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A molecular perspective on the evolution of microteiid lizards (Squamata, Gymnophthalmidae), and a new classification for the family

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A molecular phylogeny was reconstructed for 26 recognized genera of the Gymnophthalmidae using a total of 2379 bp of mitochondrial (12S, 16S and ND4) and nuclear (18S and *c-mos*) DNA sequences. We performed maximum parsimony (MP) and maximum likelihood (ML) analyses, and data partitions were analysed separately and in combination under MP. ML analyses were carried out only on the combined sequences for computational simplicity. Robustness for the recovered nodes was assessed with bootstrap and partitioned Bremer support (PBS) analyses. The total molecular evidence provided a better-resolved hypothesis than did separate analysis of individual partitions, and the PBS analysis indicates congruence among independent partitions for support of some internal nodes. Based on this hypothesis, a new classification for the family is proposed. *Alopoglossus*, the sister group of all the other Gymnophthalmidae was allocated to a new subfamily Alopoglossinae, and *Rhachisaurus* (a new genus for *Anotosaura brachylepis*) to the new Rhachisaurinae. Two tribes are recognized within the subfamily Gymnophthalmidae: Heterodactylini and Gymnophthalmini, and two others within Cercosaurinae (Ecpleopini and Cercosaurini). Some ecological and evolutionary implications of the phylogenetic hypothesis are considered, including the independent occurrence of limb reduction, body elongation, and other characters associated with fossoriality.

ADDITIONAL KEY WORDS: phylogeny – DNA sequences – mitochondrial – nuclear – fossoriality – limb reduction – maximum parsimony – maximum likelihood – combined analysis – karyotypes.

INTRODUCTION

The Teiioidea is an assemblage of exclusively Neotropical lizards comprised of the families Teiidae and Gymnophthalmidae (Estes, de Queiroz & Gauthier, 1988), informally referred to as macroteiids and microteiids, respectively, due to marked difference in body size (some macroteiids grow to a metre in length, Ruibal, 1952). Although much work is still needed to understand intrageneric affinities, relationships among macroteiid genera are relatively well known. Two subfamilies are presently recognized: the Tupinambinae, comprised of genera *Callopistes*, *Dracaena*, *Tupinambis* and *Crocodilurus*, and the Teiinae, including *Teius*, *Dicrodon*, *Ameiva*, *Cnemidophorus* and *Kentropyx* (Presch, 1974; Denton & O'Neill, 1995; Sullivan & Estes, 1997).

In contrast to macroteiids, the small to medium-sized Gymnophthalmidae (about 4–15 cm snout–vent length) are much more diversified and far from taxonomically well known at specific, generic or supra-generic levels. They occur from Southern Mexico to Argentina, in the Caribbean, and on some islands of the continental shelves of South and Central America. Presently, 178 species, 10 of them polytypic and including a total of 26 subspecies, have been assigned

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to 36 genera, most of them exclusive to South America (Table 1). The complex taxonomy of Gymnophthalmidae derives not only from the rarity of many taxa in collections, but also from the presence of convergent morphological adaptations to specialized habitats. Limb reduction, body elongation, loss of eyelids and/or of external ear openings, or presence/absence of some head scales, are some of the characters that contribute to the present difficulty of resolving relationships among microteiids at all hierarchical levels.

Gymnophthalmids occur in habitats ranging from open areas in the high Andes to lowland tropical rainforests. Most species are terrestrial and lizard-like in general appearance, but some are semi-aquatic, as are those in the genus *Neusticurus*, and others show limb reduction to various degrees. Limb reduction has apparently occurred many times within microteiids, and it is accompanied by body elongation. *Bachia* and *Calyptommatus* are good examples of these processes (Rodrigues, 1991a, 1995) but, in species of *Bachia*, reduction is more pronounced in the hindlimbs than in the forelimbs, while in *Calyptommatus*, forelimbs are entirely lacking and hindlimbs are vestigial. *Nothobachia* and *Psilophthalmus* are examples of the *Calyptommatus*-like process of forelimb reduction, whereas *Heterodactylus*, *Anotosaura*, *Colobosaura* and *Colobodactylus* have been referred to as examples of the *Bachia*-like hindlimb reduction (Rodrigues, 1991a; Kizirian & McDiarmid, 1998). These lizards are often secretive or burrowing species in tropical forests or open areas (*Bachia*), or occupy specialized sand dune habitats in the semiarid Brazilian Caatinga (as *Calyptommatus*, Rodrigues, 1991a, 1995). The wide geographic distribution of many taxa, coupled with different degrees of limb reduction and body elongation, loss of eyelids or external ear openings, considerable variation in head squamation, the presence of parthenogenesis in species of *Gymnophthalmus* and *Leposoma*, conspicuous chromosome variation (Cole *et al.*, 1990; Cole, Dessauer & Markezich, 1993; Yonenaga-Yassuda *et al.*, 1995, 1996a; Pellegrino, 1998; Yonenaga-Yassuda & Rodrigues, 1999; Pellegrino, Rodrigues & Yonenaga-Yassuda, 1999a, b), and unresolved relationships among most genera, make this an ideal group for phylogenetic studies.

The early history of herpetology is marked by several attempts to allocate gymnophthalmids in suprageneric groups but, due to the characters related to limblessness or the presence of quincuncial scales in some taxa, several genera were originally placed close to the presently recognized lizards of the families Teiidae, Lacertidae or Scincidae (Gray, 1827, 1845, 1838, 1839; Merrem, 1820; Wagler, 1830).

The first robust taxonomic proposal for Gymnophthalmidae was presented by Boulenger (1885), who recognized only one family (Teiidae), and split it into four groups based upon characters of external morphology. Species later known as macroteiids (Teiidae) were included in his first group, and the microteiids (Gymnophthalmidae) in the other three groups. Several studies followed Boulenger's proposal in attempting to subdivide his groups into smaller monophyletic clades (Presch, 1980), or to raise the status of microteiids to an independent subfamily or family distinct from Teiidae (MacLean, 1974; Presch, 1983; Estes, 1983; Presch, 1988; Estes *et al.*, 1988). Although important revisions and descriptions of new genera of microteiids have been made since Boulenger, there is as yet no phylogenetic proposal based on a large number of taxa and characters. Therefore, Boulenger's work remains a basic reference due to the lack of a more complete study of the family (Harris, 1985).

Furthermore, evidence for monophyly of Gymnophthalmidae is still ambiguous. Harris (1985) analysed the infralingual plicae of 30 microteiid genera, and suggested that they be retained in the Teiidae, as proposed by Boulenger. Harris' data confirmed that Teiidae and Gymnophthalmidae are monophyletic only because they are unique in sharing infralingual plicae; his work does not provide evidence to contradict the hypothesis of monophyly for microteiids. Hoyos (1998) concluded that there is not enough data to support monophyly of Gymnophthalmidae, but his study was based on limited character and taxonomic sampling (15 osteological and myological characters from 11 genera, assigned to 16 species).

More recently, a group of eight genera previously proposed as monophyletic by one of us (Rodrigues, 1991b), was studied on the basis of analysis of 71 characters of osteology, external morphology and hemipenial anatomy (Rodrigues, 1995). The suggested relationships for this group are: (*Tretioscincus* (*Micrablepharus* (*Gymnophthalmus* ((*Procellosaurinus*, *Vanzosaura*) (*Psilophthalmus* (*Calyptommatus* and *Nothobachia*)))))). Some genera of this radiation show the most striking characteristics associated with psamophily and fossorial habitat so far reported for lizards, including forelimb reduction, body elongation, and loss of eyelids accompanied by the differentiation of an ocular scale covering the eye.

Allozymes, mitochondrial DNA restriction-site and chromosome data have also been collected for this radiation (Martins, 1997; Benozzati & Rodrigues, submitted; Yonenaga-Yassuda *et al.*, 1995, 1996a; Yonenaga-Yassuda, Pellegrino & Rodrigues, 1996b; Yonenaga-Yassuda & Rodrigues, 1999; Pellegrino *et al.*, 1999a). The phylogenetic analyses based on allozymes and restriction-site data also supported the monophyly of this group, and the topologies show some degree of congruence with morphological data. The only published nucleotide sequences for Gymnophthalmidae are those

Table 1. List of recognized genera of Gymnophthalmidae including the number of recognized species (sp) and subspecies (ssp), and the outgroup taxa. Localities and voucher/field numbers are given for the species used in this study, along with the gene regions successfully sequenced (+) for each taxon. Political units (under 'localities') of Brazil are: AC – Acre; RO – Rondônia; MG – Minas Gerais; PB – Paraíba; MT – Mato Grosso; BA – Bahia; SP – São Paulo; PR – Paraná; GO – Goiás; CE – Ceará; RR – Roraima; AP – Amapá; PE – Pernambuco; RJ – Rio de Janeiro; PA – Pará

Known genera/known species (sp)/ subspecies (ssp)/this study	Localities	Voucher/field no.[1]	Range of the genus	mtDNA			nuclear	
				12S	16S	ND4	*c-mos*	18S
Alopoglossus Boulenger, 1885 (7 sp)								
A. atriventris	Porto Walter, AC	LSUMZ H13856		+	+	+	+	+
A. carinicaudatus	Guajará Mirím, RO	LG1026	Amazonia and Pacific forests	+	+	+	+	+
A. copii	Reserva Faunística Cuyabeno Sucumbios, Ecuador	LSUMZ H12692	of Ecuador	+	+	+	+	+
Amapasaurus Cunha, 1970 (monotypic)	—	—	Upper Maracá River, AP			—		
Anadia Gray, 1845 (14 sp)	—	—	Northern South America			—		
Anotosaura Amaral, 1933 (3 sp)								
A. brachylepis	Serra do Cipó, MG	MRT 887336	Espinhaço range, eastern	+	+	+	+	+
A. vanzolinia	Cabaceiras, PB	MRT 907989	Brazil, Caatingas and	+	+	+	−	+
* *A.* spn.	Mamanguápe, PB	MRT 05060	northern Atlantic Forest	+	+	+	−	+
Arthrosaura Boulenger, 1885 (5 sp)								
A. kockii	Vila Rica, MT	MRT 978011	Throughout Amazonia to	+	+	+	−	+
A. reticulata	Juruena, MT	MRT 976977	Venezuelan tepuis	+	+	+	+	+
Bachia Gray, 1790 (19 sp/7 ssp)								
B. bresslaui	Bataguaçu, MT	MRT 916883	Northern South America,	−	+	+	+	+
B. dorbignyi	Juruena, MT	MRT 977273	Amazonia and Cerrados	+	+	+	+	+
B. flavescens	Agropecuária Treviso, Santarém, PA	LSUMZ H12977		+	+	+	+	+
Calyptommatus Rodrigues, 1991 (3 sp)								
C. leiolepis	Queimadas, BA	MRT 05055	Sand dunes of middle São	+	+	+	+	+
C. nicterus	Vacaria, BA	MRT 05053	Francisco River, BA	+	+	+	+	+
C. sinebrachiatus	Santo Inácio, BA	MRT 05054		+	+	+	+	+
Cercosaura Wagler, 1830 (1 sp/3 ssp)								
C. ocellata ocellata	Aripuanã, MT	MRT 977406	Cerrados and Amazon and Atlantic Forests	+	+	+	+	+
Colobodactylus Amaral, 1933 (2 sp)								
C. dalcyanus	Campos de Jordão, SP	LG 761	Itatiaia mountains of	+	+	+	+	+
C. taunayi	Serra da Prata, PR	LG 646	eastern Brazil and Atlantic Forest of southern Brazil	+	+	−	+	+
Colobosaura Boulenger, 1862 (3 sp)								
C. modesta	Niquelândia, GO	LG 1145		+	+	+	+	+
C. mentalis	Morro do Chapéu, BA	MRT 906448	Cerrados, Caatingas and	+	+	+	+	+
* *C.* spn.	Una, BA	MD 1106	Atlantic Forest	+	+	+	+	+

continued

Table 1 – *continued*

Known genera/known species (sp)/ subspecies (ssp)/this study	Localities	Voucher/field no.[1]	Range of the genus	mtDNA			nuclear	
				12S	16S	ND4	*c-mos*	18S
Colobosauroides Cunha & Lima Verde, 1991 (2 sp)								
C. cearensis	Pacoti, CE	LG 1348	Caatingas	+	+	+	+	+
Echinosaura Boulenger, 1890 (1 sp/3 ssp)	—	—	Transandean South America from Ecuador to Panamá			—		
Ecpleopus Dumeril & Bibron, 1839 (monotypic)								
E. gaudichaudii	Boissucanga, SP	LG 1356	Atlantic Forest of southern Brazil	+	+	+	+	+
Euspondylus Tschudi, 1845 (7 sp)	—	—	Venezuela, Brazil, Peru and Bolivia			—		
Gymnophthalmus Merrem, 1820 (7 sp)								
G. leucomystax	Fazenda Salvamento, RR	MRT 946613	Western South America to	+	+	+	+	+
G. vanzoi		MRT 946639	northern Central America	+	+	+	+	+
Heterodactylus Spix, 1825 (2 sp)								
H. imbricatus	Serra da Cantareira, SP	LG 1504	Atlantic Forest and mountains of eastern Brazil	+	+	+	+	+
Iphisa Gray, 1851 (1 sp/2 ssp)								
I. elegans elegans	Aripuanã, MT	MRT 977426	Amazonia	+	+	+	+	+
Leposoma Spix, 1825 (13 sp)								
L. percarinatum	Iwokrama Forest Reserve, Rupunini, Guyana	USNM 531665	Eastern Brazil to southern Central America	+	+	+	−	+
L. oswaldoi	Aripuanã, MT	MRT 977435		+	+	+	+	+
Macropholidus Noble, 1921 (monotypic)	—	—	Peruvian Andes			—		
Micrablepharus Dunn, 1932 (2 sp)								
M. maximiliani	Barra do Garças, MT	LG 1017	Cerrados and Caatingas,	+	+	+	+	+
M. atticolus	Santa Rita do Araguaia, GO	LG 854	north-eastern Brazil	+	+	+	+	+
Neusticurus Dumeril & Bribon, 1839 (11 sp/ 2 ssp)								
N. bicarinatus	Apiacás, MT	MRT 968462		+	+	−	+	+
N. ecpleopus	Apiacás, MT	MRT 0472	Costa Rica to Amazonia	+	+	+	+	+
N. rudis	Serra do Navio, AP	MRT 926008		+	+	+	−	+
N. juruazensis	Porto Walter, AC	LSUMZ H13823		+	+	+	+	+
Nothobachia Rodrigues, 1984 (monotypic)								
N. ablephara	Petrolina, PE	LG 897	Sand dunes of middle São Francisco River, BA	+	+	+	+	+
Opipeuter Uzzell, 1969 (monotypic)	—	—	Eastern Andes of Bolivia			—		
Pantodactylus Dumeril & Bribon, 1839 (2 sp/ 3 ssp)								
P. quadrilineatus	Caldas Novas, GO	LG 936	Open areas in northern	+	+	+	+	+
P. schreibersii schreibersii	São Paulo, SP	LG 927	South America, south to	+	+	+	+	+
P. schreibersii albostrigatus	São Paulo, SP	LG 1168	the Amazon River	+	+	+	+	+

Pholidobolus Peters, 1862 (7 sp)									
P. montium	Cotopaxi, Ecuador	KU 196355	Northern Andes	+	+	+		+	+
Placosoma Tschudi, 1847 (3 sp/2 ssp)									
P. glabellum	Iguape, SP	LG 940	South-eastern Atlantic	+	+	+		+	+
P. cordylinum	Teresópolis, RJ	LG 1006	Forest	+	+	+		+	+
Prionodactylus O'Shaughnessy, 1881 (6 sp/ 2 ssp)									
P. eigenmanni	Juruena, MT	MRT 976979	Amazonian and	+	+	+		+	+
P. oshaughnessyi	Porto Walter, AC Reserva Faunística	LSUMZ H13584	transitional forests from Panama to Bolivia	+	+	+		+	+
P. argulus	Cuyabeno, Sucumbios, Ecuador	LSUMZ H12591		+	+	+		+	+
Procellosaurinus Rodrigues, 1991 (2 sp)									
P. tetradactylus	Alagoado, BA	MRT 05056	Sand dunes of middle São	+	+	+		+	+
P. erythrocercus	Queimadas, BA	MRT 05057	Francisco river, BA	+	+	+		+	+
Proctoporus Tschudi, 1845 (27 sp)	—	—	Tropical South America				—		
Psilophthalmus Rodrigues, 1991 (monotypic)									
P. paeminosus	Santo Inácio, BA	MRT 05058	Sand dunes of middle São Francisco river, BA	+	+	+		+	+
Ptychoglossus Boulenger, 1890 (15 sp)									
P. brevifrontalis	Porto Walter, AC	LSUMZ H13603	Tropical areas of Central and South America	+	+	−		+	+
Riolama Uzzell, 1973 (monotypic)	—	—	Mount Roraima (RR)				—		
Stenolepis Boulenger, 1887 (monotypic)	—	—	North-eastern Atlantic Forest, Brazil				—		
Teuchocercus Fritts & Smith, 1969 (monotypic)	—	—	Ecuador				—		
Tretioscincus Cope, 1862 (3 sp/2 ssp)									
T. agilis	Vila Rica, MT	MRT 978177	Amazonian South	+	+	+		+	+
T. oriximinensis	Poção, PA	MRT 926415	America	+	+	+		+	+
Vanzosaura Rodrigues, 1991 (monotypic)									
V. rubricauda	Vacaria, BA	MRT 05059	Cerrados and Caatingas, north-eastern Brazil	+	+	−		+	+
† *Cnemidophorus*									
C. ocellifer	Barra do Garças, MT	MRT 946089	North America to Argentina	+	+	+		+	+
† *Kentropyx*									
K. calcarata	Vila Rica, MT	MRT 978224	Southern South America	+	+	+		+	+
† *Tupinambis*									
T. quadrilineatus	Niquelândia, GO	LG 1132	Southern South America	−	+	+		+	+

* *Anotosaura* spn. and *Colobosaura* spn. are species not yet formally described; generic allocation is tentative.

[1] Field numbers: MRT from Miguel Trefaut Rodrigues (IBUSP and MZUSP, São Paulo, Brazil); LG from the Laboratory of Cytogenetics of Vertebrates (IBUSP, São Paulo, Brazil), and MD from Marianna Dixo (IBUSP, São Paulo, Brazil).

† Alternative outgroup taxa.

in Kizirian & Cole (1999), but their aim was primarily to use mitochondrial sequences to elucidate the origin of parthenogenesis in *Gymnophthalmus underwoodii*.

In summary, the Gymnophthalmidae offers a number of fascinating biological problems for study, but lack of detailed phylogenetic knowledge has so far limited the feasibility of other studies. To provide a better knowledge of the phylogenetic relationships of Gymnophthalmidae, we conduced a molecular study of 26 genera using mitocondrial and nuclear DNA sequences. Based on total molecular evidence, we propose a new classification for Gymnophthalmidae reflective of the phylogeny recovered for these lizards, and discuss some ecological and evolutionary implications of this hypothesis.

MATERIAL AND METHODS

TAXON SAMPLING

Fifty species (including two not yet formally described) and four subspecies, assigned to 26 recognized genera of Gymnophthalmidae, were used to reconstruct the molecular phylogeny of the family. Table 1 summarizes all recognized genera, the number of species and subspecies currently recognized in each genus, and the appropriate distributional information for the taxa included in this study. The teiids *Cnemidophorus ocellifer* and *Kentropyx calcarata* (Teiinae), and *Tupinambis quadrilineatus* (Tupinambinae) (Teiidae is considered the sister group of Gymnophthalmidae; Estes *et al.*, 1988), were used to root the trees. These taxa were also employed to provisionally test the monophyly for the family, and to evaluate the sensitivity of the topologies to alternative outgroups.

LABORATORY PROCEDURES

Total genomic DNA was extracted from frozen tissues (liver or tail) or tissues preserved in 95% ethanol, following the protocol developed by Fetzner (1999). Regions from three mitochondrial genes, including the ribosomal 12S and 16S and the protein-coding ND4 regions, and two nuclear genes, *c-mos* and 18S rDNA, were selected to reconstruct the phylogeny. Approximately 420 bp of 12S, 550 bp of 16S, 800 bp of ND4 (including three tRNAs), 400 bp of *c-mos*, and 400 bp of 18S, were amplified via polymerase chain reaction (PCR) in a cocktail containing 2.0 μl of template DNA (approximate concentration estimated on a 2% agarose gel), 8 μl of dNTPs (1.25 mM), 4 μl of 10x buffer, 4 μl of each primer (10 μM), 4 μl of MgCl (25 mM), 24 μl of distilled water and 0.25 μl of *Taq* DNA polymerase (5 U/μ) from Promega Corp., Madison, WI. The primer sequences and the thermocycling conditions for all genes are given in Table 2. Double-stranded PCR amplified products were checked by electrophoresis on a 2% agarose gel (size of the target region estimated using a molecular weight marker), purified using a GeneClean III Kit (BIO 101, INC., Vista, CA), and directly sequenced using the Perkin-Elmer ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction (PE Applied Biosystems, Foster City, CA). Excess of dye terminator was removed with CentriSep spin columns (Princeton Separations Inc.). Sequences were fractionated by polyacrylamide gel electrophoresis on an ABI PRISM 377 automated DNA sequencer (PE Applied Biosystems, Foster City, CA) at the DNA Sequencing Center at Brigham Young University. Sequences were deposited in GenBank under accession numbers AF420656 to AF420914, and the aligned data sets are available at the following website: http://bioag.byu.edu/zoology/Sites-lab/alignments

SEQUENCE ALIGNMENT AND PHYLOGENETIC ANALYSES

Most sequences were edited and aligned using the program Sequencher 3.1.1 (Gene Codes Corp., Inc., 1995). The alignment for 12S and 16S sequences was performed manually following Kjer (1995) on the basis of secondary structure models of Gutell (1994) and Gutell, Larsen & Woese (1994). This was necessary because of the poor resolution obtained with manual or computer alignments due to the extremely variable nature of some regions of these sequences (see also Kjer, 1997 for criticisms of conventional alignment methodology and advantages of the secondary structure approach for rRNA sequences). Regions of ambiguous alignment for the 12S (84 bp) and 16S (96 bp) rRNA sequences were excluded from the resulting partitions used for the analyses. Although a fragment of about 800 bp was amplified using the ND4 primers (Arévalo, Davis & Sites Jr, 1994), only a protein-coding region (630 bp) for this gene was included in the analysis to avoid similar alignment problems of the sequences for three tRNAs downstream from the ND4 gene.

Phylogenetic analyses under the optimality criteria of maximum parsimony (MP) and maximum likelihood (ML) were performed with PAUP* (version 4.0b4a, Swofford, 1998). For MP, all characters were equally weighted and each data set was analysed separately and in the following combinations: mitochondrial sequences, nuclear sequences and all data combined. For all MP analyses, we used heuristic searches with 100 replicates of random addition with tree bisection reconnection branch rearrangement (TBR) and gaps coded as missing data. In some searches, gaps were considered a fifth state for 18S and nuclear partitions.

Alternative phylogenetic hypotheses were compared with the most parsimonious phylogenetic topologies. These alternative topologies were constructed using

Table 2. List of PCR and sequencing primers used in this study, and a summary of the PCR conditions for all five gene products

Primer label	Sequence (5′-3′)	PCR conditions: denaturation/annealing/ extension
12Sa[a]	CTG GGA TTA GAT ACC CCA CTA	94°C (1:00), 45–48°C (1:00), 72°C (1:00) × 45
12Sb[a]	TGA GGA GGG TGA CGG GCG GT	
16SL[a]	CGC CTG TTT AAC AAA AAC AT	94°C (1:00), 45–48°C (1:00), 72°C (1:00) × 45
16SH[a]	CCG GTC TGA ACT CAG ATC ACG T	
16SF.0[b]	CTG TTT ACC AAA AAC ATM RCC TYT AGC	
16SR.0[b]	TAG ATA GAA ACC GAC CTG GAT T	
ND4F[c]	CAC CTA TGA CTA CCA AAA GCT CAT GTA GAA GC	95°C (:25), 52°C (1:00), 72°C (2:00) × 40
ND4R[c]	CAT TAC TTT TAC TTG GAT TTG CAC CA	
G73[d]	GCG GTA AAG CAG GTG AAG AAA	94°C (3:00), 48°C (:45), 72°C (1:00) × 1 and 94°C (:45), 48°C (:45), 72°C (1:00) × 37 or 95°C (:45), 53°C (:45), 72°C (1:00) × 45
G74[d]	TGA GCA TCC AAA GTC TCC AAT C	
18S 1F[e]	TAC CTG GTT GAT CCT GCC AGT AG	94°C (1:00), 54°C (1:00), 72°C (1:00) × 40
18Sb7.0[e]	ATT TRC GYG CCT GCT GCC TTC CT	

Reference for primers are: [a] Harris *et al.* (1998); [b] primers designed by A. S. Whiting; [c] Arévalo *et al.* (1994); [d] Saint *et al.* (1998); [e] primers designed by M. F. Whiting.

MacClade 3.08a (Maddison & Maddison, 1992) and analysed as constrained trees in PAUP* (100 heuristic searches with TBR).

For computational feasibility, ML analyses were performed only on the combined data partition, using heuristic searches with 10 replicates of random stepwise addition with branch-swapping TBR. When estimating phylogenetic relationships among sequences using distance or ML methods, one assumes an explicit model of evolution. Determining which model to use given one's data is a statistical problem (Goldman, 1993), and here we tested alternative models of evolution employing PAUP* and MODELTEST version 3.0 (Posada & Crandall, 1998). PAUP* uses an uncorrected neighbour-joining tree to estimate likelihood scores for various models of evolution, and then MODELTEST statistically compares different models using likelihood ratio tests (hierarchical likelihood tests—LRTs—and the Akaike Information Criterion—AIC) with degrees of freedom equal to the difference in free parameters between the models being tested. This program iteratively evaluates paired alternative models, from the simplest to the more complex, so as to optimize the fit of data to a model. Table 3 summarizes these paired likelihood tests for our combined data partition, and shows the GTR + Γ + I model (Rodríguez *et al.*, 1990) as the best fit for our data.

Each of the three outgroup taxa (*Cnemidophorus ocellifer, Kentropyx calcarata* and *Tupinambis quadrilineatus,* Teiidae) was used as a single alternative (Donoghue & Cantino, 1984), while the other two were allowed to 'float' among the genera of Gymnophthalmidae. This sequential substitution of alternative outgroups provides an assessment of monophyly of the ingroup (Sites *et al.*, 1996).

Confidence in resulting nodes on the MP topologies was evaluated by non-parametric bootstrap analysis (Felsenstein, 1985) using 1000 standard replicates, and 100 000 replicates with the fast stepwise-addition search for the 16S, *c-mos* and 18S data partitions to circumvent long computational time. For ML searches, 100 standard replicates were performed. Partitioned Bremer support values (Baker & DeSalle, 1997), representing the contribution of each specified data partition, were calculated for nodes of the combined data partition topology using the program TreeRot version 2 (Sorenson, 1999). Conflict between topologies estimated from separate data partitions was examined, following the qualitative approach outlined by Wiens (1998), in order to evaluate the suitability of conducting a combined analysis of different partitions (see also Wiens & Reeder, 1997).

RESULTS

MONOPHYLY OF THE GYMNOPHTHALMIDAE

The monophyly of the Gymnophthalmidae was provisionally assessed in this study by alternative rooting to the Teiidae taxa *C. ocellifer, K. calcarata,* and *T.*

Table 3. Tests of paired hierarchical substitution models for the combined data partition using the program MODELTEST v.3.0 (Posada & Crandall, 1998). The significance level of rejection of the null hypothesis is adjusted via the Bonferroni correction to $\alpha = 0.01$ due to the performance of multiple tests

Null hypothesis	Models compared	$-\ln L_0$ $-\ln L_1$	df	*P*
Equal base frequencies	H_0 JC[a]	36743.3945	3	<0.000001
	H_1 F81[b]	36462.3789		
Ti = Tv	H_0 F81[b]	36462.3789	1	<0.000001
	H_1 HKY[c]	35583.9297		
Equal Ti rates	H_0 HKY[c]	35583.9297	1	<0.000001
	H_1 TrN[d]	35535.0977		
Equal Tv rates	H_0 TrN[d]	35535.0977	1	<0.000001
	H_1 TIM[e]	35519.7500		
Only two Tv rates	H_0 TIM[e]	35519.7500	2	<0.000001
	H_1 GTR[f]	34850.3398		
Equal rates among sites	H_0 GTR[f]	34850.3398	1	<0.000001
	H_1 GTR + Γ[g]	29317.3711		
No invariable sites	H_0 GTR + Γ[g]	29317.3711	1	<0.000001
	H_1 GTR + Γ + I[h]	29055.5840		

Models: [a] JC, Jukes & Cantor (1969); [b] F81, Felsenstein (1981); [c] HKY, Hasegawa, Kishino & Yano (1985); [d] TrN, Tamura & Nei (1993); [e] TIM and [f] GTR, Rodríguez *et al.* (1990); [g] Γ = shape parameter of the gamma distribution; [h] I = proportion of invariable sites; df = degrees of freedom.

quadrilineatus. MP searches performed on the combined data partition, with a sequential substitution of the three alternative outgroups, recovered a monophyletic Gymnophthalmidae with all of them. Of these three outgroups, the tree recovered from rooting to *Cnemidophorus* provided strongest support for most internal nodes. Furthermore, we could not amplify the 12S region for *T. quadrilineatus*, so *C. ocellifer* was selected as the only outgroup for all other phylogenetic analyses performed under MP and ML optimality criteria.

PATTERNS OF VARIATION

Table 4 summarizes patterns of variation for the separate and combined partitions used in this study. The combined mitochondrial partition contained a large number of parsimony informative sites, with the proportion of these relative to the total number of variable sites ranging from 79% for 16S to 90% for ND4. Among the nuclear partitions, the proportion of invariable/variable sites for *c-mos* is also high (77%), whereas the larger 18S partition (438 bp) has the lowest number of informative sites of any of the genes used.

MAXIMUM PARSIMONY ANALYSES

Separate MP analyses were carried out for all data sets and compared for conflict, following the approach employed by Wiens (1998). In all partitions, MP trees recovered were either topologically similar (examples are 12S, ND4, *c-mos*), or unresolved for many nodes (18S, Table 5). For example, a clade of eight genera was recovered in all analyses of *c-mos*, 12S and ND4 partitions, with moderate to strong bootstrap support (60–93%). Analyses of the16S and 18S partitions revealed no strongly supported alternative topology for these genera, so we considered these partitions to be without serious conflict. Furthermore, the mtDNA partitions contained a large number of informative sites (Table 4) and, because these genes are linked and inherited as a unit, we first proceeded with a combined analysis of these three partitions.

Figure 1 represents the strict consensus of the two most parsimonious solutions (Table 5) estimated from the combined mitochondrial partition. Four major patterns are evident. First, *Alopoglossus* was resolved as the sister taxon to all the other gymnophthalmids, and second, the other genera were divided into three deeply divergent clades (named I, II and III). Third, several genera are recovered as paraphyletic (*Anotosaura*, *Colobosaura*, *Neusticurus*, *Pantodactylus* and *Prionodactylus*), and a fourth major clade consisting of eight genera, some confined to the Cerrado/Caatinga region of Brasil, is strongly supported as monophyletic (93% bootstrap proportion) within Clade I.

Clade I includes the genera *Anotosaura*, *Colobosaura*, *Iphisa*, *Heterodactylus*, *Colobodactylus* and the eight genera suggested to be monophyletic by

Table 4. Summary of the patterns of variation for separate and combined data partitions analysed under MP criterion in this study. Nucleotide base frequencies (mean) and uncorrected pairwise distances (calculated with PAUP* 4.0b4a) are also presented

Data partition	12S	16S	ND4	mtDNA[a]	18S	*c-mos*	ncDNA[b]	Combined[c]
Character no. (bp)	403	502	630	1535	438	406	844	2379
No. variable sites (V)	192	162	384	738	39	210	258	961
No. informative sites (I)[c]	155	129	347	631	21	161	198	802
Ratio I/V sites	0.8	0.79	0.90	0.85	0.53	0.77	0.76	0.83
% A	0.34	0.31	0.31	0.32	0.23	0.29	0.26	0.30
% C	0.25	0.23	0.28	0.26	0.27	0.19	0.23	0.25
% G	0.18	0.21	0.12	0.16	0.26	0.22	0.24	0.19
% T	0.21	0.23	0.27	0.24	0.22	0.27	0.25	0.24
% Pairwise distance (uncorrected)	0.5–23%	0.6–14%	5–30%	2–22%	0–2%	0.2–26%	0–13%	1–17%

[a] Combined mitochondrial partition: 12S + 16S + ND4.
[b] Combined nuclear partition: 18S + *c-mos*.
[c] Combined partition: mtDNA[a] + ncDNA[b].

Table 5. Results of separate and combined data partitions analysed under the MP criterion used in this study

Data partition	# Trees	Length	CI	RI
12S	6	1066	0.32	0.69
16S	30	811	0.35	0.61
ND4	3	3415	0.21	0.40
mtDNA[a]	2	5425	0.25	0.46
18S	14 484	57	0.70	0.88
c-mos	118	501	0.56	0.79
ncDNA[b]	31 655	661	0.54	0.80
Combined[c]	2	6079	0.27	0.49

[a] Combined mitochondrial partition: 12S + 16S + ND4.
[b] Combined nuclear partition: 18S + *c-mos*.
[c] Combined partition: mtDNA[a] + ncDNA[b].

Rodrigues (1995), and named herein informally as the 'Rodrigues' Clade. Clade II included *Ecpleopus*, *Leposoma*, *Arthrosaura*, *Colobosauroides*, *Anotosaura vanzolinia* and *Anotosaura* spn., and was the most strongly supported of the major clades interior to *Alopoglossus* (99% bootstrap). Clade III included the genera *Bachia*, *Neusticurus*, *Placosoma*, *Pholidobolus*, *Ptychoglossus*, *Pantodactylus*, *Cercosaura* and *Prionodactylus*, but it is not well supported (bootstrap <50%). Clades I and II were weakly supported (bootstraps proportions <50%), but interior to *Anotosaura brachylepis*, the other taxa from Clade I are strongly supported (91% bootstrap).

More nested nodes were also recovered with strong support from the combined mitochondrial partition analysis. In Clade I a (*Heterodactylys* + *Colobodactylus*) clade is strongly supported (97%), the 'Rodrigues' Clade (93%) and within it, the (*Nothobachia* + *Calyptommatus*) clade (88%); in Clade II: a (*Colobosauroides* (*Anotosaura vanzolinia*, *Anotosaura* spn.)) clade with 100% bootstrap support; and in Clade III: a ((*Neusticurus bicarinatus*, *Neusticurus rudis*) *Placosoma*) with 97% bootstrap, (*Neusticurus ecpleopus* + *Ptychoglossus*) clade (88%), and a ((*Pholidobolus* (*N. ecpleopus*, *Ptychoglossus*)) + (((*Pantodactylus quadrilineatus* (((*Cercosaura*, *Prionodactylus eigenmanni*) (*Pantodactylus schreibersii albostrigatus*, *P. s. schreibersii*) (*Prionodactylus oshaughnessyi*, *P. argulus*)))) clade, with 95% bootstrap support.

Figure 2 represents the strict consensus of 31 655 equally parsimonious trees obtained from the combined nuclear partition (Table 5), and recovers a largely unresolved topology. However, the genus *Alopoglossus* is also recovered as monophyletic, with the same topology as in the mtDNA partition, and with high bootstrap support (94%). Furthermore, the 'Rodrigues' Clade was again recovered, albeit with weak support (55% bootstrap proportion), and within it a strongly supported (*Nothobachia* + *Calyptommatus*) clade (89% bootstrap). These results are largely congruent with the results of the combined mtDNA analysis (Fig. 1). A single exception is that monophyly of *Tretioscincus* in the 'Rodrigues' Clade was not recovered, but no alternative topology is strongly supported by the nuclear partition.

We are aware that a combination of strongly incongruent data sets can reduce phylogenetic accuracy relative to individual partitions, even when those partitions have identical histories (Bull *et al.*, 1993). How-

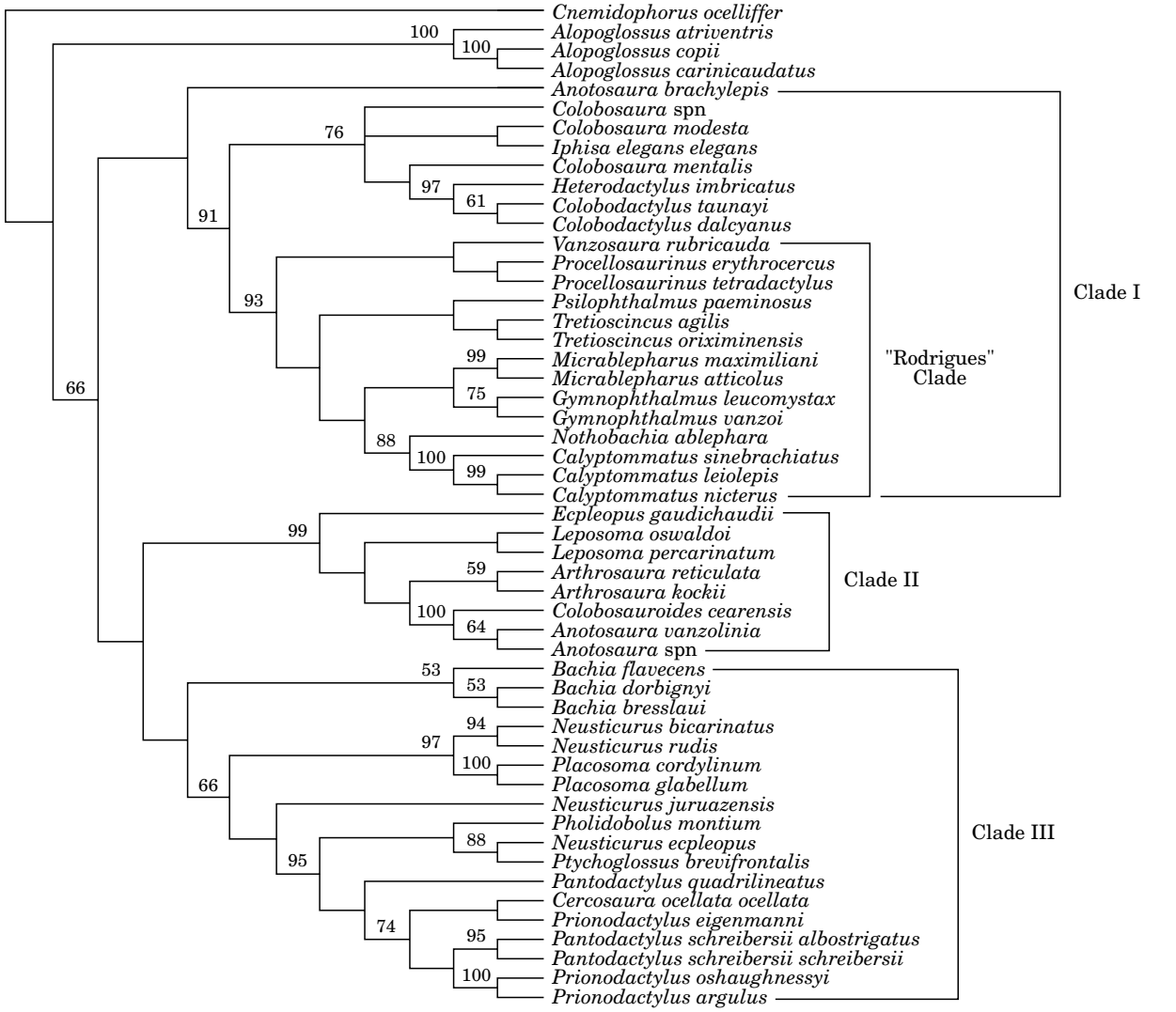

Figure 1. Strict consensus of two equally parsimonious trees (L = 5425, CI = 0.25, RI = 0.46) recovered from the combined mtDNA partition (12S + 16S + ND4); numbers above nodes are the bootstrap proportions (>50%).

ever, in the absence of strong conflict among the five individual data partitions, we performed a simultaneous analysis of the mitochondrial and the nuclear partitions combined. Our approach is based on the following advantages of combined analysis, which have been demonstrated in several empirical studies (for more details see Cunningham, 1997a, b; Wiens, 1998; de Queiroz, Donoghue & Kim, 1995; Nixon & Carpenter, 1996): (1) independent partitions may complement each other because, if they evolve at different rates, they will be better suited to resolve nodes at different hierarchical levels (Hillis, 1987); (2) weak signals that are 'suppressed' by noise in individual data sets may be 'activated' when added to the weak signals of the other data sets (Barrett, Donoghue & Sober, 1991), and (3) nodes that are weakly supported by conventional indicators (bootstrap, Bremer support) may be improved by increased congruence of independent characters (Flores-Villela *et al.*, 2000).

Simultaneous analysis of all data partitions recovered two equally parsimonious trees (Table 5), the strict consensus of which is presented in Figure 3 (support values in Table 6). These two trees differed only in the positions of *Psilophthalmus* and *Gymnophthalmus* in the 'Rodrigues' Clade, which remain unresolved in the combined analysis. With this exception, the topology presented in Figure 3 is better resolved and contains stronger nodal support than the phylogenies previously estimated from separate partitions, and we consider the results of the combined analysis to be our best working hypothesis of Gymnophthalmidae phylogeny based on molecular evi-

Figure 2. Strict consensus of 31 655 equally parsimonious trees (L = 661, CI = 0.54, RI = 0.80) recovered from the combined ncDNA partition (18S + *c-mos*); numbers above nodes are the bootstrap proportions (>50%).

dence. We estimated partitioned Bremer support for each node in the strict consensus topology (Table 6), which permits the evaluation of individual contributions from each data partition to the total Bremer support for each node. The major influence of the 12S and 16S partitions is evident; these sequences combined contribute 73% of the total Bremer support to all nodes, followed by the nuclear *c-mos* gene with 15%.

From the MP combined analysis, *Alopoglossus* was again recovered as the sister taxon to all the other Gymnophthalmidae, with strong support for its monophyly and for the monophyly of its sister clade (nodes 47 and 45, respectively; Table 6). Within the large clade, the same three clades (I, II and III) were also recovered. Clade II and Clade I (interior to *Anotosaura brachylepis*) are the most strongly supported as in previous analysis, with bootstrap proportions of 75% and 99%, and Bremer supports of 6 and 15, respectively (Table 6). There is also strong support for monophyly of the 'Rodrigues' Clade (bootstrap 100% and Bremer support of 15; Table 6), and no resolution of the five genera (*Anotosaura, Colobosaura, Neusticurus, Pantodactylus* and *Prionodactylus*; Fig. 3) recovered as paraphyletic in the mtDNA partition (Fig. 1).

Within each of the three major clades recovered by the combined analysis, internal topologies differed from those recovered by the mtDNA partition (Fig. 1). In Clade I, the node (*Colobosaura mentalis* (('*C.* spn.' (*C. modesta, Iphisa*))) is better resolved with moderate support (69% bootstrap and Bremer support 2) in the combined analysis; and in the 'Rodrigues' Clade, the

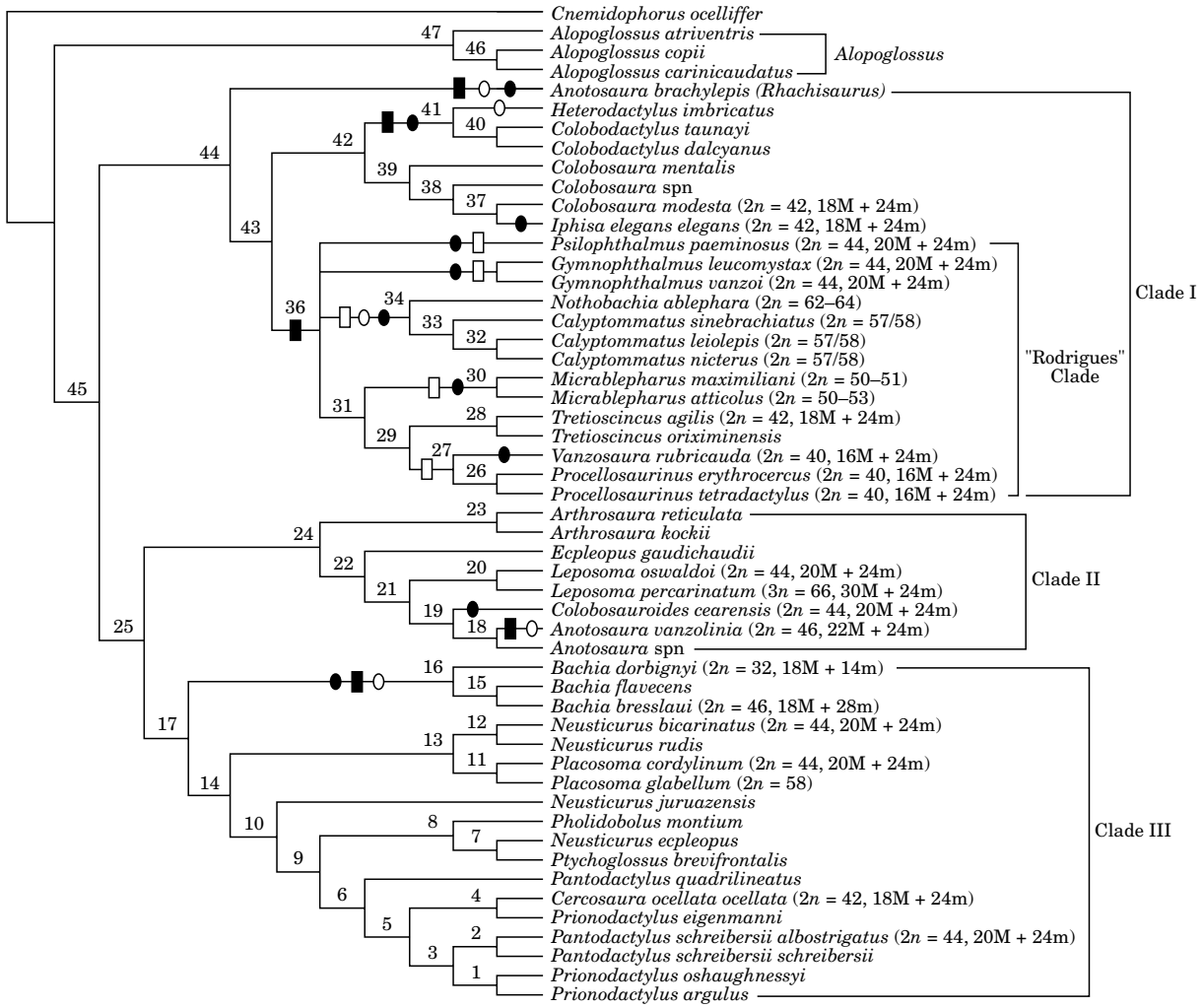

Figure 3. Strict consensus of two equally parsimonious trees (L = 6079, CI = 0.27, RI = 0.49) recovered from the combined analysis of mtDNA and ncDNA partitions. The internal nodes are numbered (above the branches) and support indexes are summarized in Table 6 for each node. The karyotypes are given for the taxa for which these data are available (in parenthesis, with 2*n* numbers, followed by the number of macro [M] and micro [m] autosomes), and other symbols on the branches indicate the following: (■) limb reduction; (□) loss of eyelids; (●) body elongation; (○) loss of external ear openings.

node (*Vanzosaura* + *Procellosaurinus*) is also better supported (66% bootstrap and Bremer support 3), but the placement of *Psilophthalmus*, *Gymnophthalmus* and the (*Nothobachia* + *Calyptommatus*) clade is unresolved. In Clade II *Arthrosaura* is the sister taxon of all the other genera in the combined analysis, whereas *Ecpleopus* is recovered in this position in the mtDNA partition (Fig. 1). In Clade III, the combined analysis recovers a (*Bachia flavescens* + *B. bresslaui*) clade that is strongly supported (bootstrap 89% and Bremer support 11) relative to a weakly supported (*B. dorbignyi* + *B. bresslaui*) clade (53% bootstrap proportion) in the mtDNA partition (Fig. 1).

A comparison of alternative hypotheses with our two most parsimonious solutions obtained from the combined data partition (strict consensus depicted in Fig. 3) was also carried out. The genera recovered as paraphyletic (*Anotosaura*, *Colobosaura*, *Neusticurus*, *Pantodactylus* and *Prionodactylus*) were constrained to be monophyletic. All the trees recovered from these analyses were longer than the MP consensus tree (Fig. 3) by two (*Colobosaura* monophyletic) to 63 steps (*Anotosaura* monophyletic) (Table 7).

Lastly, the topology in Figure 3 requires a minimum of three independent origins of limb reduction; one in the common ancestor of the *Bachia* clade, a second in the common ancestor of the 'Rodrigues' Clade, and a third time in the ancestor of (*Colobodactylus* + *Hetero-*

Table 6. Measures of support for all internal nodes of the strict consensus tree recovered from a combined analysis of all molecular data sets (Fig. 3). Columns present the bootstraps proportions, and total partitioned Bremer; positive and negative partitioned values indicate support for a given relationship in the combined analysis over the alternative relationship in separate analyses, and contradictory evidence for a particular relationship in the combined analysis, respectively. The nodes highlighted in bold font are those defining the major clades in Fig. 3; nodes underlined correspond to relationships recovered exclusively in the combined analysis (see text for details)

Node #	Bootstrap support	Bremer support	Partitioned Bremer					Node #	Bootstrap support	Bremer support	Partitioned Bremer				
			12S	16S	ND4	18S	*c-mos*				12S	16S	ND4	18S	*c-mos*
1	100	29	7.0	6.0	9.0	1.0	6.0	**25**	**<50**	**2.0**	**3.5**	**0.5**	**−3.0**	**0.0**	**1.0**
2	98	14	3.0	9.0	−1.0	0.0	3.0	26	100	14	5.5	2.7	−0.2	0.0	6.0
3	<50	2.0	0.0	5.0	−2.0	0.0	−1.0	27	66	3.0	−2.5	3.0	−0.5	0.0	3.0
4	<50	2.0	0.0	5.0	−2.0	0.0	−1.0	28	99	14	6.0	4.0	2.0	0.0	2.0
5	86	9.0	3.0	6.0	−3.0	0.0	3.0	29	<50	2.0	−2.3	1.4	−0.7	−0.2	3.8
6	57	8.0	−1.0	1.0	4.0	0.0	4.0	30	99	12	5.0	2.0	0.0	0.0	5.0
7	75	9.0	3.0	7.0	0.0	0.0	−1.0	31	<50	2.0	−2.9	1.2	0.3	−0.4	3.8
8	<50	9.0	−0.5	1.0	7.8	0.0	0.8	32	100	11	−0.5	4.0	5.5	0.0	2.0
9	83	16	15.3	8.6	−10.3	2.0	0.4	33	100	29	1.5	11	−0.5	3.0	14
10	64	4.0	−4.2	−4.3	10.8	0.0	1.7	34	99	16	−3.5	7.0	4.0	4.0	4.5
11	100	55	26	17	0.0	0.0	12	35	100	17	−13.5	15	−0.5	−1.0	17
12	97	12	5.5	7.7	−2.2	0.7	0.3	**36**	**100**	**15**	**−0.5**	**0.5**	**1.0**	**0.0**	**14**
13	98	13	9.5	9.0	−7.5	2.0	0.0	37	<50	1.0	2.5	4.0	−5.5	0.0	0.0
14	85	12	0.5	4.7	5.2	3.0	−1.3	38	<50	1.0	2.5	4.0	−5.5	0.0	0.0
15	89	11	0.0	0.0	−4.0	0.0	15	39	69	2.0	−3.3	0.6	−1.1	−0.2	6.0
16	<50	3.0	−0.8	2.3	5.5	1.0	−5.0	40	70	3.0	1.2	−1.0	1.5	0.0	1.3
17	**<50**	**4.0**	**4.5**	**3.0**	**−6.5**	**2.0**	**1.0**	41	99	13	−0.5	3.0	9.5	0.0	1.0
18	56	2.0	5.5	4.0	−8.5	0.0	1.0	**42**	**71**	**2.0**	**0.8**	**−1.2**	**4.8**	**0.0**	**−2.5**
19	100	21	4.0	1.0	16	0.0	0.0	**43**	**99**	**15**	**3.4**	**3.7**	**2.1**	**1.0**	**4.9**
20	100	23	6.0	5.0	12	0.0	0.0	**44**	**<50**	**4.0**	**2.5**	**3.7**	**−1.8**	**0.7**	**−1.0**
21	76	6.0	1.2	3.0	0.2	0.0	1.7	**45**	**86**	**12**	**1.5**	**8.0**	**−0.5**	**0.0**	**3.0**
22	55	2.0	5.5	4.0	−8.5	0.0	1.0	46	100	27	4.3	3.4	16.7	0.2	2.4
23	<50	2.0	5.5	4.0	−8.5	0.0	1.0	**47**	**100**	**62**	**44**	**41**	**0.0**	**−4.0**	**−19**
24	**75**	**6.0**	**13.5**	**7.0**	**13.5**	**3.0**	**−31**	Total		553	166.7	237.5	47.6	17.8	83.8
								%			30.13	42.92	8.60	3.22	15.15

Table 7. Tree lengths for the combined data partition for alternative hypotheses, relative to the MP consensus tree (Fig. 3)

Constraint tree	# Trees	Parsimony steps
MP consensus	2	6079
Anotosaura monophyletic	1	6142
Colobosaura monophyletic	1	6081
Neusticurus monophyletic	6	6130
Pantodactylus monophyletic	2	6101
Prionodactylus monophyletic	4	6094

dactylus) clade. Less parsimonious alternatives for Clade I, would postulate limb reduction in the ancestor of the group followed by reversals to the limbed condition again in one more genera. There are other possible independent origins of limb reduction, and we return to this issue in the Discussion.

MAXIMUM LIKELIHOOD ANALYSES

Analysis using the ML optimality criterion was only conducted on the combined data partition for constraints of computation time. The topology presented in Figure 4 was estimated using the general time reversible substitution model (Rodríguez *et al.*, 1990), with a gamma correction [Γ] and a proportion of invariable sites [I]. The GTR + Γ + I was the selected model in both the LRTs and AIC likelihood tests implemented in MODELTEST (Table 3). Parameters estimates for the ML topology were: R (A–C) = 2.5930, R (A–G) = 5.4557, R (A–T) = 2.7742, R (C–G) = 0.6429, R (C–T) = 17.5994, R (G–T) = 1.0; freq(A) = 0.3590, freq(C) = 0.2656, freq(G) = 01558, and freq(T) = 0.2196, and I = 05335, and Γ = 0.6597.

The ML analysis recovered a topology similar to the total molecular evidence MP analysis: there is strong support for monophyly of *Alopoglossus* (100% bootstrap) and its sister clade (85% bootstrap; Fig. 4), and within the latter clade, bootstrap support is high for monophyly of Clades I, II, and the 'Rodrigues' Clade (81%, 83% and 100%, respectively). However, the ML topology shows three major differences relative to the MP strict consensus topology (Fig. 3). First, within Clade I, the genera *Colobosaura*, *Iphisa*, *Heterodactylus* and *Colobodactylus* were not recovered as a monophyletic group (these genera are recovered as monophyletic with 71% bootstrap in the combined data MP analysis). ML analysis supports two distinct clades: (*Colobosaura* + *Iphisa*) 93% bootstrap, and (*Heterodactylus* + *Colobodactylus*) 100% bootstrap proportion. Still within Clade I, *Colobosaura modesta* grouped with *C. mentalis*, with *Iphisa* as the sister group but with low support (bootstrap <50%). Second, the 'Rodrigues' Clade is better resolved regarding the placement of *Psilophthalmus*, *Gymnophthalmus* and the (*Nothobachia* + *Calyptommatus*) clade. Third, within Clade II, *Arthrosaura* is recovered as paraphyletic, although the alternative sister relationship (*Arthrosaura kockii* + *Leposoma*) is only weakly supported (51% bootstrap). Finally, Clade II itself is more strongly supported (83% bootstrap) by the ML than the MP analysis (75% bootstrap, Fig. 3).

A PHYLOGENETIC CLASSIFICATION FOR THE GYMNOPHTHALMIDAE

This study is the most extensive to date for the Gymnophthalmidae, both with respect to character and taxon sampling, and our results show clearly that the current taxonomy of microteiids does not reflect the recovered phylogenetic structure (Fig. 3). We provide reasonably strong support for monophyly of the Gymnophthalmidae, and strong support for monophyly of several major groups. We propose several taxonomic changes in order to make the classification consistent with the evolutionary history of the group (de Queiroz & Gauthier, 1992). Except for *Anotosaura brachylepis*, for which we propose a new genus (*Rhachisaurus*) to eliminate non-monophyly for *Anotosaura* as originally defined, and because discovery of new species is still occurring at a rapid pace (Table 1, Kizirian & McDiarmid, 1998; Rodrigues, ms. in preparation), we confine taxonomic changes to the subfamily and tribe levels to accommodate the major clades identified in this study. Furthermore, because several of the presently recognized genera are almost certainly not monophyletic, we prefer to be prudent here and wait for better characterization of some of these species complexes in order to undertake a more strongly based rediagnosis for them. For example, among the genera *Colobosaura* and *Heterodactylus*, the taxonomic diversity given in Table 1 is an underestimate, and more information is needed on other populations and species (some not yet described) of both genera. We also need more information on several species of *Neusticurus* and *Placosoma*, and on their relationships to *Anadia*, *Echinosaura* and *Teuchocercus*, in order to properly re-diagnose those genera. The same applies to the relationships of several other extremely complex and diverse genera entirely missing from our taxonomic sampling (*Euspondylus*, *Macropholidus*, *Opipeuter* and *Proctoporus*), or species-rich groups represented by only a few taxa (*Prionodactylus* and especially *Ptychoglossus*; Table 1).

Although the examples above show that a lot of additional work is necessary to improve generic definitions and to define and allocate correctly many species complexes, we proceeded with subfamilial and tribal allocation of the 10 genera missed in our analysis on

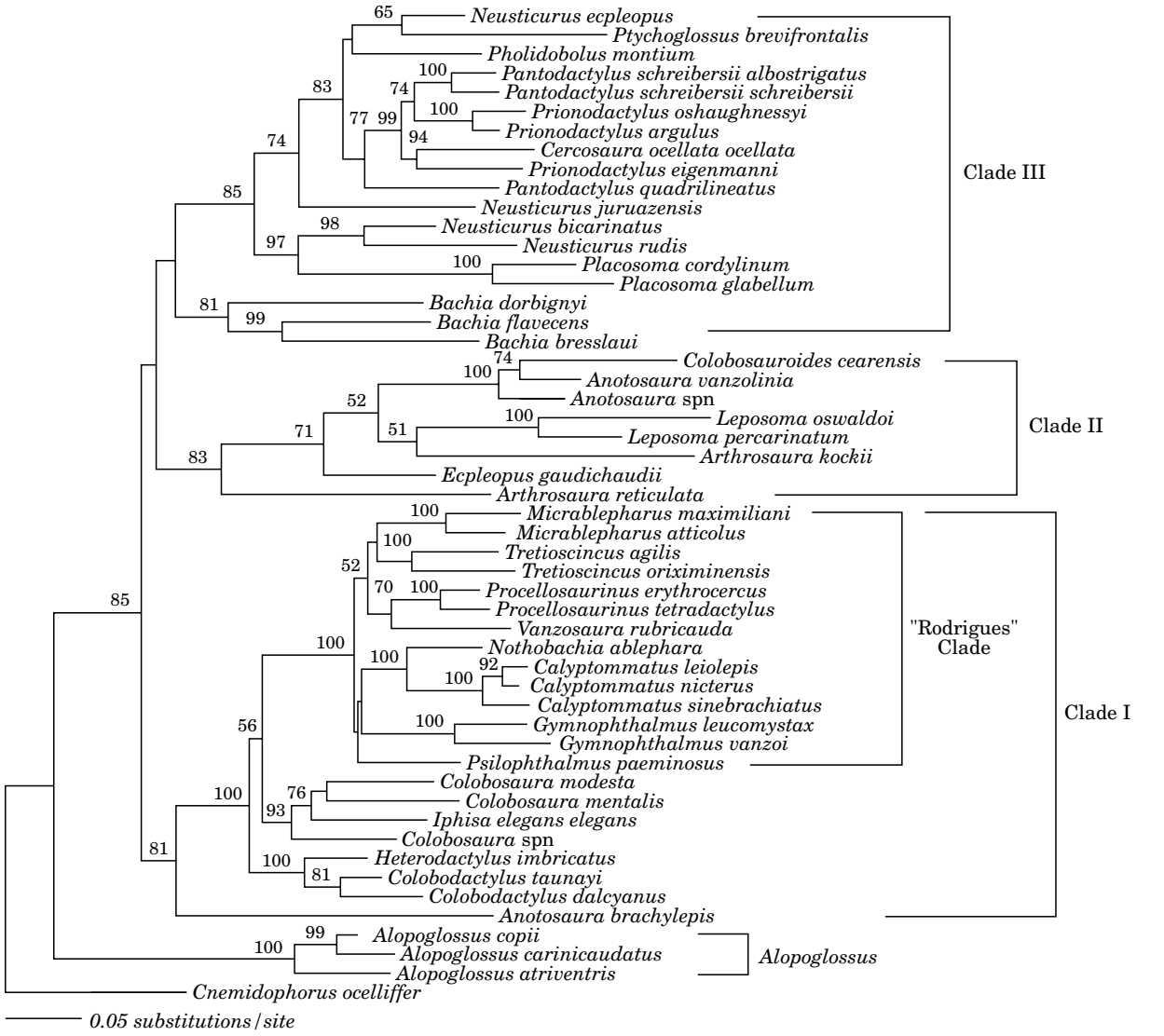

Figure 4. Phylogenetic hypothesis recovered by maximum likelihood criterion for the combined analysis of mtDNA and ncDNA partitions, under a GTR + Γ + I model of nucleotide substitution; −1n L = 27906.94978.

the basis of their proposed relationships to other genera included in this study. The genus *Amapasaurus* closely resembles *Leposoma* (Cunha, 1970; Rodrigues, 1997; Ávila-Pires, 1995), and *Leposoma* is deeply nested in Clade II (Fig. 3). *Anadia* shares many morphological similarities to a paraphyletic complex of species that have been associated with *Euspondylus*, *Ptychoglossus*, *Prionodactylus* and *Placosoma* (Oftedal, 1974; Presch, 1980). *Echinosaura* and *Teuchocercus* have been, since their original descriptions, considered close relatives to *Neusticurus* (Boulenger, 1890; Uzzell, 1966; Fritts & Smith, 1969). *Proctoporus* was recently reviewed and shown to be non-monophyletic (Kizirian, 1996), and this genus, as well as *Euspondylus*, *Macropholidus*, *Opipeuter*, *Proctoporus* and *Riolama*, have been traditionally associated with *Prionodactylus*, *Ptychoglossus* and *Pholidobolus* (all three represented in this study). Furthermore, earlier workers have also suggested a close relationship between *Pantodactylus*, *Prionodactylus* and *Cercosaura* (Ruibal, 1952; Montanucci, 1973; Uzzell, 1973). So, even considering that the diagnoses and content of several of these genera will change in the future, its seems clear from the above that their relationships can be provisionally placed in the gymnophthalmid grouping recovered in Clade III.

The genus *Stenolepis* cannot be placed with as much confidence. It is a poorly known monotypic genus that Boulenger (1888) suggested as intermediate between *Arthrosaura* and *Heterodactylus*. Presch (1980) sug-

gested that *Stenolepis* had affinities with his *Gymnophthalmus* group (*Iphisa, Tretioscincus, Gymnophthalmus, Bachia* and *Heterodactylus*), specifically with *Tretioscincus*. His hypothesis was based on a reduction of the digits on the first finger of the forelimb, and the keeled ventrals in *Stenolepis*. Pending future studies, we place *Stenolepis* provisionally with the species of the *Heterodactylus* clade, favouring the *Colobosaura* relationship proposed by Boulenger.

Considering the evidence above, all ten genera missed in this study can be credibly although tentatively allocated to one of the three major clades recovered in our analysis. A detailed morphological analysis of all recognized gymnophthalmid genera is presently underway by one of us (MTR), and that will combine an extended molecular data set with a morphological one.

This study provides enough resolution to offer a reasonably complete 'big picture' phylogenetic hypothesis, and both its topology and the generic content of the groups proposed here are predictive and therefore testable with additional sampling of taxa and data. The proposal of this hypothesis, and its attendant classification, will serve to focus attention on the most poorly resolved phylogenetic and taxonomic issues within the Gymnophthalmidae, while permitting other kinds of evolutionary studies on better known groups to proceed with the benefit of an available phylogenetic context.

The cladogram shown in Figure 5 depicts a hypothesis of relationships of subfamilial and tribal levels within the Gymnophthalmidae. Stem 1 clade (all Gymnophthalmidae, except *Alopoglossus*), remains unnamed, as well as stem 2 clade which includes the Rhachisaurinae and Gymnophthalminae (Heterodactylini + Gymnophthalmini). Because this study was not designed to assess higher-level relationships within the Teiioidea, we prefer to leave these branches unnamed, and preserve the present concept of Gymnophthalmidae. As a working hypothesis toward a phylogenetic classification of the Teiioidea, we suggest the following taxonomic arrangement for the Gymnophthalmidae:

Gymnophthalmidae Merrem, 1820

Alopoglossinae New subfamily
Content: *Alopoglossus* Boulenger, 1885.

Gymnophthalminae Merrem, 1820

Heterodactylini New Tribe
Content: *Colobodactylus* Amaral, 1933, *Colobosaura* Boulenger 1887, *Heterodactylus* Spix, 1825, *Iphisa* Gray, 1851, and probably *Stenolepis*, Boulenger 1888.
Comment: Gray (1838) described Chirocolidae based on the unjustified new generic name *Chirocolus,* Wagler, 1830, monotypic, for *Heterodactylus imbricatus,* Spix, 1825. *Chirocolus,* was subsequently recognized as a synonym of *Heterodactylus* and Chirocolidae was used by Gray (1838, 1845) until placed definitively in the synonymy of Boulenger's Teiidae (1885).

Gymnophthalmini Merrem, 1820
Content: *Calyptommatus* Rodrigues, 1991; *Gymnophthalmus* Merrem, 1820; *Micrablepharus* Dunn, 1932; *Nothobachia* Rodrigues, 1984; *Procellosaurinus* Rodrigues, 1991; *Psilophthalmus*, Rodrigues, 1991; *Vanzosaura* Rodrigues, 1991; and *Tretioscincus* Cope, 1862.

Rhachisaurinae New Subfamily
Content: *Rhachisaurus*, new genus for *Anotosaura brachylepis* Dixon, 1974.
Diagnosis: as given for *Anotosaura brachylepis* Dixon, 1974.
Etymology: from Greek 'rhachis', an allusion to 'Espinhaço' (backbone), a single-word reference for the Portuguese noun 'Serra do Espinhaço', an extensive mountain range of eastern Brazil from where most specimens of *Rhachisaurus brachylepis* are known.

Cercosaurinae Gray, 1838
Ecpleopini Fitzinger, 1843
Content: *Anotosaura* Amaral, 1933, *Arthrosaura* Boulenger, 1885, *Colobosauroides* Cunha & Lima Verde, 1991, *Ecpleopus* Duméril & Bibron, 1839, *Leposoma* Spix, 1825, and probably *Amapasaurus* Cunha, 1970.

Cercosaurini Gray, 1838
Content: *Bachia* Gray, 1845, *Cercosaura* Wagler, 1830, *Neusticurus* Duméril & Bibron, 1839, *Pantodactylus* Duméril & Bibron, 1839, *Pholidobolus* Peters, 1862, *Placosoma* Tschudi, 1847, *Prionodactylus* O'Shaugnessy, 1881, *Ptychoglossus* Boulenger, 1890, and probably *Anadia* Gray, 1845, *Echinosaura* Boulenger, 1890, *Euspondylus* Tschudi, 1845, *Macropholidus* Noble, 1921, *Opipeuter* Uzzell, 1969, *Proctoporus* Tschudi, 1845, *Riolama* Uzzell, 1973, and, *Teuchocercus* Fritts & Smith, 1969.

DISCUSSION

PHYLOGENETIC RELATIONSHIPS AND A NEW CLASSIFICATION FOR GYMNOPHTHALMIDAE

This study based on molecular data represents the first step toward a better understanding of the relationships of the Gymnophthalmidae, and we present a phylogenetic hypothesis for 26 genera based on a combined analysis of five different gene regions.

The probable convergence of characters related to fossoriality among several taxa is one of the reasons for the present unstable status of microteiid systematics at

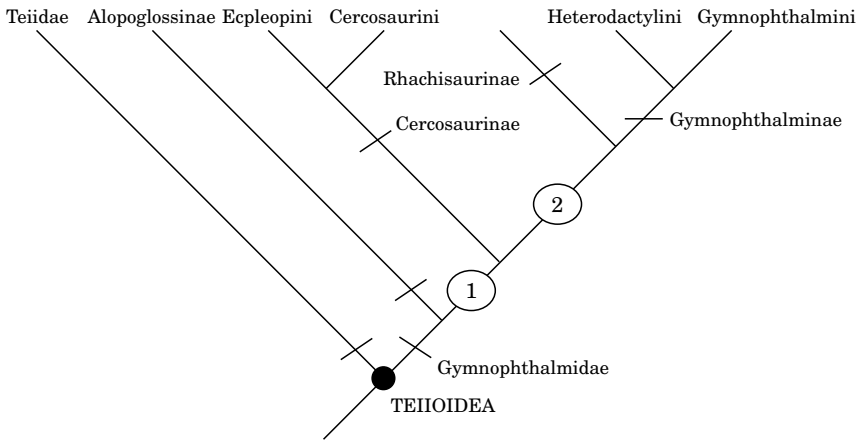

Figure 5. Phylogenetic hypothesis of relationships of subfamilial and tribal levels within the family Gymnophthalmidae based on the total molecular evidence phylogeny depicted in Figure 3. Stems 1 and 2 remain unnamed.

all hierarchical levels. On the basis of the hypothesis depicted in Figure 3, and on the suggested relationships for the 10 genera not included in this study, we propose a new classification for the family Gymnophthalmidae. The taxonomic changes were limited to subfamilial and tribal levels (Fig. 5) in order to accommodate the major clades recovered in our combined analysis. *Alopoglossus*, the sister taxon of all other gymnophthalmids, was allocated to a new subfamily Alopoglossinae (node 47; bold font, Table 6), while the deeply divergent Clade I was formally recognized as two subfamilies: the new Rhachisaurinae (to include the new genus *Rhachisaurus*), and Gymnophthalminae (node 43; bold font, Table 6). Two tribes are recognized within the Gymnophthalminae: the new Heterodactylini (node 42; bold font, Table 6), and the Gymnophthalmini (for the 'Rodrigues' Clade; node 36; bold font, Table 6). Clades II and III were included in the subfamily Cercosaurinae (node 25; bold font, Table 6), with the tribes Ecpleopini (for Clade II, node 24; bold font, Table 6) and Cercosaurini (to accommodate the large Clade III, node 17; bold font, Table 6). The support for these major clades ranged from very strong (Gymnophthalminae and Gymnophthalmini; bootstrap = 99 and 100, and Bremer indexes = 15, respectively) to moderate (Ecpleopini, bootstrap = 75 and Bremer index = 6.0) or weak (Cercosaurinae and Cercosaurini; support indexes <50% and <5.0; Table 6).

There is no general consensus about whether different data sets should always be combined in a simultaneous analysis, but in this study, the total molecular evidence approach yielded a better-resolved and more strongly supported phylogeny than the individual trees from any of the separate data partitions (Fig. 3). Although several nodes presented only weak or moderate bootstrap proportions in the combined analysis (nodes 6, 8, 16, 17, 18, 21–25, 27, 40 and 44; Table 6), they were supported by multiple independent data sets, as revealed by the partitioned Bremer support (PBS) analysis (Table 6).

The PBS approach is one way of assessing the support provided by different data partitions within a simultaneous analysis. It has an advantage over the taxonomic congruence approach because the secondary signals hidden in separate analyses may be recovered with a simultaneous analysis, as a result of interaction of independent characters. Positive values for PBS indicate that within a combined analysis of different partitions any given partition may provide support for that particular relationship over the alternative relationship specified in the tree(s) without the given node (in a separate analysis). Negative values mean contradictory evidence for the relationship recovered in the simultaneous analysis, and a zero score indicates the indifference of a given data set at a specific node (Baker & DeSalle, 1997; Gatesy & Arctander, 2000).

As previously mentioned, several nodes were supported by multiple partitions in the combined hypothesis, even though they are only weakly or moderately supported by conventional indexes. For instance, node 17 (Cercosaurini, Fig. 3), is weakly supported by bootstrap (<50%) and Bremer index (4.0), but two mitochondrial genes (12S and 16S) and the two nuclear genes (18S and *c-mos*) support this node, indicating congruence among independent data sets on that node. This applies also to node 25 (Cercosaurinae) and node 44 (the sister group relationship Gymnophthalminae + Rhachisaurinae), which are supported by mitochondrial and nuclear genes (Table 6).

The 12S and 16S gene regions make a major contribution to support of nodes in the MP combined phylogeny, and they seem suitable to resolve relationships at intrafamilial and intrageneric levels, as pointed out by studies such as those in Lacertidae

(Harris, Arnold & Thomas, 1998; Fu, 1998, 2000), the second outgroup to Gymnophthalmidae following Teiidae (Estes *et al.*, 1988). Among the nuclear regions used in this study, the lower support provided by the 18S partition in most of the nodes may reflect the previously noted small number of parsimony informative characters (Table 4), although this partition provides some support for selected deeper nodes (14, 17 and 24). For instance, node 14 was only moderately supported (66% bootstrap) in the mtDNA analysis (Fig. 1), but its bootstrap support was increased to 85% in the combined analysis (Fig. 3). Two mtDNA gene regions and the 18S region provide support for this node (Table 6), and this congruence of characters in the combined analysis may be responsible for increasing the bootstrap support. By contrast, the *c-mos* partition, after 12S and 16S, has the largest influence on the support for both recent and more divergent nodes in the simultaneous analysis, confirming its use for assessing deep divergence relationships, as demonstrated in previous studies in Squamata (Saint *et al.*, 1998; Harris *et al.*, 1999).

It seems that the difference in support among partitions is not simply a function of size of the data set (Baker & DeSalle, 1997). The ND4 partition has the highest number of informative sites of the mtDNA regions in our study, but the PBS analysis indicates a low contribution (8.60%, Table 4) to the total support for nodes in Figure 3. So, although the ND4 partition has the highest proportion of parsimony informative characters (Table 4), its contributions do not overwhelm the other data partitions in the combined analysis.

The combination of different data partitions may allow some relationships, absent in the separate analyses, to emerge in a simultaneous framework (Baker & DeSalle, 1997). This is the case for the sister taxa relationships (*Leposoma* + *Colobosauroides* + *Anotosaura*) and (*Leposoma* + *Colobosauroides* + *Anotosaura* + *Ecpleopus*) which are unique to the combined analysis (nodes 21 and 22, respectively; underlined in Table 6).

The topology recovered by the ML analysis for all sequences combined (Fig. 4) was largely congruent with that derived from MP analysis (Fig. 3), but recovered one major conflicting clade which deserves comment. The tribe Heterodactylini was recovered as a non-monophyletic group, but the alternative sister group relationship (Gymnophthalmini + (*Colobosaura–Iphisa*) group) is only weakly supported (56% bootstrap) by the ML analysis. The stability of Heterodactylini as a monophyletic assemblage may be sensitive to different assumptions of character evolution, which may not be accommodated in a combined analysis of all sequences under the same model of evolution. The ideal situation would include separate analyses for each data partition based on appropriate models, but this would require an enormous computational effort.

A recent example is given by Flores-Villela *et al.* (2000), who showed extensive heterogeneity in among-site-rate-variation between mtDNA protein, tRNA and nuclear aldolase sequences. These investigators accommodated rate heterogeneity by two methods; first they estimated instantaneous rates of all possible symmetrical substitutions individually on each of the three DNA partitions. These rates were estimated under a general reversible likelihood model on an imported tree, then normalized to down-weight the more common substitutions, and converted to whole numbers for inclusion in a step-matrix that was then implemented in a weighted parsimony analysis. Second, Flores-Villela *et al.* (2000) implemented a ML analysis by combining all gene sequences, estimating parameters across six different tree topologies (which permitted assessment of sensitivity of likelihood searches to the range of parameters used), and then implemented ML searches (under a GTR model derived as in this paper) after constraining all nodes supported by 100% bootstrap proportions, and 5^{+} Bremer indexes derived from a previous MP analysis. The study of Flores-Villela *et al.* (2000) included 34 ingroup taxa, fewer total base pairs, and fewer data partitions than this study, and it was still not feasible to carry out a full ML estimation with an adequate search strategy. We mention these points only to indicate that it is beyond the scope of this paper to fully explore the possible cause(s) of the conflict between the MP and ML topologies. We can only highlight the issue here, and continue on the basis of the MP topologies (Fig. 3).

COMPARISON WITH PREVIOUS HYPOTHESES

After Boulenger (1885), the first attempt to split the Gymnophthalmidae into groups of genera was that made by Presch (1980). He recognized six major groups of microteiids based on osteology and myology, working with 20 of the 30 genera recognized at that time. The groups were:

(I) *Ptychoglossus, Alopoglossus, Proctoporus, Opipeuter* and *Prionodactylus*).
(II) *Euspondylus* and *Pholidobolus*.
(III) *Ecpleopus, Anadia* and *Placosoma*.
(IV) *Echinosaura, Leposoma, Neusticurus, Cercosaura* and *Arthrosaura*.
(V) *Pantodactylus*.
(VI) *Iphisa, Tretioscincus, Gymnophthalmus, Bachia* and *Heterodactylus*.

Presch's arrangement for microteiids was very similar to the Boulengerian scheme: Groups I to V corresponded to group 2 of Boulenger, while group VI to

Boulenger's groups 3 and 4. Although Presch's groups I and II were considered closely related, a polytomy was recovered for groups I–II, III, IV, and V, suggesting uncertain relationships within Boulenger's group 2.

Nevertheless, some of Presch's groups expressed relationships already suggested for smaller groups of genera. Ruibal (1952) suggested that *Cercosaura* was closely related to *Pantodactylus* and that the last genus might be indistinguishable from *Prionodactylus*. This view was endorsed by Uzzell (1973) who added *Pholidobolus* to the (*Cercosaura*+*Pantodactylus*+*Prionodactylus*) group. In an effort to clarify the content of *Prionodactylus*, the genera *Opipeuter* and *Riolama* were also described by Uzzell (1969, 1973). A close relationship between *Neusticurus* and *Echinosaura* had already been suggested (Uzzell, 1966), and Uzzell (1969) also suggested a close relationship between *Ecpleopus* and *Leposoma* based on a number of shared characters, and contrary to the Presch (1980) proposal affiliating *Ecpleopus* to *Anadia* and *Placosoma*. Uzzell & Barry (1971) later suggested a relationship between *Arthrosaura* and *Leposoma*, and Fritts & Smith (1969) suggested a close affinity between *Teuchocercus* and *Echinosaura*. Dixon (1973) considered *Bachia* and *Heterodactylus* closely related, and later added *Anotosaura* to this group (Dixon, 1974); Vanzolini & Ramos-Costa (1979) subsequently considered *Colobodactylus* and *Colobosaura* also to be close to this same group. Finally, following the description of several new genera related to the eyelid-less radiation of gymnophthalmids, which was considered monophyletic, *Iphisa* and *Colobosaura* were admitted sequentially as the more closely related outgroups for that eyelid-less radiation (Rodrigues, 1991a, b, 1995).

Except for *Alopoglossus*, Presch's groups I–V correspond to our Cercosaurinae and, except for *Bachia*, his group VI is included in our Gymnophthalminae. We should mention also that, in separate analysis of 12S and 16S partitions, *Alopoglossus* was recovered as the sister taxon of *Neustiurus juruazensis* (77% and 89% bootstrap proportions, respectively, data not shown), and also *Alopoglossus* and *Ptychoglossus* grouped together for 18S and *c-mos* (bootstrap <50% and 99%, data not shown) and in the nuclear partition (91% bootstrap, Fig. 2).

The agreement among many of these early studies, which were not strictly phylogenetic (=cladistic), may reflect recovery of correct phylogenetic signal because a high proportion of shared derived character states were included in these early projects.

EVOLUTION OF FOSSORIALITY

Although it was previously assumed that body elongation, limb reduction, loss of external ear openings, or loss of scutes has occurred more than once in Gymnophthalmidae (Presch, 1980; Rodrigues, 1991a, b, 1995; and many others), this study offers the most comprehensive historical context in which to evaluate the multiple origins of these character complexes. The molecular data base is almost certainly independent of morphology and, from this perspective, our preferred phylogenetic hypothesis (Fig. 3) suggests that convergence affecting morphological adaptations to fossoriality may have been frequent enough in the history of Gymnophthalmidae virtually to ensure that the current taxonomic confusion was unavoidable, given the sampling limitations (for characters and taxa) of previous studies.

Assuming that the ancestor of all Gymnophthalmidae except *Alopoglossus* was an *Alopoglossus*-like lizard (i.e. four-limbed and pentadactylous, no body elongation, with eyelids and external ear openings), then the 'best hypothesis' requires a minimum of five independent losses of external ear openings. One loss characterizes *Rhachisaurus brachylepis*, a second occurred among the Heterodactylini (*Heterodactylus imbricatus*), a third within Gymnophthalmini (the ancestor of *Calyptommatus/Nothobachia*), a fourth in the Ecpleopini (the ancestor of *Anotosaura vanzolinia/collaris*), and a fifth within the Cercosaurini (genus *Bachia*).

On the basis of the same assumptions, a minimum of five independent events leading to body elongation occurred among Gymnophthalmidae (defined as an increase of the number of presacral vertebrae to beyond 27; MacLean, 1974; Presch, 1980; Rodrigues, 1995). These shifts occur in the same or slightly more inclusive suprageneric groupings that lacked external ear openings: *Rhachisaurus brachylepis*, the Heterodactylini and the Gymnophthalmini among Gymnophthalminae and the Ecpleopini and the Cercosaurini among Cercosaurinae (Fig. 3). In the Cercosaurini (sister clade *Bachia*), body elongation has occurred many times, but the exact number of events cannot be resolved, and must await clarification of the presently unsatisfactory generic arrangement, and the fact that some species of *Anadia*, *Euspondylus/Ptychoglossus* and *Proctoporus* have more than 27 presacral vertebrae (MacLean, 1974).

In addition, at least six independent events leading to limb reduction characterized the history of Gymnophthalmidae. One loss occurred in *Rachisaurus brachylepis*, a probable autapomorphy because its sister group includes pentadactylous species showing no body elongation. Another case of limb reduction occurred in some Heterodactylini (*Colobodactylus* and *Heterodactylus* only), and a third in Gymnophthalmini. Two losses occurred in the Ecpleopini: one in the *Anotosaura* radiation and another within the genus *Leposoma*. In *Leposoma*, the species *L. nanodactylus* differs from all congeners in reduction in fingers and toes (Rodrigues, 1997) and *Amapasaurus*, its putative sister taxon,

has only four fingers (Cunha, 1970; Ávila-Pires, 1995; Rodrigues, 1997; Rodrigues & Borges, 1997). Finally, a sixth episode occurred in the Cercosaurini and characterizes the genus *Bachia*. The occurrence of independent losses of limb elements has been previously suggested in the *Bachia/Colobodactylus/Heterodactylus/Anotosaura* assemblage of genera (Kizirian & McDiarmid, 1998).

Contrary to the frequent convergence of the other morphological adaptations towards secretive habitats, our phylogeny reveals that loss of eyelids occurred only in Gymnophthalmini. Unexpectedly, the recovered molecular topology places *Tretioscincus*, the only genus of that radiation with eyelids, as deeply nested within Gymnophthalmini. This hypothesis implies either multiple losses among the other genera (as shown in Fig. 3), or a reversal to the presence of eyelids in *Tretoscincus* in a clade in which absence of eyelids is inferred to be ancestral. However, an extensive morphological data set (Rodrigues, 1995) strongly supports a basal position of *Tretioscincus* in this clade. The molecular data leave an incompletely resolved topology for this clade but, if *Tretioscincus* really is the sister genus to all others in this group (see also Rodrigues, 1991b; Fig. 4), then loss of eyelids may have occurred only in the ancestor of the remaining seven genera. Considering this conflict, and the non-monophyly of *Tretioscincus* recovered by the combined nuclear data (Fig. 2), we defer this discussion until we have completed a more detailed study of this group (now underway).

ECOLOGICAL IMPLICATIONS OF THE PHYLOGENETIC RELATIONSHIPS

Another interesting result from this study is the relationships among the semiaquatic genus *Neusticurus*. Uzzell (1966) recognized two different radiations in the genus mainly based on hemipenial structure: the *ecpleopus* and *bicarinatus* groups. *Echinosaura* was admitted as a terrestrial *Neusticurus* derivative, most closely related to the *Neusticurus* of the *ecpleopus* group. Similarly, *Teuchocercus*, like *Echinosaura,* was considered close to the *Neusticurus* of *ecpleopus* group (Fritts & Smith, 1969). Despite the apparently deep divergence reported in *Neusticurus*, the close relationship of the three genera was accepted without question. Our data confirm that the external similarity in *Neusticurus* did not result from a common history, but is the result of convergent adaptation to aquatic habitats. *Neusticurus rudis* and *N. bicarinatus*, placed with *N. tatei* by Uzzell (1966) in his *bicarinatus* group, are recovered in our cladogram as the sister group of *Placosoma*, one of the most arboreal of the gymnophthalmid genera. The two other species we studied, *N. juruazensis* and *N. ecpleopus,* share with all the other species of *Neusticurus*, *Echinosaura* and *Teuchocercus*, the hemipenial structure of the *ecpleopus* group, and are recovered here as a paraphyletic assemblage (Fig. 3). Considering the diversity of *Neusticurus* (11 species, two subspecies), *Ptychoglossus* (15 species), *Pholidobolus* (seven species) and those of other Cercosaurini not available for this study, it is imperative to improve the characterization of these species complexes. A special emphasis should be given to understanding the relationships of *Anadia*. Like *Placosoma*, several species of *Anadia* are arboreal and bromelicolous, and knowledge of their relationships should aid interpretation of the history of *Placosoma* and *Neusticurus*. Our hypothesis implies that adaptations to life in water occurred at least three times in Cercosaurini, but only after a much more inclusive study of their relationships will we be able to answer more precisely such questions as: (1) how many times have adaptations towards a semiaquatic life occurred in the Cercosaurini radiation? and (2) which was the original habitat of the ancestors (terrestrial, arboreal or semifossorial)?

It was difficult to understand why *Neusticurus*, a genus widespread in central and western Amazonia and in Central American forests, and typical in forest streams in all of these regions, never successfully colonized the presumably older Atlantic forests of eastern Brazil. Our hypothesis shows *Neusticurus* and the endemic Atlantic Forest *Placosoma* as sister groups with strong support in MP and ML combined analyses. This sheds light on one puzzle in South American lizard biogeography, but it does not resolve whether the most recent common ancestor was likely to have been a semiaquatic lizard that became bromelicolous and arboreal, or the reverse. An interesting parallel puzzle was resolved by Mendelson, Silva & Maglia (2000), in their study of the relationships of marsupial hylid frogs of the genus *Gastrotheca*. This genus is represented in Central American forests, western South American, Andean slope forests and Atlantic forests, but not in Amazonia, and the phylogenetic study showed that the Amazonian radiation of *'Gastrotheca'* was represented by the highly differentiated genus *Hemiphractus*.

CHROMOSOME VARIABILITY IN GYMNOPHTHALMIDAE AND ITS POTENTIAL FOR PHYLOGENETIC STUDIES

Chromosome data have been collected extensively for gymnophthalmids (Cole *et al.*, 1990, 1993; Yonenaga-Yassuda *et al.*, 1995, 1996a, b; Pellegrino, 1998; Yonenaga-Yassuda & Rodrigues, 1999; Pellegrino *et al.*, 1999a, b); total karyotypes have been described for 26 species assigned to 18 genera (Fig. 3). These studies have revealed remarkable chromosome variability among these lizards (diploid numbers ranging from

$2n = 32$ in *Bachia dorbignyi* to $2n = 62$–64 in *Nothobachia ablephara*), probably one of the highest in Squamata.

The extensive variability is not limited to variation in diploid number alone; some taxa are characterized by the presence of supernumerary chromosomes (*Micrablepharus* and *Nothobachia*; Yonenaga-Yassuda & Rodrigues, 1999; Pellegrino *et al.*, 1999a), different mechanisms of sex determination (Yonenaga-Yassuda *et al.*, 1996b; Yonenaga-Yassuda & Rodrigues, 1999), and triploidy (in the parthenoform *Leposoma percarinatum*; Pellegrino, Rodrigues & Yonenaga-Yassuda, ms. submitted).

Two different types of karyotypes have been found among gymnophthalmids: those with a clear distinction between macrochromosomes and microchromosomes, and those with chromosomes decreasing gradually in size. In some genera (*Gymnophthalmus, Placosoma* and *Leposoma*), very distinct kinds of karyotypes have been described for closely related species. The highest diploid numbers were found in species of *Calyptommatus, Micrablepharus, Leposoma* and *Placosoma,* and were not associated with the presence of macro- and microchromosomes, but with gradually decreasing size of chromosomes. The presence of these distinct complements in the same monophyletic radiation, along with the range of diploid numbers and other classes of variation, suggest characters that represent some synapomorphies useful in a phylogenetic context. However, karyotypes need to be obtained from more taxa, and banding techniques extended to all of these so that inferences of homology, and the kinds of rearrangements that might diagnose historical entities, are unambiguous. These classes of high-resolution chromosomal data can then be coded on the basis of individual characters, and included in an extended phylogenetic analysis (see Borowik, 1995; Flores-Villela *et al.*, 2000, for recent examples).

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