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Phylogenetic Relationships of Chelid Turtles (Pleurodira: Chelidae) Based on Mitochondrial 12S rRNA Gene Sequence Variation

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Conflicting phylogenies have been proposed for the Chelidae (Testudines: Pleurodira), a family of side-necked turtles found only in Australasia and South America. Sequence data from the mitochondrial 12S rRNA gene were used to test these phylogenies. In total, 411 nucleotides were sequenced for each of 16 chelid species, including all 11 recognized chelid genera and, as outgroups, 5 genera of Pelomedusidae (Testudines: Pleurodira). Analyses using parsimony and neighbor joining algorithms strongly support the division of Australian Chelidae into the three monophyletic groups initially suggested by Burbidge *et al.* (1974; *Copeia* 2: 392–409): *Chelodina* (bootstrap value 99%), the *Emydura* group (87%), and *Pseudemydura*. The analyses suggest that the Australian chelids are a monophyletic lineage (64%), with the Australian long-necked turtles, *Chelodina*, more closely related to the Australian short-necked chelids than to the long-necked South American species. These relationships are in contrast to those of Gaffney (1977; *Am. Mus. Novitates* 2620: 1–28). The species of Australian long-necked chelids consistently form a monophyletic clade, with *Chelodina longicollis* and *Chelodina oblonga* as sister taxa. The data failed to resolve relationships among the Australian short-necked taxa: *Emydura*, the *Elseya latisternum* group, the *Elseya dentata* group, *Rheodytes*, and *Elusor*. Unlike Gaffney (1977), we find some weak support (58%) for *Pseudemydura* as the closest relative of the other Australian short-necked taxa. With the exception of *Hydromedusa*, the South American taxa are monophyletic and the subgenera of *Phrynops* are paraphyletic. © 1997 Academic Press

INTRODUCTION

Side-necked turtles of the suborder Pleurodira comprise two families, Pelomedusidae and Chelidae, which have been clearly separated on the basis of morphological and molecular data (Gaffney, 1977, 1991; Pritchard, 1979; Bull and Legler, 1980; Gaffney and Meylan, 1988). The Chelidae occur only in South America, Australia, New Guinea, and the Indonesian island of Roti. Fossils chelids have not been found outside these regions (Ernst and Barbour, 1989), suggesting that this family has been restricted to the Southern hemisphere and is of Gondwanan origin. Eleven chelid genera are represented: *Chelus, Hydromedusa, Platemys, Acanthochelys,* and *Phrynops* in South America and *Chelodina, Emydura, Elseya, Rheodytes, Elusor,* and *Pseudemydura* in Australasia.

Phylogenies of the Chelidae have been inferred from morphology (Burbidge *et al.*, 1974; Gaffney, 1977, 1991; Pritchard, 1979; Legler, 1981; McDowell, 1983; Gaffney and Meylan, 1988), karyotypes (Bull and Legler, 1980), serology (Frair, 1962, 1980; Burbidge *et al.*, 1974), and electrophoresis (Georges and Adams, 1992). There has been little agreement among these phylogenies and two main conflicting hypotheses have been proposed, typified by the phylogenies of Burbidge *et al.* (1974) and Gaffney (1977).

Based on an analysis of morphological and serological data, Burbidge *et al.* (1974) concluded that all Australian forms were more closely related to each other than to any of the South American species examined, suggesting a monophyly of the Australian chelids. In contrast, Gaffney's (1977) cladistic analysis of chelid relationships, based principally on cranial characters, concluded that the long-necked chelids, *Chelodina* (Australia), *Hydromedusa* (South America), and *Chelus* (South America), formed a monophyletic group spanning the two continents. Gaffney (1977) also concluded that the Australian chelid *Pseudemydura* was the sister taxon to all other chelids.

Neither hypothesis has gained wide acceptance, but several other studies have contributed to further understanding of the relationships of the long-necked chelids. For example, Pritchard (1984) regarded the elongated head and neck structure of *Hydromedusa* and *Chelo-*

Sequence data from this article have been deposited with the GenBank/EMBL Data Libraries under Accession Nos. U40392 and U40633–U40651.

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dina to have arisen, not from a close phylogenetic relationship, but from parallel evolution as they became specialized for piscivory. The origins of the South American and Australasian chelids, in particular of the long-necked species, require further examination.

Within Australasia, three groups have been consistently identified: *Pseudemydura,* the *Chelodina* species, and the *Emydura* group (consisting of *Emydura, Elseya, Rheodytes,* and *Elusor*) (Burbidge *et al.,* 1974; Gaffney, 1977; Bull and Legler, 1980; Webb, 1978). However, within the latter two groups there has been conflict among hypothesized phylogenetic relationships. For example, *Elusor* and *Rheodytes* cannot be consistently placed within the Australian radiation with available data (Legler and Cann, 1980; Georges and Adams, 1992; Cann and Legler, 1994), *Elseya* is reportedly a paraphyletic assemblage (Boulenger, 1889; Pritchard, 1967; Legler and Cann, 1980; Georges and Adams, 1992), and the affinities of *Chelodina oblonga* within *Chelodina* are obscure (Burbidge *et al.,* 1974; Legler, 1981; Georges and Adams, 1992).

We use 12S rRNA mitochondrial DNA sequences to resolve more fully the phylogenetic relationships of the Chelidae. Mitochondrial DNA sequence data have been widely applied to phylogenetic studies, examining taxa of varying divergence times. Its wide applicability has been attributed to the high, but internally variable, rate of sequence evolution. The divergence time of the Chelidae within the Pleurodira has been estimated at 65 MY (Chen *et al.,* 1980) and the earliest fossil chelid is eocene (Benton, 1993). A pilot study revealed 10.8% sequence divergence among chelid species to 19.2% sequence divergence between chelid species and a pelomedusid outgroup for 12S rRNA, indicating its suitability for elucidating the phylogenetic relationships among the Chelidae (Baverstock and Moritz, 1990; Mindell and Honeycutt, 1990).

MATERIALS AND METHODS

Blood or liver was obtained from 16 species, representing each of the recognized Australasian and South American genera of chelids, the three subgenera of *Phrynops,* the two generic groups of *Elseya* (Legler, 1981), and three subspecies groups of *Chelodina* (Table 1).

DNA was extracted using proteinase K digestion, phenol:chloroform extraction, and ethanol precipitation (after Bothwell *et al.,* 1990). A portion of the 12S rRNA gene was amplified from genomic DNA (gDNA) by the polymerase chain reaction (PCR; Mullis and Faloona, 1987) using the "universal" primers of Kocher *et al.* (1989). PCR products were purified using the

TABLE 1

Specimens of Chelidae and Pelomedusidae Examined

Taxon	Origin[a]	Tissue[b]	Collection[c]
Acanthochelys pallidopectoris	Chaco Region, Argentina, SAM	Blood	McCORD
Chelodina longicollis	Hunter River, NSW, Aus	Liver	AM R123056
Chelodina oblonga	Perth, WA	Blood	AM R125478
Chelodina rugosa	Darwin, NT	Liver	NTM R13437
Chelus fimbriata	Guyana, SAM	Blood	McCORD
Emydura macquarii	Murray River, VIC	Liver	AM R120956
Elseya dentata	Victoria River, NT	Liver	NTM R13521
Elseya latisternum	Tweed River, NSW	Liver	AM R123032
Elusor macrurus	Mary River, QLD	Liver	AM R125485
Erymnochelus madagascarensis	Madagascar	Blood	McCORD
Hydromedusa tectifera	Uraguay, SAM	Blood	McCORD
Pelomedusa subrufa	AFR	Blood	McCORD
Peltocephalus durmerilliana	SAM	Blood	McCORD
Pelusios sinnuatus	Tanzania, AFR	Blood	McCORD
Phrynops (Batrachemys) nasuta	Surinam/Guyana, SAM	Blood	McCORD
Phrynops (Mesoclemmys) gibbus	Surinam/Guyana, SAM	Blood	McCORD
Phrynops (Phrynops) geoffroannus	Bolivia, SAM	Blood	McCORD
Platemys platycephala	SAM	Blood	RMZG
Podocnemis expansa	Brazil, SAM	Blood	McCORD
Pseudemydura umbrina	Perth, WA	Liver	KUCHLING
Rheodytes leukops	Fitzroy River, QLD	Liver	AM R125481

[a] Location abbreviations: Australia: NSW, New South Wales; WA, Western Australia; NT, Northern Territory; QLD, Queensland; VIC, Victoria; SAM, South America; AFR, Africa.

[b] Liver specimens of Australian chelids were provided by the frozen tissue collection of Georges and Adams (1992). Turtles were identified and blood collected for the South American chelids, except *Platemys,* and for pelomedusids by one of us (W.M.). For *Platemys,* blood was collected from a specimen housed at Royal Melbourne Zoological Gardens.

[c] Museum abbreviations: AM, Australian Museum, Sydney; McCORD, Live Collection held by Bill McCord, Hopewell Junction; NTM, Museums and Art Galleries of the Northern Territory, Darwin; RMZG, Royal Melbourne Zoological Gardens, Melbourne.

Wizard PCR Preps DNA Purification system (Promega). Sequencing was performed using the PRISM Ready Reaction Dye-Deoxy Terminator Cycle Sequencing kit (Applied Biosystems), with 50 fmol (20 ng) of purified PCR product as template and the Applied Biosystems Model 373A DNA Sequencing system. Both strands were sequenced with repeat sequencing of at least one strand of each sample. Sequences have been deposited with GenBank (Accession Nos. U40392, U40633–U40651).

The sequences of the 12S rRNA gene were aligned with Clustal-W (Thompson *et al.,* 1994) using default settings, and the consequent alignment was adjusted by eye to improve inferred homology. Phylogenetic analyses were undertaken using parsimony in PAUP Version 3.0 (Phylogenetic Analysis Using Parsimony; Swofford, 1991) with the default settings unless otherwise indicated. Nucleotide positions were treated as unordered discrete characters and indels coded as missing data. Heuristic searches were performed using tree-bissection-reconnection branch swapping. Although heuristic searches do not guarantee to find the most parsimonious tree, computer power and time necessitated their use for analysis of a large number of species. A distance method, neighbor joining, was also employed to derive a phylogeny for the Chelidae. Neighbor joining analysis was performed using Kimura's two parameter distances, calculated using a pairwise deletion of gaps and missing data, in MEGA 1.01 (Molecular Evolutionary Genetics Analysis; Kumar *et al.,* 1993).

RESULTS

Sequence Data

In total, 411 nucleotides were sequenced for all taxa and 41 nucleotides removed as regions of questionable homology (Fig. 1). Of the remaining 370 sites, 242 are variable and 159 are informative under parsimony.

Parsimony Analysis

A single most parsimonious tree was obtained from an heuristic search of 21 taxa (Fig. 2). This tree supports monophyly of both the Australian chelids and the South American chelids. Within the Australian taxa, *Pseudemydura* is the closest relative of a clade containing the other short-necked Australian chelids, *Rheodytes, Elseya dentata, Emydura, Elseya latisternum,* and *Elusor.* The species of *Chelodina* form a monophyletic group, with *Chelodina longicollis* the sister species of *C. oblonga.* The South American taxa are monophyletic; however, this monophyly is supported by only two unambiguous character changes.

Hydromedusa is placed outside a clade containing the other South American taxa. The subgenera of *Phrynops* are paraphyletic on this tree: *Phrynops* (*Mesoclemmys*) is given as the sister taxon to *Phrynops* (*Batrachemys*) but *Phrynops* (*Phrynops*) is most closely related to *Chelus.*

The robustness of the tree was determined by the bootstrap resampling method (Felsenstein, 1985). In the 50% majority rule tree resulting from 1000 bootstrap replicates and an heuristic search (Fig. 3), the relationships of *Hydromedusa* and those among the *Emydura* group (containing *Emydura, El. dentata, El. latisternum, Elusor,* and *Rheodytes*) are unresolved. There is strong support (>90%) for the monophyly of the *Chelodina* clade (99%), the placement of *C. longicollis* as the sister taxon to *C. oblonga* (97%), the linking of *Phrynops* (*Mesoclemmys*) with *Phrynops* (*Batrachemys*) (99%), and the linking of *Platemys* with *Acanthochelys* (94%). There is also moderate support (>80%) for a monophyly of *Emydura, Rheodytes, Elusor,* and *Elseya* species (87%) and for the monophyly of the South American taxa, except *Hydromedusa* (84%).

Neighbor Joining Analysis

The most significant difference between the neighbor joining tree (Fig. 4) and the parsimony tree is the placement of *Hydromedusa* as the closest relative of the monophyletic Australian chelids. In addition, the neighbor joining tree places the *El. latisternum* group as the sister taxon to *Emydura,* with the *El. dentata* group outside this clade. In the tree resulting from 1000 bootstrap replicates of the neighbor joining analysis (not shown), this relationship of *Hydromedusa* with the Australasian chelids remains with weak support (50% bootstrap value). In comparison with the parsimony analysis, the bootstrapped neighbor joining tree lends moderate support (83%) to a monophyly of *Pseudemydura* with the other short-necked Australasian chelids.

Constrained Phylogenies

Constraining the tree to Gaffney's (1977) phylogeny (with the addition of *Elusor* and *Rheodytes* to the position of *Emydura* in the phylogeny), an heuristic search produced a tree which has a length of 616, an increase of 23 steps over the unconstrained tree (length 593).

Legler (1981) described several features shared by *Pseudemydura* and *Platemys* that suggest a close relationship. Imposing the constraint of a monophyly of *Pseudemydura, Platemys,* and *Acanthochelys* within the South American chelids, an heuristic search produced a most parsimonious tree of length 612, 19 steps longer than that of an unconstrained search (length 593).

DISCUSSION

Chelid Phylogeny

Parsimony and neighbor joining analyses have established the following phylogenetic relationships:

- the short-necked Australian chelids, *Elusor, Rheodytes, Emydura,* and *Elseya,* form a monophyletic group,

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                                                 50                                                100
Pum CCTTAAACC- TAGATTTTTT T----ATATA T-AAAAATTT ACC-AGAGAA -CT-ACAAAC TAAACGTTTA AAATTCAAAG GACTTGGCGG -TACCTCAA- ACCCACCTAG
Rle ..C....... .......... A..TTT...- -........G G......... A......... C...T...GG ...C....G. .......... C.G..C.... ..........
Elm ..C...T... .......... A..AA.C.C- -.......C. G......... .......... C...A....G ...C...... .......... ..G..C.... ..........
Ema ..C...T... ..A....... A..AG...C- -.....T..G G......... .......... C...T...GG ...C....G. .......... ..G..C.... ..........
Ela ..C....... ..A.....G. A..AN...C- -..GN..... G......... .......... C......... N..C....G. .......... ..GT.C.... ..........
Ede ..C....... ..AN...... A....--... .A.......G G.......T. ...T...... C...T....G ...C....G. .......... ..G....... ..........
Clo .......... ...TA..-CC ACAGCCC.C. .A.GG...CC G......... .......... C......... ...C....G. .......... ..G.T..... ..........
Cob ..C.....A. ......--CC ACAACCC.C. .A.GG...CC G......... .......GN. C.N....... N.......G. ....G..G.. ..GTTC.... ..........
Cru ..C.....T. C.....CCAC AACCC--.C. .A..G...CC G......... .......... C......... ...C...... .......... ..G.TN.... ..........
Cfi ..CC..TTA. C........C .CCA.--.C. ........CC G......... .......C.. CCTGAA..AG ...C...... ......A... ..GT...... .....A....
Ppl ..C...TAA. .........C .CAA.-..C. ......T.CC G.......C. AN....T.TT C.T..A..NG ..GC...GG. .TT.N..T.. ..TT...... C....A....
Apa ..C.....A. .G.......C .NTACG..C. ........CC G......... AT.T-TT..T C.C.AA.... ..TC...GG. .TT...CG.. ..GT...... .T...T....
Pge ..C.....A. ..T......C -CCA.-..C. ........CC G.......G. AT.T-.C... C.CGAA..GG ...C....G. ..T....G.. ..GTT..... .....A....
Pgi ..C.....A. .......... -CCA.-..C. A.....T.CC G.......TT ...T-TTN.. A...AA..GG G..N....G. .GN....... .GGT...... .....AT.T.
Pna ..C.....A. .......... -CAA.-N.A. A.....T-CC G..C....T- ..NT-TTT.. CC..AA...G G..C...... .G........ .GGN...... ....CA..N.
Hte ..C....... A........A ACA..-..C. .....GG.CC G......... A.....C..T C....A...G ...C....G. ......A... ..GT...... -.........
Psu ..C...N... AT...A...A ACATA.C.C- -....T..CC G..C-..... ......G.G. CT.CA.C... ...C.T..G. .......... ..GT.C..T. .....A....
Erm ..C...TT.T C....A...A -.CT.-..C. ..G..T..CC G......... ........G. A...T.C.CG ...C....G. ......A... ..GT...... ....C.....
Psi ..C....T.. AT...A..CC ACAT.-A.C. .....T..CC G..C-..... ......G.G. .T.TA.C... ...C...... .......... ..GT.C..TN ....TA....
Pex ..C....... C....A--CC CACT.-..C. A..G.T..CC G......... ........G. C...T.C... ...C....G. ......A... ..G....... ..........
Pdu ..C.....A. .T...A.--C .ATT.-..C. ...G.C..CC G......... ......G.G. -.CC..C..G ...C...... .......... ..GT..T... .....T....
Cin ..C....... A........A AAA..-ACA. AA..TTC.-G G...G.G... ...T-..C.. C........G G..C...... .GT....... .GG..CT... ........G.
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                                150                                                200
Pum AGGAGCCTGT TCTATAATCG ATAACCCACG ATTAACCTCA CCACCCCTTG CCCT--CAGC CTATATACCT CCGTCTACAG CTCACTCTAC AANGAGAT-A AAAGTAAGCC
Rle .......... .......... ....T..... ...C.....G .....T..C. ...A...... .......... .....C.... ..T..C..G. T.A.GA.... .........A
Elm .......... .......... ....T..... ...T.....G .....T..C. ...A...... .......... .....C.... ..T..C.... T.G.G..C.. .........A
Ema .......... ...N...... ....T..... ..NC...... .....T..C. ...A...... .......... ...N.C.... ..T..C.... T.A.GA.N.. .........A
Ela .......... .......... ....T.N... ...C...... .....T..C. ...A...... .......... .....C.... ..T..C.... C.C.GAGA.. .........A
Ede .......... .......... ....T..... .NCT...... .....T..C. ...A...... .......... .....C.... ..T..C.... T.A.GA.C.. .........A
Clo .......... .......... ....T..... ..AC...... ...T.T..A. ...A...... .......... .......... ..T..C..GT G.A.GA..T. .........A
Cob .......... .......C.. ....T..... ...TG..... ...T.T..A. ...A...... .......... ...N..C... ..T..C...T G.A.GA.C.. ....C....A
Cru .......... .......... ....T..... ..CT...... ...T.T..A. ...A...... .......... .......... ..T..C...T G.A.G..... .........A
Cfi .......... C.C.A..C.. ....T..... ..AC....A. ....TT..A. ...A...... .......... ......C... .CT..CT..T G.AAGT.C.. ......G..A
Ppl ....N..... .......T.. ...CT..... ..CT...... ......A.AN ...ATN...T A.....N... ..T...C... .CTT....TT GGGNG...A. T.......GA
Apa G.......T. .......... ...CT..... ..CTC..... ......A.G. ...NC..... ......C..C ..C...T... .CT..CT..T G.A.GTT... ..G...G.NA
Pge .......... ...TC..C.. ..T.T..... ...T...... ........A. ..AAT..... .......... ......T... .CT..CT..T G.AA-..C.. ......G..A
Pgi G..G....T. ...T.T.... G..CT..... ...T...... ......T.A. G..A.....G ..T.T.T..C ..C...T... .CTC.C..GG G.AAGAT..T ..G..GG..A
Pna .....N.... .......... ....T..... ...T...... ......T.A. ...C...... ......N... ......T... .CT..C..GT G.AAG....T ......G..A
Hte .......... .......... ....T..... ...C...... ...T.T.... .......... .......... .......... T.T..CT..T G.AAGT.... .......A.A
Psu .......... .......G.. ..T.T..... ..C.....T. ......T... ..A.C..... .........G .......A.. .....CT..T G.AAGCTA.. .........A
Erm .......... .......G.. ....T..... ..A.....T. .....T.... ...AT..... .........G ......CT.. .CT..CT.GT G.AAGATC.. .....T...A
Psi .......... ....C..G.. ....T..... ..A.....T. ......T... ..A.N..... .........G .......... ..T..CT..T G.AAGCTA.. .........A
Pex .......... .......G.. ....T..... ..A.....T. ...T.TT... ...CC..... .........G ......C... .CT..CT.GT G.GAG..A.. G........A
Pdu .......... .......G.. .C...A.... ..A.....T. .....T..C. ..A....... .........G ...C.GC... .CT..CT.GT G.AAGC.C.. T........G
Cin ...G...... ...T...... ....T..... ...C.....N .....T.... G..A.....G ...T.T.... ...G...... G.G..C...N T.G.G..... .........A
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                                250                                                300
Pum AANCAATC-- TACATAATTA ATAAGTCAGG TCAAGGTGTA GCCAA-TGGG GTGGAAGAAA TGGGCTACA- --TTTTCTAA ATTAGAAATA -ACTAA---- -CGGAAAGAA
Rle C.AT.G.T.. C.TNC..C.. NC........ .......... .....C..A. .......... .......... .........C .C........ .TTC.-.... .........G
Elm T.AT.G.T.. ..T.C..C.. .C........ .......... .....C..A. A......... .......... .........C CCC....... .TTC.-.... .........G
Ema C.A..G.... .N..C..C.. .C........ .......... .....C..A. .......... .......... .........C .C........ .TTC.-.... .........G
Ela C.AT.G.... ....C..C.. .C........ .......... T....C..A. .......... .......... .........C .C........ .TTC.-.... .........G
Ede C.AT.G.T.. ....C..C.. .......... ......N... .....C.... ....N..... ...C...... .........C .C........ .TTA.-.... ..........
Clo T.A..G.TA. -...C.GC.. .C........ .......... ....NC..A. A......... .......... .........T .C.....T.. TTT....... .........G
Cob T.C..G.TA. -T..C.GC.. GC........ .......... .....C..A. A......... .......... .........T .......C.. CTTC.-.... .........G
Cru T.AT.G.TA. -...C..C.. .C........ .......... .....C..AA A......... .......... .........T .......T.. ....C-.... A........G
Cfi C.A..G.TAA C---C..C.. .C........ .......... ........AA A...TG..G. .......... .........C .A......C. TCT..CACTA A.A...C.G.
Ppl CCA..GC.AN C..---.C.C .C....G..C .........N C..CN..... ....N....G .....N.... ........CC CA........ C...TTA... A.....G.G.
Apa T.ATNGC.AA C.AGC.-C.. .C........ ...G...... ...C...... .N........ .C.N...... ........CC CAA..G..AT CT.CTTA... A.A.G.G.GG
Pge C.AT.G..AC A.A.AC.C.. .C..A..... .....T.... N...T..NA. .......... .......... ........TC CA...TC... CC.CCTA... A....TT.G.
Pgi C.AT.G..AC C.A-GC.C.. TC........ .......... .......TA. C...G..... .......... .........C .A........ .TTCTT.... A....GG.C.
Pna C.AT.G..AA C.A-GC.C.. .C........ .......... T......TA. .......... .......... .........T .A........ .TTCTT.... A....GG.C.
Hte C.AT.G.TAA C...A..C.. .......... .......... ...T.T..A. A......... .........T TT.C.A...T .GA.AT.T.T ..ACG-.... .--....ACC
Psu ..AT.GCT.. ACT.C.GC.. GA........ ..C....... .......A.. .....T.T.. .......... .......... .A........ .TTC.-.... .....C...C
Erm C.AT.G..C. ACT.C..C.. .A........ ..C....... ........A. .......... .......... .......... CA....T... .GTC.-.... ..........
Psi ..AT.GC.C. ATT.C.GC.. GA........ ..C....... .......A.. ....GT.C.. .......... .......... .A.......N .TTC.-.... ...A.C...C
Pex C.AT.G..C. A-T.C..C.. GA.C...... ..C...C... ........A. A......... .......... .......... TA........ .GTC.-.... .....G...G
Pdu ..AT.G.AT. A-A.CC.C.. .......... ..C....... ........A. ....C..... .......... .......... .A........ .TT..-.... A....G...C
Cin T.AT.G..T. ----A..C.. C...C.A..C .G.TC....T ..ATCC..NT .A..T..G.G ...T.GG..A NA.C.....C .CC.A..... ..TCG-.... .GA......C
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                      350                                                400        411
Pum CCTTGAAAT- -ATGGGTCT- AAAAATAGGA TTTAGCAGTA ATGTGTGAAC AGAGAGCCCA CATTAACCCG GACCTGA-GA T
Rle .......... .....AC... ....G..... .......... .....AA.N. .....N.... .......... ..T...G..- C
Elm ..C....... .....AC..T ....G..... .......... .....A..G. .......... .......T.. ..T...G.AG C
Ema ..C....... .....AC... ....G..... .......... .....AA.G. .......... .......T.. ..T...G... C
Ela .TC....... ...A.AC... ....GC.... .......... .....AA.G. .......... .......T.. ..T...G... C
Ede ..C....... .....ACT.. ....G..... .......... .....AA.G. .......... ........A. ..T....... C
Clo ........A. .CATT.CTCT ....GC.... .......... ..A..A.... ...A...... T......AA. ........AG C
Cob ........A. .CATT.CTCT T...GC.... .......... ..A..A.... .......T.. T......AA. .......TAG C
Cru ..C.....A. C..T.C.... ....GC.... ....T..... ..A..A...T .......T.. T......AA. .......CAG C
Cfi TT........ ..AACCCGA. ....GA.... .......... .....A...T .......TT. ........A. .......... C
Ppl ........-. .CAA.AC.CA .C..GC.... ........C. .....A.... .......... .......T.A. ..T....... .
Apa ..C..G.... .TA..AC.CA T...GC.... .......... ....AA.... ........T. ......TTA. ..-....... C
Pge T.A.....C. .CAATCC.CA ....GC.... .A..T..... ....AA...T .......TT. .......T.A. .......... C
Pgi .T......-. .CA..AC.CA .T..GC.... .......... ....AGA.G. ........T. ....C.TTA. .......... C
Pna .T......-. .CA..AC.CA .T..GC.... .......... ....AGA.G. .......... ......TTA. .......... C
Hte .........T T......T.A T...GC.... .....T.... ....AA.... ........T. ........A. ..T....... C
Psu ........-. .CA...A..A .T..G.T... .......... ..C.AA...T ...A....T. T.....A... .C....G... C
Erm ..C.....-. .CAA.....A .G..GCT.A. .......... ..T.TGA... ......T.A. A.....GTA. ..TA..G... C
Psi ........-. TTA......T .G..G..... .......... ..T.AG...T ........T. T.....G... .C....G... C
Pex ..C....T-. ..AA..C..C ....GC..A. .......... ..T.TG...T ..T.....A. A.....A.A. ..AA....AG C
Pdu T.C.....-. .TAA.A...C T...GC.... .......... ..T.TG...T ........A. A..G..A.A. .CAA...... C
Cin .TC.....-. T.....C..C T...GC.... .......... .....A.... .......... .......T.. ..T...G.AG C
```

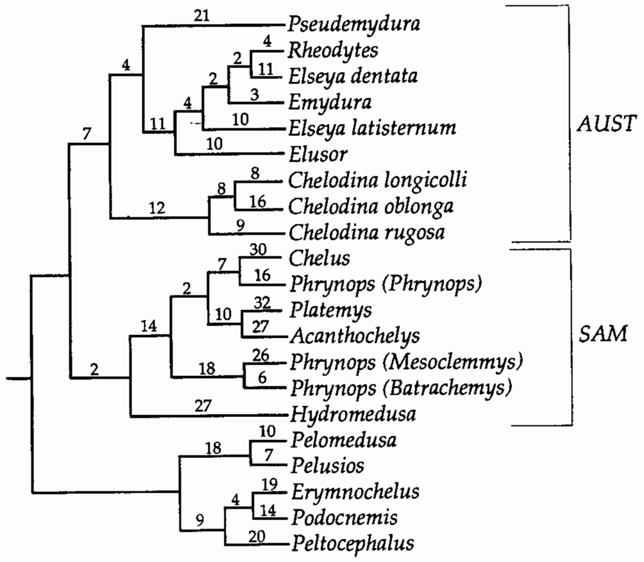

FIG. 2. Most parsimonious tree of chelid turtles based on mitochondrial 12S rRNA sequence data. The tree resulting from an heuristic search has a length of 593 steps and is rooted by the Pelomedusidac genera. The numbers represent the number of characters changing unambiguously on each branch (MacClade; Maddison and Maddison, 1992). Abbreviations: AUST, Australasian; SAM, South American.

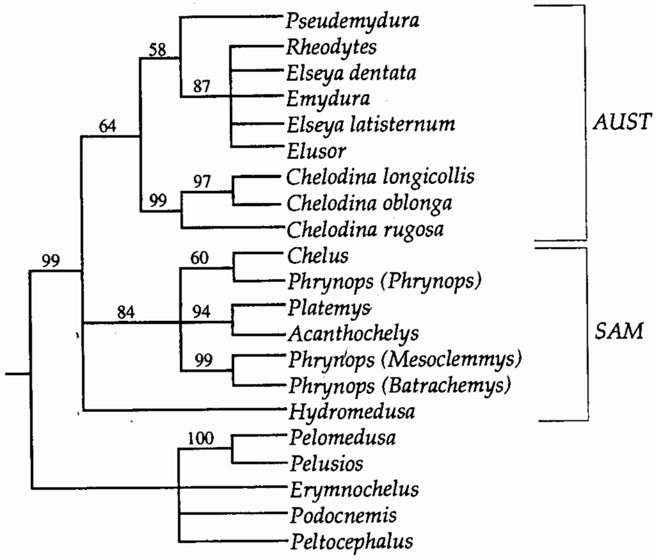

FIG. 3. 50% Majority rule consensus tree of chelid taxa based on 12S rRNA sequence data following bootstrap resampling. Percentages of 1000 bootstrap replicates (heuristic searching) are shown above branches. Tree is rooted by Pelomedusidae genera. Abbreviations: AUST, Australasian; SAM, South American.

- the Australian long-necked chelids, the species of *Chelodina,* are monophyletic, with *C. longicollis* the sister species to *C. oblonga,*
- the South American chelids, *Chelus, Platemys, Acanthochelys,* and the three subgenera of *Phrynops,* form a monophyletic group,
- *Acanthochelys* is the closest relative of *Platemys,* and
- *Phrynops* (*Batrachemys*) is the sister taxon to *Phrynops* (*Mesoclemmys*) but the subgenera of *Phrynops* are paraphyletic.

However, several other relationships within the Chelidae are less well supported in bootstrap analyses: the Australian chelids as a monophyletic assemblage (64%), *Pseudemydura* as the sister taxon to the other Australasian short-necked taxa (58%), and *Chelus* as the closest relative of *Phrynops* (*Phrynops*) (60%).

Our results suggest that the Australasian long-necked chelids, *Chelodina,* are more closely related to the Australian short-necked chelids than to any of the South American species. Such a placement supports a previous phylogeny based on a phenetic examination of morphology and serology (Burbidge *et al.,* 1974) and is consistent with an independent radiation of the Chelidae following the separation of the Australian and South American continents. Monophyly of the Australian chelids contradicts the phylogeny presented by Gaffney (1977), which placed the long-necked chelids, *Chelodina* (Australia) and *Hydromedusa* (South America), as sister taxa with their closest relative *Chelus.* The increase in tree length with the employment of constraints further suggests that the 12S rRNA sequence data support a phylogeny in which the long-necked Australian taxa are more closely related to the other Australian chelids than to the South American long-necked chelids.

The trichotomy of Australian chelids suggested by Burbidge *et al.* (1974) is strongly supported by these sequence data, with the three monophyletic groupings of the *Chelodina* species (99% bootstrap value, Fig. 3), *Pseudemydura,* and the *Emydura* group (87%). *Pseudemydura,* the endangered Western Swamp Tortoise, is found only in southwestern Australia, although fossils

FIG. 1. Alignment of 12S rRNA gene sequences of 16 chelid taxa and 6 outgroup taxa. (.) identical base; (-) alignment gap; (N) nucleotide unknown. Species abbreviations: *Pum, Pseudemydura umbrina; Rle, Rheodytes leukops; Elm, Elusor macrurus; Ema, Emydura macquarii; Ela, Elseya latisternum; Ede, Elseya dentata; Clo, Chelodina longicollis; Cob, Chelodina oblonga; Cru, Chelodina rugosa; Cfi, Chelus fimbriata; Ppl, Platemys platycephala; Apa, Acanthochelys pallidopectoris; Pge, Phrynops (Phrynops) geoffroannus; Pgi, Phrynops (Mesoclemmys) gibbus; Pna, Phrynops (Batrachemys) nasuta; Hte, Hydromedusa tectifera; Psu, Pelomedusa subrufa; Erm, Erymnochelus madagascarensis; Psi, Pelusios sinnuatus; Pex, Podocnemis expansa; Pdu, Peltocephalus durmerilliana; Cin, Carettochelys insulpta.* Sequences are numbered from the first base in the reference sequence. Regions of questionable homology removed prior to analysis are indicated by underlining in the reference sequence.

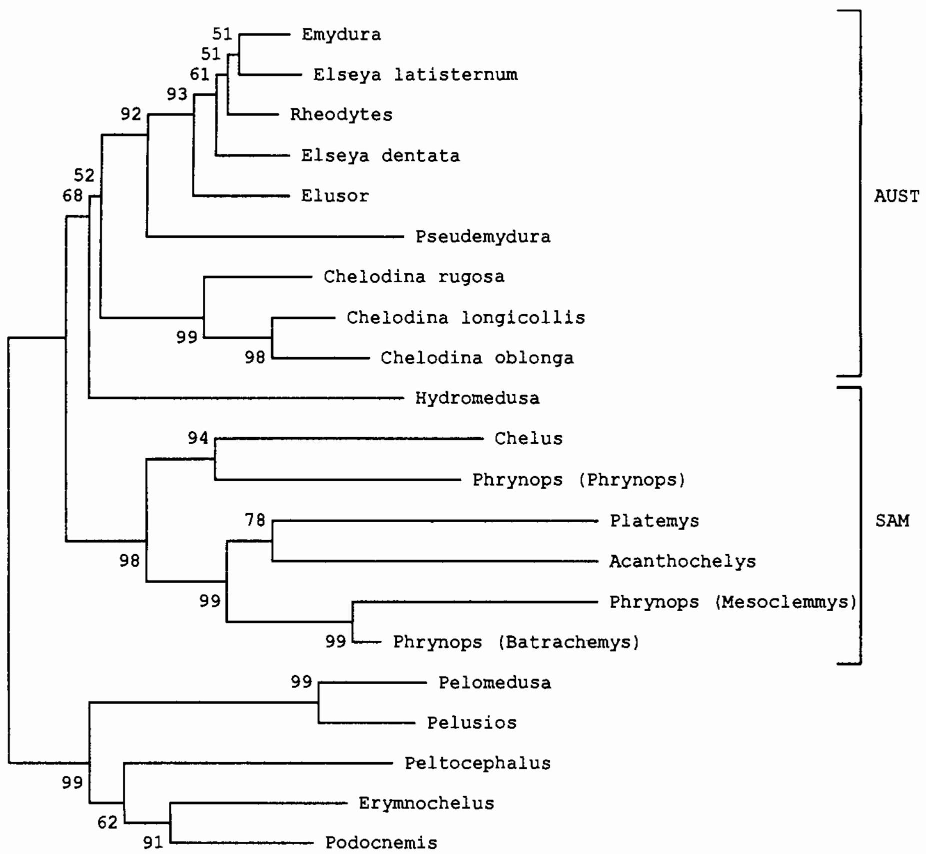

FIG. 4. Neighbor joining tree for chelid taxa based on 12S rRNA sequence data. The tree is based on Kimura's two-parameter distances and is rooted by Pelomedusidae genera. Confidence probabilities are given. Abbreviations: AUST, Australasian; SAM, South American.

recovered in Queensland suggest it was once more widespread (Gaffney, 1991). The relationships of *Pseudemydura* among the other Chelidae have not been well determined. Gaffney (1977) suggested *Pseudemydura* as the sister to all other chelids. However, this relationship was based on one character, the separation of the frontals by the nasal bones, which was considered a defining primitive feature. Several shared features have suggested a close relationship between *Pseudemydura* and the South American chelid *Platemys* (Legler, 1981). The 12S rRNA sequence data are unable to confidently establish the relationships of *Pseudemydura* but suggest that it is the sister taxon only to the other short-necked Australasian chelids, giving a relatively recent divergence of *Pseudemydura*. The relationships within the *Emydura* group could not be resolved by the sequence data. However, neither of the analyses resolve a monophyletic *Elseya,* suggesting that it is paraphyletic.

Chelodina oblonga is restricted in distribution to the southwest corner of the Australian continent and has been considered morphologically and serologically distinct (Burbidge *et al.,* 1974). Burbidge *et al.* (1974) suggest that the Australian chelids were divided into eastern and western populations by the Cretaceous sea, which precluded movement across the Australian continent, leaving *C. oblonga* phylogenetically distinct from the other species. However, other relationships also have been hypothesized. Goode (1967) classified *C. oblonga* as the sister species of *Chelodina rugosa,* and this grouping was supported by morphological and ecological features (Legler, 1981). In contrast, allozyme electrophoretic data support *C. oblonga* and *C. longicollis* as sister species (Georges and Adams, 1992). Analyses of the 12S rRNA sequence data support the allozyme data of Georges and Adams (1992), placing the *C. longicollis* group as the closest relative of *C. oblonga* with 97% bootstrap support (Fig. 3).

Our sequence data provide strong support for the inclusion of *Chelus* in a *Platemys, Acanthochelys,* and *Phrynops* clade, in contrast to the conclusions of a phenetic analysis of karyotypic data (Bull and Legler, 1980). The sequence data consistently support a paraphyly of the subgenera of *Phrynops,* with two of the subgenera, *Phrynops* (*Batrachemys*) and *Phrynops* (*Mesoclemmys*), as sister taxa. The third subgenus, *Phrynops* (*Phrynops*), is more distant and is placed (with limited bootstrap support) as the closest relative to *Chelus*. None of the subgenera of *Phrynops* are closely related to *Hydromedusa,* as suggested by karyotypic similarities (Bull and Legler, 1980).

The relationships of the South American long-necked chelid, *Hydromedusa,* have not been resolved by this study. A close relationship with the Australian chelids (Fig. 4) would be consistent with the radiation of Australian chelids from a long-necked ancestor which also gave rise to *Hydromedusa.* However, Pritchard's (1984) suggestion of multiple independent origins of the long neck in *Chelodina* and *Hydromedusa* cannot be discounted. Pritchard (1984) argued that the expan-

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