. xenodactylus*, *A.xenodactyloides*, *A. schubotzi*). The discovery of a new, small, and cryptic *Arthroleptis* species from the Eastern Arc Mountains is hardly surprising as many species of amphibians continue to be described from this region

Table 7
Intraspecific uncorrected percent pairwise sequence divergences for *Arthroleptis* and *Cardioglossa*

Species	# Comparisons	Min.	Max.	Median	Mean
Arthroleptis adelphus	15	0.00	0.02	0.02	0.015
Arthroleptis affinis	10	0.00	0.05	0.005	0.021
Arthroleptis francei	1	0.00	0.00	0.00	0.000
Arthroleptis nikeae	1	0.00	0.00	0.00	0.000
Arthroleptis "*poecilonotus*" (All)	105	0.00	0.03	0.03	0.021
Arthroleptis "*poecilonotus*" (Cameroon)	10	0.00	0.01	0.00	0.004
Arthroleptis "*poecilonotus*" (Togo Hills)	3	0.00	0.00	0.00	0.000
Arthroleptis "*poecilonotus*" (Western)	15	0.00	0.03	0.00	0.010
Arthroleptis reichei	6	0.01	0.04	0.03	0.028
Arthroleptis schubotzi	1	0.00	0.00	0.00	0.000
Arthroleptis stenodactylus	15	0.00	0.06	0.06	0.035
Arthroleptis sylvaticus	**15**	**0.01**	**0.13**	**0.10**	**0.091**
Arthroleptis tanneri	1	0.00	0.00	0.00	0.000
Arthroleptis variabilis	21	0.00	0.02	0.01	0.013
Arthroleptis xenodactyloides	15	0.00	0.04	0.01	0.016
Arthroleptis xenodactylus	1	0.00	0.00	0.00	0.000
Arthroleptis sp. nov. 1	6	0.00	0.00	0.00	0.000
Arthroleptis sp. nov. 2	6	0.00	0.00	0.00	0.000
Cardioglossa gracilis	3	0.00	0.02	0.02	0.013
Cardioglossa leucomystax	3	0.00	0.00	0.00	0.000
Cardioglossa manengouba	6	0.00	0.00	0.00	0.000
Cardioglossa melanogaster	3	0.00	0.00	0.00	0.000
Cardioglossa oreas	15	0.00	0.00	0.00	0.000
Cardioglossa pulchra	3	0.00	0.01	0.01	0.007
Cardioglossa schioetzi	10	0.00	0.00	0.00	0.000

(e.g., Poynton, 2003b; Loader et al., 2006; Menegon et al., 2004, 2007; de Sá et al., 2004; Pickersgill, 2007).

This work provides the first phylogenetic evidence that populations from Western and Central Africa recognized here as *Arthroleptis* "*poecilonotus*" may comprise multiple unnamed species. Previous authors have discussed the possibility that these populations represent a complex of cryptic species (e.g., Perret, 1991; Rödel, 2000a; Rödel and Agyei, 2003; Rödel and Bangoura, 2004; Rödel et al., 2005). While divergence among populations is low (Table 7), these results provide strong support for three genetically and geographically distinct clades among the *A.* "*poecilonotus*" included in this analysis: (1) Western Ghana + Sierra Leone, (2) Togo Hills of Eastern Ghana, and (3) Cameroon + Equatorial Guinea. Previous authors (Rödel and Agyei, 2003; Rödel et al., 2005) have suggested that populations from Western Ghana and the Togo Hills of Eastern Ghana represent different species. This analysis provides phylogenetic support for the existence of morphologically cryptic, but genetically distinct, clades in Western and Eastern Ghana. Based on these data, populations from the Togo Hills are more closely related to populations found in Cameroon. Perhaps the most surprising result of this analysis is the inclusion of a morphologically distinct taxon from the mountains of Bioko Island (Equatorial Guinea) within the clade comprising *A.* "*poecilonotus*". This undescribed taxon is arguably easier to diagnose morphologically than most other *Arthroleptis* species (unpublished data), yet it is firmly embedded within *A.* "*poecilonotus*", which while exhibiting distinct genetic clades is morphologically uniform. An integrative analysis combining multiple forms of data is needed to resolve species boundaries in this clade, especially among the populations from Western Africa.

Arthroleptis sylvaticus exhibits a high level of genetic diversity (as much as 13% uncorrected pairwise divergence). The maximum and mean divergences between and among *A. sylvaticus* are much greater than for any other species examined in this analysis (Table 7). One possibility is that there are multiple cryptic species within the taxon currently recognized as *A. sylvaticus*. In support of this hypothesis, two adult specimens from Nsoung in Cameroon exhibit an uncorrected pairwise divergence of 11%, an amount of divergence similar to interspecific divergences found in this study. There are no striking morphological differences between these specimens, which indicates that if these represent cryptic species, then other information, such as call data, may be needed to resolve species boundaries.

4.6. Historical biogeography: out of Central Africa

Most species of *Arthroleptis* and *Cardioglossa* have distributions restricted to one of the geographic zones used in this study. Eight *Cardioglossa* species have small montane or submontane distributions in Cameroon and Nigeria and one *Cardioglossa* is found only in the Albertine Rift Mountains. Lowland *Cardioglossa* species exhibit larger distributions but are restricted to either Central or Western Africa. Only three of the 37 *Arthroleptis* species have a geographic distribution spanning more than one of the five zones used in this study. Two of these species, *A.* "*poecilonotus*" and *A. variabilis*, may represent two or more cryptic species with more restricted geographic distributions (e.g., Rödel, 2000b; Rödel and Bangoura, 2004; Ernst et al., 2008). The third species, *A. stenodactylus*, is a widespread species occurring in lowland and montane areas from Kenya south to northeastern South Africa.

The most recent common ancestor (*mrca*) of extant *Arthroleptis* and *Cardioglossa* is reconstructed as occurring in Central Africa (Fig. 4; Supplementary materials). Because of ambiguity in the reconstruction of ancestral ranges at the base of *Arthroleptis*, it is also possible that the *mrca* occurred in East Africa. This result is similar to that of studies of both angiosperms and wood mice in which large clades of Central African taxa were found to be paraphyletic with respect to taxa occurring in Western and Eastern Africa (Davis et al., 2002; Plana et al., 2004; Nicholas et al., 2006). Given that the vast majority of *Cardioglossa* species, as well as lineages more basal to *Arthroleptis* and *Cardioglossa* such as *Scotobleps* and astylosterniines (sensu Frost et al., 2006) occur only in Cameroon and neighboring countries, the Lower Guinean Forest Zone is probably the location of the *mrca* of *Arthroleptis* and

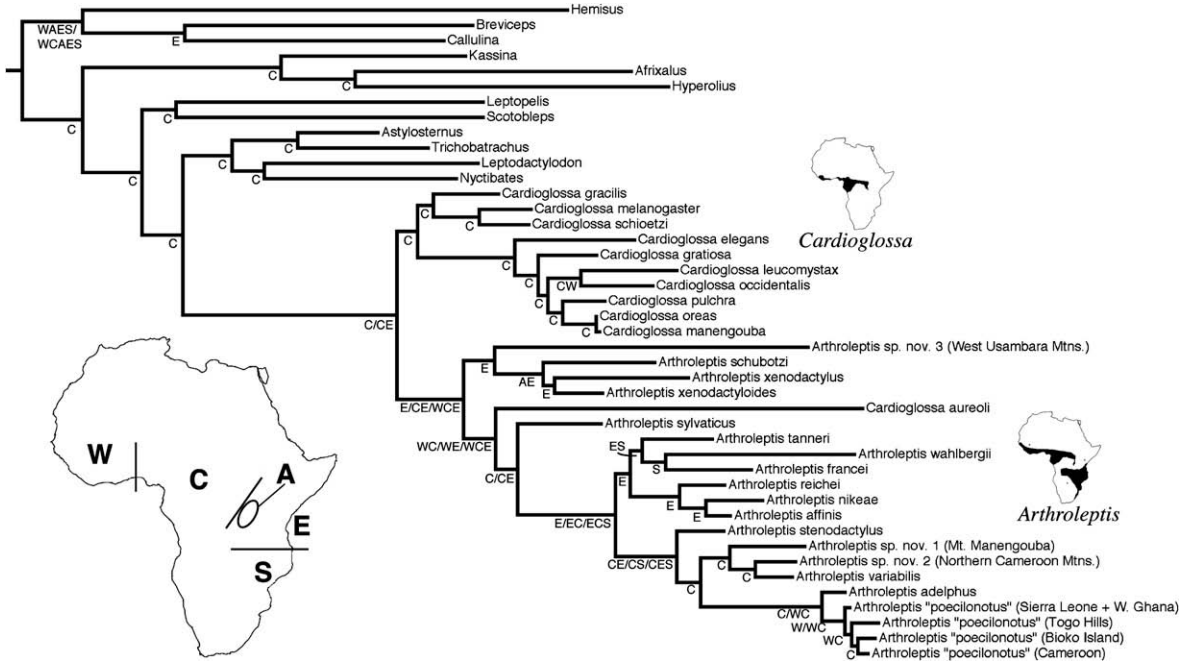

Fig. 4. Historical biogeography of *Arthroleptis* and *Cardioglossa* reconstructed on the gc15 ML topology (reconstructions using other topologies are given in Supplementary materials). Letters below branches indicate reconstruction of historical biogeography; all possible reconstructions for a node are given in order to illustrate ambiguity in reconstruction. Range maps of *Arthroleptis* and *Cardioglossa* are provided to aid in interpretation of historical biogeography.

Cardioglossa. Based on these phylogenetic data, *Cardioglossa* has dispersed to Western Africa (i.e., *C. occidentalis*) only recently in its evolutionary history. As the relationships differ based on the alignment used for phylogenetic analysis, the reconstruction of historical biogeography within *Arthroleptis* is not clear-cut. However, all topologies agree on a few salient points. The *mrca* of *Arthroleptis* is reconstructed as having a broader range than the *mrca* of *Arthroleptis* and *Cardioglossa*, although the reconstructions are somewhat ambiguous and dependent on the alignment. At least some ambiguity is expected when reconstructing historical biogeography, especially as these reconstructions are based solely on the distribution of extant species. However, because the outgroups to *Arthroleptis*, including *Cardioglossa*, are restricted almost entirely to Central Africa, and the basal node in *Arthroleptis* separates species found in West, Central, and Eastern Africa, it seems a safe conclusion that *Arthroleptis* expanded out of Central Africa early in its phylogeny.

The topology produced from analysis of the consensus alignment suggests that larger *Arthroleptis* (*mrca* of *A. walhbergii* and *A.* "*poecilonotus*") diversified in Eastern Africa and then dispersed into Central and Western Africa. However, analyses of the gc10, gc15, and gc20 alignments suggest two sister clades, one of which contains the *mrca* of *A. wahlbergii* from Southern Africa and all large montane *Arthroleptis* from the Eastern Arc and the other of which contains the *mrca* of *A. stenodactylus* and *A.* "*poecilonotus*" (Fig. 4; gc10 and gc20 analyses not shown). The major points of disagreement among the different biogeographic reconstructions are at basal nodes in *Arthroleptis* and especially near the base of the clade containing larger *Arthroleptis* species. While somewhat ambiguous, these results seem to indicate that the *mrca* of *A. variabilis* and *A.* "*poecilonotus*" dispersed from Eastern Africa to Central Africa. Additional taxon sampling within *Arthroleptis* may aid in resolving these reconstructions. Within larger *Arthroleptis*, the possible paraphyly of Eastern African species with respect to Central and West African species is similar to the biogeographic pattern found for forest robins by Roy et al. (2001). The reconstruction of historical biogeography in *Arthroleptis* and *Cardioglossa* suggests that this clade originated in and dispersed from Central Africa, diversified elsewhere, and then subsequently recolonized Central Africa.

Previous analyses of sub-Saharan frogs have differed in their ability to resolve the historical biogeography of the groups under study. Wieczorek et al. (2000) and Vences et al. (2004) were generally unable to resolve the historical biogeography of the African genera *Hyperolius* or *Ptychadena*, respectively. In the former study, this may in part be the effect of low nodal support, and both studies had low taxonomic sampling (<30% of the extant species diversity was included in either analysis). In an analysis of phylogenetic relationships of clawed frogs in the genera *Silurana* and *Xenopus*, which included nearly all described species of both genera, Evans et al. (2004) resolved the ancestral ranges of these genera. The *mrca* of *Silurana* was restricted to Central Africa and later dispersed to West Africa, whereas *Xenopus* originated in Eastern Africa and subsequently dispersed into Southern, Central, and even Western Africa. In both genera, dispersal into Western Africa appears to have occurred relatively recently in the evolutionary history of the clade (Evans et al., 2004). Interestingly, among the species of *Arthroleptis* and *Cardioglossa* analyzed here, there is a similar pattern in that the species present in Western Africa, including *A.* "*poecilonotus*" and *C. occidentalis*, are nested deep within the phylogeny of each genus. In addition, in both this phylogeny and the one of pipid frogs, a basal taxon is found in Western Africa (*C. aureoli*, this study; *Hymenochirus*, Evans et al., 2004). These results lead to the hypothesis that cladogenic events producing Western African species are either disproportionally young or old. I speculate that this may be a phylogenetic signal of one or several periods of extreme aridity in Western Africa. The historical range of the Saharan Desert is known to have encompassed regions now occupied by equatorial forests (Nichol, 1999). The expansion of the Sahara may have resulted in regional extinction of amphibians, which then persisted in refugia (as demonstrated by old cladogenic events) or only recolonized the forests and/or savannas of Western Africa relatively recently (ca. 20 million years ago; based on Evans et al., 2004).

Phylogenetic studies of African amphibians indicate a complex history of African faunas characterized by dispersal between several centers of diversification (e.g., Evans et al., 2004; Loader et al., 2007; Measey et al., 2007; Uyeda et al., 2007; this study). Most studies discussing the historical biogeography of African amphibians are hampered by low taxon sampling of the group of interest (generally <30% of the species diversity; e.g., Wieczorek et al., 2000; Drewes and Wilkinson, 2004; Vences et al., 2004; Measey et al., 2007; Uyeda et al., 2007) and uneven sampling across the geographic zones in which the group occurs (e.g., Wieczorek et al., 2000; Drewes and Wilkinson, 2004; Measey et al., 2007; Uyeda et al., 2007). Possibly as a consequence, several studies have found somewhat puzzling relationships between taxa from different regions, such as a close phylogenetic relationship between amphibians of the Gulf of Guinea islands (São Tomé and Príncipe) and Eastern Africa (e.g., Drewes and Wilkinson, 2004; Loader et al., 2007; Measey et al., 2007; Uyeda et al., 2007). While these results should not be considered spurious, it seems likely that inadequate taxonomic and geographic sampling obscures the complexity of spatial and temporal diversification of these clades. As suggested in this study of *Arthroleptis* and *Cardioglossa*, clades can originate and diversify in one region, then disperse to and diversify in another region, and then later recolonize the region of origin. Future studies of African historical biogeography should work towards better sampling across many geographic zones and improved taxon sampling, including outgroups.

4.7. Evolution of life history and body size

Body size and life history have figured importantly in previous discussions of the taxonomy of *Arthroleptis* and *Cardioglossa*. The phylogeny presented here sheds light on the evolution of these traits across the diversity of these genera. The most parsimonious reconstruction of life history evolution, based on the topologies from ML and Bayesian analyses, suggests that direct development has evolved only once and at the base of the *Arthroleptis* phylogeny (Fig. 5). *Cardioglossa aureoli* is believed to be a direct-developer (e.g., IUCN et al. 2006), which is in concordance with the placement of this species as either the sister to or embedded within *Arthroleptis*. As other *Cardioglossa* and nearly all of the outgroup taxa have free-living and feeding tadpoles, this phylogeny suggests that the *mrca* of *Arthroleptis* and *Cardioglossa* had a tadpole. These results reject the hypothesis of Laurent and Fabrezi (1985), who suggested that direct development evolved independently in *Arthroleptis* and *Schoutedenella*.

There is more than a fourfold difference in body size between adults of the smallest and largest *Arthroleptis* species. While previous authors have suggested that species with small body size are essentially "small versions" of those with large body size, and thus nested within the diversity of large species, the phylogeny indicates a more complex relationship. Indeed, there is a surprising result if body size is mapped onto the phylogenetic topology obtained through ML and Bayesian analyses. Large-bodied species appear to have evolved from ancestors with much smaller body sizes (Fig. 5). The *mrca* of *Arthroleptis* and *Cardioglossa* was smaller than related outgroup taxa and a transition to even smaller body size occurred at the base of *Arthroleptis*. Then, within *Arthroleptis*, there is a general trend towards larger body sizes. The species with

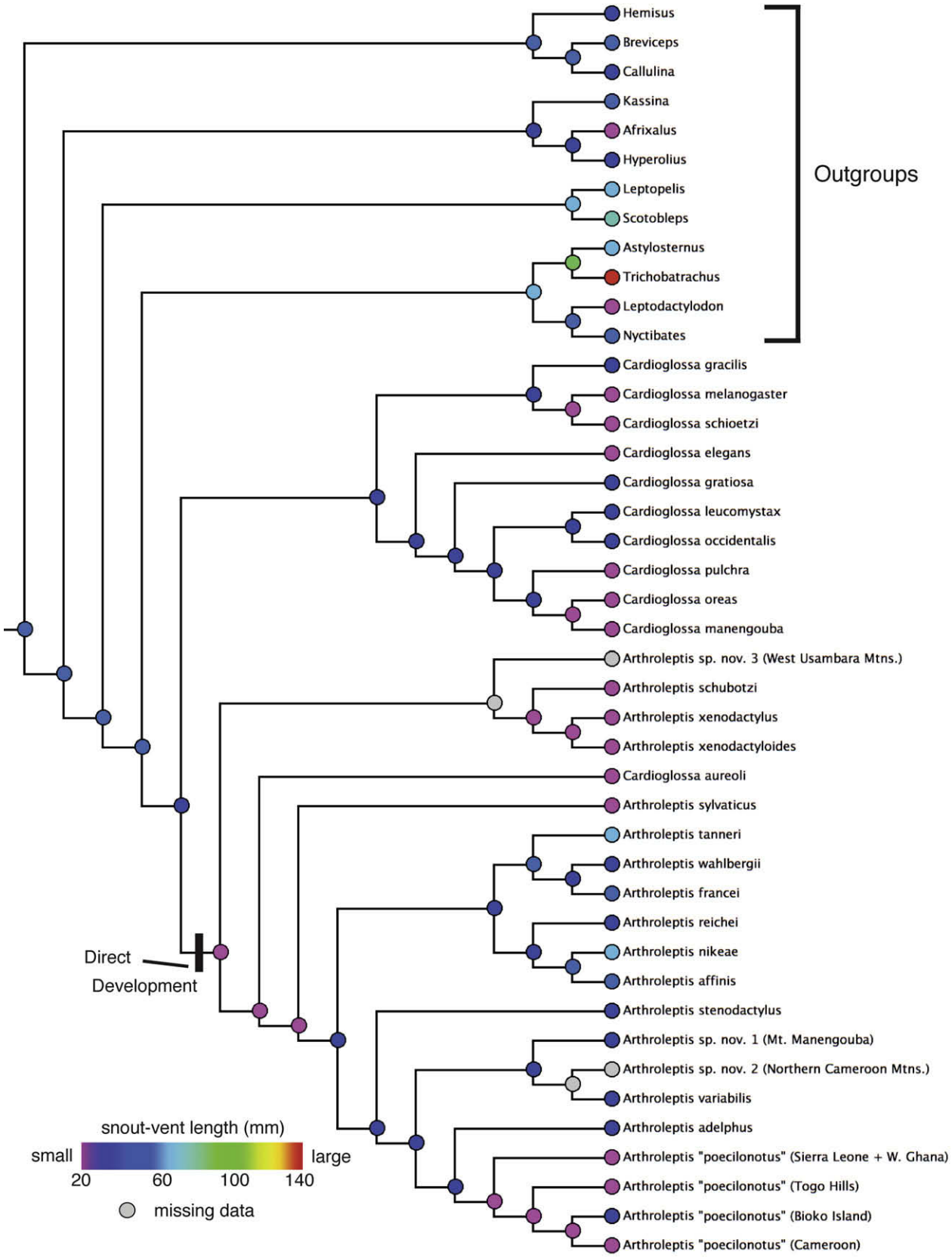

Fig. 5. Evolution of life history and body size reconstructed on the gc15 ML topology. As results are generally similar for ML topologies resulting from analyses of all alignments, only the results using the gc15 alignment are shown. The most parsimonious reconstruction of the evolution of direct development is mapped on the phylogeny. Ancestral state reconstructions for body size (maximum snout-vent length) are mapped on each node using squared-change parsimony; color scheme corresponds to 12 bins (each appx. 10 mm) in which continuous snout-vent length values have been placed. Gray circles indicate missing data for those species known only from juveniles as well as internal nodes for which reconstructions are not possible.

the largest body sizes (*A. nikeae* and *A. tanneri*) are not closely related, and thus their similar body sizes represent convergence.

Phylogenetic reduction of body size (i.e., miniaturization; Hanken and Wake, 1993) has dramatic effects on organismal biology. Within vertebrates, miniaturization may have played a causative role in the origin and diversification of large clades such as snakes, amphibians, and mammals (Rensch, 1959; Hanken and Wake, 1993; Luo et al., 2001). In extant amphibians, miniaturization has dramatic effects producing novel morphologies (Hanken, 1985; Hanken and Wake, 1993), increased morphological variation (Hanken, 1984), radical changes to anatomical organization (Rensch, 1959; Hanken, 1983) and novel life histories and reproductive biology (Hanken and Wake, 1993; Salthe and Duellman, 1973). In anurans, small size is correlated with derived life histories, including changes in clutch and ova size and direct development, the loss of the free-living, feeding tadpole stage (e.g., Duellman and Trueb, 1994; Clarke, 1996). In addition, both the evolution of small size and derived life histories may enable dispersal into ecological niches and/or habitat types previously unavailable to a lineage (e.g., Rensch, 1959; Clarke, 1996). Thus, in this context, the results of this analysis of phylogeny, character evolution, and biogeography in *Arthroleptis* and *Cardioglossa* are very intriguing. The appearance of both small body size and direct development at the base of *Arthroleptis* phylogeny suggest that one or both of these characters may have had a causative role in the dispersal of this clade from Central African forests to more diverse habitat types, including forests, woodlands, and open grasslands, extending from Western Africa to Southeastern Africa.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2008.08.015.

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