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A phylogeny for side-necked turtles (Chelonia: Pleurodira) based on mitochondrial and nuclear gene sequence variation

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Aspects of the phylogeny of pleurodiran turtles are contentious, particularly within the Chelidae. Morphological analyses group the long-necked Australasian *Chelodina* and the long-necked South American *Chelus* and *Hydromedusa* into a single clade, suggesting a common derived origin of the long neck and associated habits that predated the separation of Australia from South America. In contrast, published analyses of *12S rRNA* and cytochrome *b* sequences suggest that the long-necked *Chelodina* are more closely related to the short-necked Australasian genera than to either *Chelus* or *Hydromedusa*. This paper adds partial sequences of *16S rRNA* and *CO1* mitochondrial genes and partial sequences of the nuclear oncogene c-*mos* to test a range of previous hypotheses on the phylogenetic relationships among chelid turtles. In total, 1382 nucleotides were available for each of 25 taxa after elimination of ambiguously aligned regions. These taxa included representatives of all the genera of the turtle families Chelidae and Pelomedusidae, the three sub-genera of *Phrynops*, and recognized sub-generic groups of *Elseya* and *Chelodina*. Of the four genes examined, *12S rRNA* was the most informative, followed by c-*mos* with *16S rRNA* and *CO1* the least informative. The molecular data support the currently accepted arrangement for pelomedusid genera, that is, a sister relationship between the African *Pelusios* and *Pelomedusa* and a clade comprising the South American *Peltocephalus* and *Podocnemis* with the Madagascan *Erymnochelys*. However, there is also support for *Erymnochelys* and *Podocnemis* as sister taxa to the exclusion of *Peltocephalus* (bootstrap values of 69–80%) which is at odds with the most commonly accepted arrangement. The South American chelids are monophyletic (76–82%). This clade includes the long-necked *Chelus* and *Hydromedusa*, but excludes the Australasian long-necked *Chelodina*. Furthermore, the South American long-necked chelids are not themselves monophyletic, with 98–100% bootstrap values for the node supporting *Chelus* and the remaining South American chelids to the exclusion of *Hydromedusa*. Hence, the hypothesis of a monophyletic grouping of the long-necked genera of South America and Australasia is not supported by the molecular data. Although reciprocal monophyly of the South American and Australasian chelid faunas was the most likely and the most parsimonious arrangement in all but one analysis, bootstrap support for the monophyly of the Australasian chelids was low (52–66%). The South American chelids, *Chelodina* and the short-necked Australasian chelids form an unresolved trichotomy. The genera *Phrynops* and *Elseya* are paraphyletic, leading to a recommendation

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to elevate the three sub-genera of *Phrynops* to generic status and support for previous suggestions to erect a new genus for *Elseya latistermum* and close relatives. A revised classification of the extant Pleurodira is presented, consistent with the phylogenetic relationships that emerge from this study.

ADDITIONAL KEY WORDS:—Turtle – mtDNA – c-*mos* – phylogeny – systematics – Chelidae – Pelomedusidae.

CONTENTS

INTRODUCTION

Two living families of side-necked turtles (sub-order Pleurodira) have traditionally been recognized. The Chelidae is represented by eleven genera—*Acanthochelys*, *Chelus*, *Hydromedusa*, *Phrynops* and *Platemys* of South America and *Chelodina*, *Emydura*, *Elseya*, *Elusor*, *Pseudemydura* and *Rheodytes* of the Australasian region. Their fossil record extends back to the Upper Cretaceous of South America (de Broin, 1987) and the Miocene of Australia (Gaffney, Archer & White, 1989), but no fossil chelids are known from outside the present range of the family (Williams, 1953a, b; Gaffney, 1991). All but one species of Australian freshwater turtle are chelids, which is the only clear Gondwanan group of Australian reptiles. The second family, Pelomedusidae, comprises five extant genera from South America (*Podocnemis*, *Peltocephalus*), Africa (*Pelusios*, *Pelomedusa*) and Madagascar (*Erymnochelys*). Its fossil record is far more extensive than that of the chelids, being drawn from deposits in Europe, North and South America, Africa, India and Asia, and their fossil record dates back to the Cretaceous (Pritchard, 1979).

Several aspects of the phylogeny of these animals are contentious, particularly within the Chelidae. The very early literature splits the Chelidae into two groups – those with a neck shorter than the shell (*Phrynops*, *Platemys*, *Elseya* and *Emydura*) and those with longer necks (*Chelus*, *Hydromedusa* and *Chelodina*) (Boulenger, 1888). More recently, Gaffney (1977) presented the first comprehensive phylogeny of chelid turtles, based on a cladistic analysis of cranial and postcranial skeletal characters. Consistent with the earlier view, his analysis grouped the long-necked Australasian *Chelodina* and the long-necked South American *Chelus* and *Hydromedusa* into a single clade. Similarly, de Broin and Fuente (1993) grouped *Chelodina* and *Hydromedusa*

together on consideration of fossil material from Argentina. This suggests a common derived origin of the long neck and associated habits that predated or was coincident with the separation of Australia and Antarctica from South America. Pritchard (1984) took a more functional approach to his interpretation of characters. For example, the development of a long neck requires expansion of the anterior plastral lobe and the anterior carapace to accommodate and protect the neck when withdrawn. Pritchard argues that turtles in the three genera have achieved this in fundamentally different ways, indicating independent origins of the long neck, with supporting evidence from a range of other characters. Pritchard does not regard any of the genera as being particularly closely related, but regards *Hydromedusa* as more closely related to *Chelus* than to *Chelodina.*

Seddon *et al.* (1997) used partial *12S rRNA* mitochondrial gene sequences to establish a phylogeny in which the Australasian chelids form a monophyletic assemblage, the long-necked *Chelodina* being more closely related to the short-necked Australasian genera than to either *Chelus* or *Hydromedusa. Hydromedusa* is ambiguously placed as one branch of an unresolved trichotomy, with the Australasian chelids representing a second branch and the other South American chelids a third. *Chelus* is the sister taxon to the subgenus *Phrynops* (*Phrynops*) (see also Kasper, 1903). In a broader analysis involving all turtle families and both molecular and morphological data, Shaffer, Meylan & McKnight (1997) found that the morphological data consistently grouped *Chelodina* with *Chelus*, whereas the molecular data (*12S rRNA* and *cytochrome b* combined) supported the monophyly of each of the Australasian and South American forms. The morphological analyses of Gaffney (1977), Pritchard (1984), de Broin and Fuente (1993) and Shaffer *et al.* (1997) are equivocal, and the molecular data are limited in terms of either the number of chelid genera examined (Shaffer *et al.*, 1997 lacked Hydromedusa) or the number of genes examined (Seddon *et al.*, 1997 examined 12S rRNA only). The issue of the monophyly of each of the Australasian and South American chelid turtles in general, and the relationships among the long-necked chelid turtles in particular, is yet to be satisfactorily resolved.

The study of Seddon *et al.* (1997) failed to resolve a series of important questions, tantalizingly suggested by the parsimony analysis but receiving insufficient support following bootstrapping and majority consensus. *Hydromedusa*, a taxon of considerable antiquity (early Eocene; Wood & Moody, 1976), could not be placed reliably in the chelid phylogeny, and remained part of a deep unresolved trichotomy. Nor could the analysis unambiguously resolve any relationships among the short-necked Australian genera *Emydura*, *Elseya*, *Rheodytes* and *Elusor*. At issue here also is the paraphyly of the genus *Elseya*, first indicated by allozyme electrophoresis (Georges & Adams, 1992), supported by the parsimony analysis of Seddon *et al.* but not their bootstrap consensus. It may well be that these genera arose from a series of rapid cladogenic events, with little time for the accumulation of character states that could potentially resolve the branching patterns. Further data are required to make a convincing case for intractable rapid cladogenesis on the one hand, or to reveal the phylogenetic relationships among these taxa on the other.

In this paper, we increase the molecular data available to establish a phylogeny for the side-necked turtles, with the addition of partial sequences of *16S rRNA* and *CO1* mitochondrial genes and partial sequences of the nuclear oncogene c-*mos* to the *12S rRNA* sequences of Seddon *et al.* (1997). Sequences of *12S rRNA* for two additional lineages of Australian chelids identified by Georges and Adams (1992, 1996) were also added. We test a range of previous hypotheses on the phylogenetic

relationships among chelid turtles, including those of Gaffney (1977), Burbidge, Kirsch & Main (1974), Seddon *et al.* (1997), and Shaffer *et al.* (1997) for consistency with this expanded data set. We attempt to resolve several polytomies in these earlier phylogenies and make recommendations for altering the current chelid classification to remove instances of paraphyly at the generic level.

MATERIAL AND METHODS

Sources of DNA

Blood or liver was available from 25 taxa representing all the currently recognized genera of the turtle families Chelidae and Pelomedusidae, the three sub-genera of *Phrynops* and the sub-generic groups within *Elseya* and *Chelodina* (*sensu* Legler, 1981). *Acanthochelys pallidipectoris* (Freiberg, 1945), *Chelus fimbriata* (Schneider, 1783), *Hydromedusa tectifera* Cope, 1869, *Phrynops (Batrachemys) nasutus* (Schweigger, 1812), *Phrynops (Mesoclemmys) gibbus* (Schweigger, 1812), *Phrynops (Phrynops) geoffroanus* (Schweigger, 1812), and *Platemys platycephala* (Schneider, 1792) of the Chelidae, and *Peltocephalus dumerilianus* (Schweigger, 1812) and *Podocnemis expansa* (Schweigger, 1812) of the Pelomedusidae are from South America. *Chelodina longicollis* (Shaw, 1794), *Chelodina oblonga* Gray, 1841, *Chelodina rugosa* Ogilby, 1890, *Elseya dentata* (Gray, 1863), *Elseya georgesi* Cann, 1997, *Elseya purvisi* Wells & Wellington, 1985, *Elseya latisternum* Gray, 1867, *Elusor macrurus* Cann & Legler, 1994, *Emydura macquarii* (Gray, 1830), *Emydura macquarii* (formerly *signata* Ahl, 1932), *Pseudemydura umbrina* Siebenrock, 1901, and *Rheodytes leukops* Legler and Cann, 1990 of the Chelidae are from Australia. Pelomedusids *Pelomedusa subrufa* (Bonaterre, 1789) and *Pelusios sinuatus* (Smith, 1838) are from Africa and *Erymnochelys madagascariensis* (Grandidier, 1867) is from Madagascar. Hereinafter, the binomial names of these taxa will be abbreviated to their genus or to their genus and subgenus, unless two or more taxa from the same genus have been sequenced.

DNA isolation, PCR amplification and sequencing

Partial sequences of *16S rRNA* and *CO1* were obtained for each of the 25 taxa. Sequences of *12S rRNA* were obtained from Seddon *et al.* (1997), supplemented by additional sequences for a sibling species pair (Georges & Adams, 1996), *Elseya georgesi* (Cann 1997) and *Elseya purvisi* (Wells & Wellington, 1985). The c-*mos* gene was sequenced for the following 12 taxa only: *Chelodina oblonga*, *C. rugosa*, *Elseya dentata*, *Elusor*, *Rheodytes*, *Hydromedusa*, *Chelus*, *Phrynops (Phrynops)*, *Phrynops (Mesoclemmys)*, and *Acanthochelys* as ingroup taxa; the pelomedusids *Pelomedusa* and *Podocnemis* as outgroup taxa. These taxa were considered sufficient to address the central hypothesis of the reciprocal monophyly of the South American and Australasian chelid faunas, and specifically the relationships amongst long-necked forms. Although they would be relevant to such hypotheses, reliable c-*mos* sequences could not be obtained for *Pseudemydura*.

Total cellular DNA was extracted from frozen liver by the standard phenol-chloroform method (Sambrook, Fritsch & Maniatis, 1989). Portions of the mitochondrial *12S rRNA*, *16S rRNA* and *cytochrome oxidase 1 (CO1)* genes were amplified

TABLE 1. Details of the PCR and sequencing primers designed in the present study. Letter in parentheses after each primer name represents strand (L, H) or direction (R,F)

Gene	Primer	Sequence
16S rRNA	M89 (L)	5′-AGGAGTGATGCCTGCCCAGTGAC-3′
16S rRNA	M90 (H)	5′-CCTTAATAGCGGCTGCACCATTAGGA-3′
CO1	M72 (L)	5′-TGATTCTTCGGTCACCCAGAAGTGTA-3′
CO1	M73 (H)	5′-CCTATTGATAGGACGTAGTGGAAGTG-3′
c-*mos*	G136 (F)	5′-AAGCAGGTGAAGAAATGCAG-3′
c-*mos*	G137 (R)	5′-TCCAATCTTGCACACACCC-3′

and sequenced with the following primer pairs: L1091/H1478 (Kocher *et al.*, 1989) for *12S rRNA*, 16sar/16sbr (Cunningham, Blackstone & Buss, 1992) and M89/90 (Table 1) for *16S rRNA*, and CO1f-L/CO1a-H (Palumbi *et al.*, 1991) and M72/M73 (Table 1) for *CO1*. Partial c-*mos* sequences were obtained with primers G73/G74 (Saint *et al.*, 1998) or G136/G137 (Table 1). The PCR conditions were: 50-100 ng of DNA, 0.25 μM of each primer, 0.2 mM of each dNTP, 4 mM of $MgCl_2$, 5 μl of 10 × Promega Taq buffer and 0.75 units of Promega Taq DNA polymerase in a reaction volume of 50 μl. PCR cycling conditions on a FTS-320 Thermal Sequencer (Corbett Research) were: cycle 1 (94°C for 3 min, 48° or 55°C for 45 sec, 72°C for 1 min); cycles 2 to 35 (94°C for 45 sec, 48° or 55°C for 45 sec, 72°C for 1 min); cycle 36 (72°C for 6 min, hold at room temperature). PCR products were purified using Bresa-Clean Nucleic Acid Purification Kit (Bresatec). Sequences of both strands were obtained with the same PCR primers by direct sequencing of the double-stranded PCR product using Perkin Elmer ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit. Sequence reactions were electrophoresed on the ABI Model 373A DNA Sequencer or the ABI Model 377 PRISM DNA Sequencer. GenBank accession numbers are: c-*mos* AF109200-9; *12S* AF095893-4; *16S* AF113620-43; *CO1* AF113644-67.

To test whether sequences were nuclear paralogues of mitochondrial genes (Zhang & Hewitt, 1996) rather than the target mitochondrial genes, we carried out the following procedures. First, mitochondrial DNA (mtDNA) was enriched from frozen liver of *Chelodina longicollis* [188, refer to Specimens Examined below] by the caesium-chloride ultra-centrifugation method of Dowling *et al.* (1996). Sequences for the three mitochondrial genes were obtained from this enriched mtDNA template and compared to sequences obtained from total cellular DNA amplifications.

Second, long-range PCR was used to amplify an almost complete copy of the mitochondrial genome for three other DNA samples (*Elseya purvisi* [130], *Emydura macquarii* (formerly *signata* [33, 34])) on the assumption that intact entire mitochondrial genomes are far less likely to be translocated to the nucleus. The long-range PCR products were then diluted and used as templates for the nested amplification of *16S rRNA* genes and *CO1* genes. Direct sequences of these products were compared with sequences obtained from total cellular DNA amplifications. The long range PCR was carried out with primer pair H1478 and M89 (*op cit.*) using the XL PCR kit (Perkin Elmer) containing the enzyme r*Tth* Polymerase. The PCR cycling conditions on a FTS-320 Thermal Sequencer (Corbett Research) were: cycles 1 to 16 (94°C for 90 sec, 68°C for 10 min); cycles 17 to 28 (94°C for 30 sec, 68°C for 10 min and increments of 15 sec per cycle for 12 cycles); cycle 29 (72°C for 10 min, hold at room temperature).

We verified that the long-range PCR product was the only template amplified in the nested PCR of the *16S rRNA* and *CO1* genes. The original total cellular DNA template was diluted to a level equivalent to that which the total cellular DNA would have undergone in the passage through the long range and nested PCR amplifications described above. When PCR was applied to this diluted solution, no amplification of *16S rRNA* or *CO1* was observed. This indicates that the original total cellular DNA did not contribute to the nested amplification from the diluted long-range product.

Finally, the protein encoding sequences of *CO1* were translated with MEGA (Kumar, Tamura & Nei, 1993)) to detect possible shifts in reading frame and premature stop codons likely to be characteristic of nuclear paralogues.

Analysis of sequence data

The mitochondrial and nuclear coding sequences were aligned by eye and non-coding sequences were aligned using CLUSTAL W (Thompson, Higgins & Gibson, 1994). For the ribosomal RNA sequences, regions where the alignment was ambiguous, that is, regions that required large numbers of indels to optimize the alignment, were deleted from the analyses (see Appendix).

The phylogenetic analysis was undertaken in three stages. The first stage involved analyses of a restricted taxon set for which c-*mos* sequences were available (the 12 taxa listed above), in addition to sequences for *12S rRNA*, *16S rRNA* and *CO1*. Analysis by Shaffer *et al.* (1997) gave strong additional support for the well-accepted notion of reciprocal monophyly of the Chelidae and its sister lineage, the Pelomedusidae. Hence, the pelomedusids were used as the outgroup for analysis of relationships within the Chelidae. Where possible, data from separate genes were combined in the phylogenetic reconstructions. The sequence data for different genes was tested for combinability using the Partition Homogeneity Test (Huelsenbeck, Bull & Cunningham, 1996) implemented in test version 4.0d60 of PAUP* written by D.L. Swofford and executed on a Sun Sparc Station and an Apple LC630. If the data partitions failed this test, they were analysed separately rather than in a combined analysis (Bull *et al.*, 1993).

The second stage of the analysis involved the 25 taxa for which sequences of mitochondrial *12S rRNA*, *16S rRNA* and *CO1* were available. For 16 taxa (those for which material was available), two individuals were sequenced to identify potential mislabeling or polymerase chain reaction (PCR) contamination. In most cases, sequences of the two specimens of the same taxon did not differ. To reduce the number of taxa in the interests of efficiency of analysis, all but one of the specimens of *Elseya purvisi* [130] were discarded from the analysis, as they differed by only one base substitution over all sequences. Similarly, *Emydura macquarii* (formerly *signata*) was removed from the analysis as it differed by only ten substitutions from *E. macquarii* proper over all sequences and in preliminary analyses they were always strongly supported sister lineages. Thus 23 taxa remained for analysis. Again, pelomedusids were used as the outgroup for the analysis of chelid relationships. Sequences for all five extant pelomedusid taxa were included. Partition homogeneity tests of combinability were again applied to determine if sequence data from different genes could be pooled for analysis.

The third stage involved analyses of monophyletic subsets of taxa for which a

clear outgroup taxon was available. Both the monophyly of the ingroup and the status of the outgroup were established in stages 1 and 2 of the analysis. This enabled us to combine the sequence data of different genes for the reduced subset of taxa, data that were heterogeneous across the full range of taxa in the stage 2 analysis.

Phylogenetic tree-building algorithms were based on both a maximum parsimony (MP) analysis, with and without weightings for transition/transversion (ts/tv) ratios, and a maximum likelihood (ML) analysis using PAUP*. The ts/tv ratios, estimated separately for each of the sequence data sets using maximum likelihood were applied to each dataset in the weighted parsimony analyses. Branch and bound searches were used for smaller data sets, and heuristic searches with the ACCTRAN option were used for larger data sets. In the ML analysis, the mean ts/tv ratio was calculated across genes in the analysis to obtain weightings. The Hasegawa-Kishino-Yano (1985) two-parameter model for unequal base frequencies was implemented using empirical base frequencies, and equal rates were assumed for all sites unless otherwise specified.

The above approach of using MP analysis, with and without weightings, and ML analysis can generate many trees, with considerable differences among topologies. Clades to emerge from these analyses were tested for their robustness using bootstrapping (2000 pseudoreplicates for MP and 100 pseudoreplicates for ML) (Felsenstein, 1985). Bootstrapping can provide a relative ranking of the degree of support in an analysis for the recovered clades (Hills & Bull, 1993). Throughout this paper, we refer to bootstrap values <50% as not supported, bootstrap values between 50 and 70% as weakly supported, values $\geq$ 70% as strongly supported. Our approach was to accept only strongly supported nodes (those with $\geq$ 70% bootstrap support), because in simulations such nodes accurately reflect true underlying nodes 95% of the time, under not too restrictive assumptions (Hillis & Bull, 1993, but see Felsenstein & Kishino, 1993). We then obtained a consensus of strongly supported results over all analyses to construct a single well-supported hypothesis of relationships among the pleurodiran turtles.

We also compared the weighted MP hypotheses obtained in the present study with published alternative phylogenetic hypotheses (e.g. Burbidge *et al.*, 1974; Gaffney, 1977; Legler, 1981; Seddon *et al.*, 1997). In cases where the published hypothesis was not a fully specified tree, the hypothesis was defined as a constraint in a subsequent search for the most parsimonious tree(s). We then compared the resultant constrained MP trees with the most parsimonious solution to emerge from our unweighted analysis of the same genes, using a Templeton test (Templeton, 1983). We typically used a one-tailed test because the constrained tree can only be equal to or longer than the branch and bound solution for the MP tree. In cases where an heuristic search was used to obtain the MP tree, it is possible for the constrained tree to be shorter, so a two-tailed test was applied.

In a final analysis, allozyme data of Georges and Adams (1992) were re-analysed, treating loci as characters and alleles as character states, using PAUP* where multistate characters were interpreted as polymorphisms. The allozyme data were then combined with the sequence data for taxa with both forms of data available.

Specimens examined

Australian Chelidae: *Chelodina longicollis*, Gwydir R., NSW, AMS R123051[188], R123052[189], R123056[194]; *Chelodina oblonga*, Perth Region, WA, AMS

R125478[229], R125479[230]; *Chelodina rugosa*, South Alligator R., NTM R13434[262], R13437[266], R13439[271]; *Elseya dentata*, Victoria River, NT, NTM R13521[258], R13523[261]; *Elseya georgesi*, Bellinger R., NSW, AMS R123046[159]; *Elseya latisternum*, Tweed R., NSW, AMS R123032[172], Burnett R., Qld, QM J48017[80], J48024[81]; *Elseya purvisi*, Manning R., NSW, AMS R120966[129], R120967[130], R123040[140]; *Elusor macrurus*, Mary R., Qld, AM R125484[280], AMS R125485[281]; *Emydura macquarii*, Murray R, NSW, AMS R120953[119], R120956[122]; *Emydura macquarii* (formerly *signata*), Brisbane R., Qld QM J48053[34], J48056[33], Clarence R., NSW, AMS R123008[137]; *Pseudemydura umbrina*, Perth, WA, no voucher specimen, [o29LIV]; *Rheodytes leukops*, Fitzroy R., Qld., AMS R125481[232]. South American chelids: *Acanthochelys pallidipectoris*, Chaco Region, Argentina, MCC [574], [548]; *Chelus fimbriata*, Guyana, MCC [839], [842], [896]; *Hydromedusa tectifera*, Rio Santa Lucia, Uruguay, MCC [575], [579], [587]; *Phrynops (Batrachemys) nasutus*, Guyana, MCC [838]; *Phrynops (Mesoclemmys) gibbus*, Surinam/ Guyana, MCC [Y021]; *Phrynops (Phrynops) geoffroanus*, Guyana, MCC [576], [598], [PG89–64]; *Platemys platycephala*, Surinam/Guyana, MCC [550], [572], [885]. Pelomedusidae: *Erymnochelys madagascariensis*, Madagascar, MCC [850]; *Pelomedusa subrufa*, Togo, West Africa, MCC [866] [898]; *Peltocephalus dumerilianus*; San Carlos, Venezuela, MCC [860]; *Pelusios sinuatus*, Tanzania, MCC [835], [879]; *Podocnemis expansa*, Brazil, MCC [874], [877]. Abbreviations: AMS, Australian Museum, Sydney; QM, Queensland Museum, Brisbane; NTM, Museums and Art Galleries of the Northern Territory, Darwin; MCC, live specimen collection of William P. McCord; NSW, New South Wales; Qld, Queensland; NT, Northern Territory. Museum voucher specimens were not lodged for specimens held by William McCord. Diagnostic photographs are held at the University of Canberra by the senior author. Numbers in square brackets are tissue label numbers.

RESULTS

Sequence data

Lengths of the aligned sequences were 394 base pairs (bp) for *12S rRNA*, 474 bp for *16S rRNA*, 345 bp for *CO1* and 365 bp for c-*mos*. Excluding ambiguously aligned sequences reduced the base pairs available for analysis to 352 for *12S rRNA* and to 368 for *16S rRNA* (Appendix). There was a 6 base-pair (two codon) indel starting at position 209 in the c-*mos* alignment. No stop codons or reading frame shifts were detected in the either of the two protein encoding sequences, *CO1* and c-*mos*.

There were no differences between the sequences of *CO1* and *16S rRNA* derived from total cellular DNA and those derived from purified mtDNA, for *Chelodina longicollis*. Similarly, sequences derived from PCR amplification of long-range PCR template and total cellular template did not differ for *Emydura macquarii* (formerly *signata*) or *Elseya purvisi*. These results, together with the absence of frame shifts or premature stop codons in the *CO1* sequence data, provide no evidence that nuclear paralogues of mitochondrial genes have been amplified in place of the target gene sequences.

Transition/transversion (ts/tv) ratios were estimated for each of the genes, using the entire data at hand for each gene. They were 2.4 for *CO1*, 1.9 for *16S rRNA*

1.55 for *12S rRNA* and 2.02 for c-*mos*. For analyses of data combined across two or more genes, we used the average ts/tv ratios of the included genes.

Phylogenetic analyses

Reduced taxon set (mitochondrial and nuclear data)

A total of 1382 characters from the *12S* and *16S rRNA*, *CO1* and c-*mos* genes were available after elimination of ambiguously aligned regions. For *CO1* sequences, the total number of sites available, the number of sites that were invariant, the number of sites that were variable but cladistically uninformative and the number of sites that were informative in the unweighted parsimony analyses were 344/214/39/91 overall, 229/202/17/10 for the first and second codon positions only and 115/12/22/81 for the third position only. For *12S rRNA* the corresponding figures were 331/161/77/93, for *16S rRNA* they were 342/230/65/47 and for c-*mos* they were 365/293/41/31. We also estimated the relative information in each gene by conducting bootstrap analyses separately on each of the genes under the same conditions as the combined dataset. The *12S rRNA* gene provided the greatest resolution (had the most concordant information) with between five (weighted and unweighted MP) and seven (ML) of eight possible nodes receiving 70% or greater bootstrap support within the chelid clade. The c-*mos* gene supported between four (unweighted MP) and five (weighted MP, ML) nodes, the *16S rRNA* gene between two (ML) and four (unweighted MP) nodes, and the *CO1* gene between two (unweighted MP) and four (weighted MP) nodes.

Partition homogeneity tests revealed significant heterogeneity when data for all four genes were included in the comparisons ($P < 0.02$). Topological comparisons revealed that two of four supported nodes in the *CO1* chelid trees were in conflict with both the *12S rRNA* and c-*mos* trees. All conflicts were amongst the South American taxa, excluding *Hydromedusa*. No significant heterogeneity among genes remained after the removal of *CO1* ($P = 0.56$). Data for the *CO1* gene were therefore analysed separately, with the analysis deferred until all taxa were considered (see below).

The weighted and unweighted parsimony analyses for the two ribosomal rRNA genes and the c-*mos* gene combined yielded the same equally most parsimonious trees (two trees only, with length of the unweighted trees = 599 steps). The strict consensus of these two MP trees, the bootstrap consensus tree for the MP analysis and the bootstrap consensus tree for the ML analysis were topologically identical (Fig. 1). Where they were significant ($\geq 70\%$), bootstrap proportions for the weighted MP analysis were usually greater than those of the unweighted MP analysis (Fig. 1), indicating that the influence of homoplasy was reduced by the down-weighting of transitions, the class of substitutions more likely to be influenced by saturation.

There are two substantive conclusions to be drawn here. First, the South American chelids are monophyletic (bootstrap values of 77/70/89% for the unweighted MP, weighted MP and ML analyses respectively – this order will be followed in presenting bootstrap values from hereon). This clade includes the long-necked *Chelus* and *Hydromedusa*, but excludes the Australasian long-necked *Chelodina*. Furthermore, the South American long-necked chelids are not monophyletic, with 99–100% bootstrap values for the node supporting monophyly of *Chelus* and the remaining South American chelids to the exclusion of *Hydromedusa*. Unfortunately, the analyses were

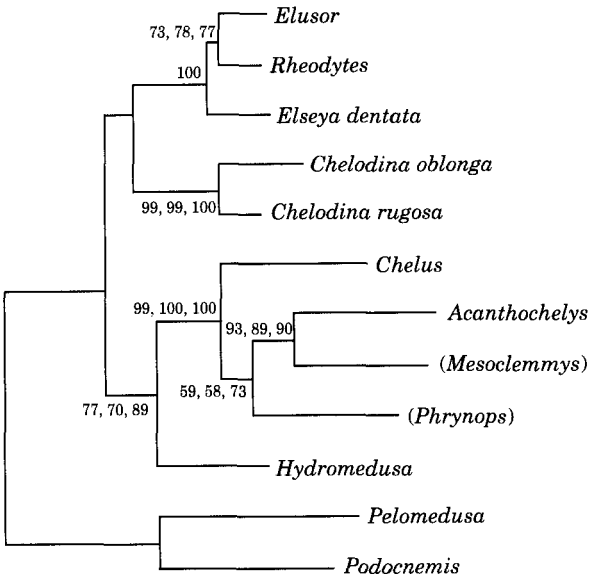

Figure 1. A phylogeny for 10 genera of pleurodiran turtles generated from partial sequences of mitochondrial *12S rRNA* and *16S rRNA* and the nuclear oncogene c-*mos* using the criterion of maximum likelihood. The three values shown on each of several nodes are the bootstrap percentages for maximum parsimony analysis (unweighted), maximum parsimony analysis (weighted) and maximum likelihood analysis respectively. Bootstrap values are shown only for nodes with 70% or greater bootstrap support in one or more analyses. Taxa shown in parentheses are subgenera of *Phrynops*.

uninformative on the question of the monophyly of the Australasian chelids (Fig. 1). One of the unweighted MP trees, the weighted MP tree and the ML tree had the Australasian and South American chelids as reciprocally monophyletic, as shown in Figure 1. The second unweighted MP tree had *Chelodina* as sister to all other chelids. Neither of these arrangements received strong bootstrap support in any analysis (52/66/65% for reciprocal monophyly), so we failed to resolve a trichotomy involving the South American chelids, *Chelodina* and the short-necked Australian chelids (excluding *Pseudemydura*). The second substantive conclusion to emerge from this analysis is that the genus *Phrynops* is paraphyletic. There was very strong bootstrap support (93/89/90%) for the node uniting *Phrynops* (*Mesoclemmys*) with *Acanthochelys* to the exclusion of *Phrynops* (*Phrynops*) in all analyses (Fig. 1).

Our unweighted, unconstrained MP trees were both significantly shorter at 599 steps than the most parsimonious trees constrained by Gaffney's (1977) hypothesis of a close relationship between *Chelodina*, *Chelus* and *Hydromedusa* (620 steps, $z = 3.01$, $P < 0.003$, $n = 37$). Our MP trees provided a better explanation for 29 characters whereas Gaffney's hypothesis provided a better explanation for eight characters. Overall, our trees were 21 steps shorter. Our MP trees were both also significantly shorter than those constrained by Gaffney's fully specified phylogenetic hypothesis (632 steps, $z = 3.70$, 3.76, $P < 0.0002$, $n = 58$, 56). Our MP trees provided a better explanation for 44–45 characters whereas Gaffney's hypothesis provided a better explanation for 12–13 characters. Overall, our trees were 33 steps shorter. Clearly, Gaffney's hypothesis of sister relationships among the long-necked chelids is not supported.

All taxa (mitochondrial data only)

A total of 1017 characters from the *CO1*, *12S rRNA* and *16S rRNA* sequences were available for analys after elimination of ambiguously aligned regions. For *CO1* sequences from the 23 taxa, the total number of sites available, the number of sites that were invariant, the number of sites that were variable but cladistically uninformative and the number of sites that were informative in the unweighted parsimony analyses were 344/182/46/116 overall, 229/175/35/19 for the first and second codon positions only and 115/7/11/97 for the third position only. For *12S rRNA* the corresponding figures were 331/118/79/134 and for *16S rRNA* they were 342/195/78/69 with a total of 203 informative sites in the combined rRNA data.

Partition homogeneity tests of the *12S rRNA* and *16S rRNA* data sets revealed no significant heterogeneity ($P=0.28$), allowing the two data sets to be combined for an overall analysis. *CO1* was excluded on the basis of significant heterogeneity in the restricted taxon dataset described above, and has been analysed separately.

Owing to the relatively large number of taxa, all searches were heuristic. For the ribosomal RNA genes, we initially used 46 random stepwise additions of the input order of the taxa to test the efficiency of the heuristic search mode. All 46 searches found the same tree, so further heuristic searches were conducted with a single taxon input order. However, random stepwise additions were implemented in all heuristic searches of the *CO1* data, as single taxon input order searches were less efficient, presumably due to the larger number of equally most parsimonious trees.

Both the weighted and the unweighted parsimony analyses for the two ribosomal rRNA genes combined yielded the same single most parsimonious tree (length 823 steps) which differed topologically from the ML tree (Fig. 2) in only one respect. In the MP tree, *Elseya dentata* was the sister taxon to a clade comprising *Emydura macquarii*, *Elseya latisternum* and *Elseya georgesi* whereas the ML tree had *Elseya dentata* as the sister taxon to all short-necked Australasian species excluding *Pseudemydura*. Neither of these arrangements received significant bootstrap support however (<54%), with the *Elseya*, *Elusor*, *Emydura* and *Rheodytes* forming an unresolved polytomy in both the MP and ML analyses. As the topological difference between the ML and MP trees is not supported by bootstrapping, only the ML tree is presented, though it shows bootstrap support from all three analyses (Fig. 2).

The South American and Australasian chelids were reciprocally monophyletic in all three analyses, although this result was not supported or only weakly supported by bootstrapping. There was strong bootstrap support for the monophyly of the South American chelids (82/76/79%) and even stronger support for the monophyly of the South American forms excluding *Hydromedusa* (98/99/100%). There was also strong bootstrap support for sister relationships between *Acanthochelys* and *Platemys* (95/95/93%) and between *Phrynops (Batrachemys) nasutus* and *P. (Mesoclemmys) gibbus* (100%).

Bootstrap support for the monophyly of the Australasian chelids was weak (62/54/66%). The short-necked Australasian forms, *Chelodina*, and the South American chelids essentially form an unresolved trichotomy. Within the Australasian chelids, a monophyletic *Chelodina* lineage was highly supported (100/99/100%). Within *Chelodina*, there was strong support for a sister species relationship between *Chelodina longicollis* and *Chelodina oblonga* (82/89/82%), despite *Chelodina oblonga* superficially being more similar to *Chelodina rugosa* on morphological grounds (Legler & Georges 1993). There was poor bootstrap support for the monophyly of the short-neck Australasian chelids in all but the ML analysis (64/58/77%). This result arose

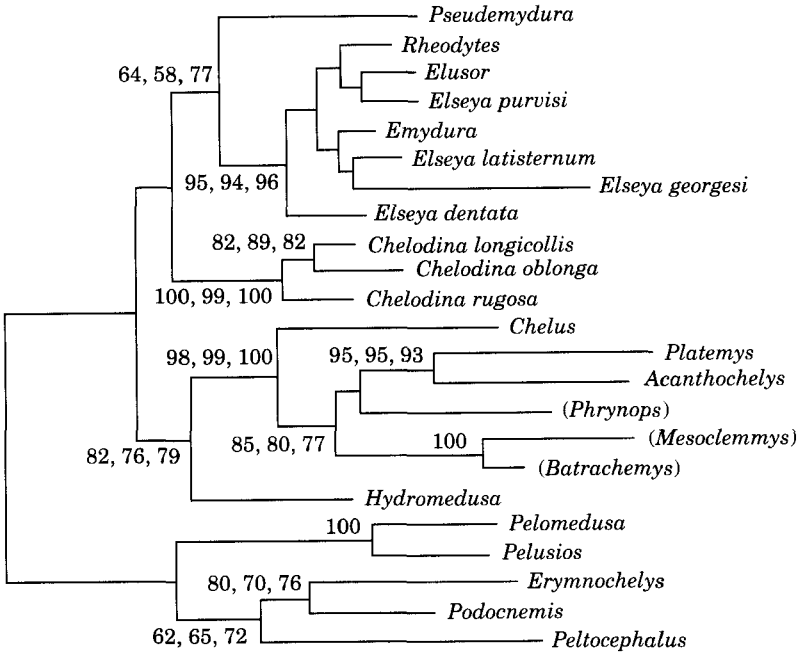

Figure 2. A phylogeny for the genera of pleurodiran turtles generated from partial sequences of mitochondrial *12S rRNA* and *16S rRNA* using the criterion of maximum likelihood. The three values shown on each of several nodes are the bootstrap percentages for maximum parsimony analysis (unweighted), maximum parsimony analysis (weighted) and maximum likelihood analysis respectively. Bootstrap values are shown only for nodes with 70% or greater bootstrap support in one or more analyses. Taxa shown in parentheses are subgenera of *Phrynops*.

primarily because of *Pseudemydura*, as bootstrap support for the monophyly of the other short-neck forms was strong (95/94/96%). None of the relationships among the short-neck forms (Fig. 2), excluding *Pseudemydura*, received strong support from bootstrapping.

Two relationships within the outgroup Pelomedusidae received strong bootstrap support from the weighted and unweighted MP analyses and the ML analysis. The first was a sister relationship between *Pelomedusa* and *Pelusios* (100% in all three cases). The second was a sister relationship between *Erymnochelys* and *Podocnemis*, which was strongly supported by bootstrapping of the unweighted MP tree (80%) and the ML analysis (76%) but with moderate support from the weighted MP analysis (69%). Monophyly of the group comprising *Peltocephalus*, *Erymnochelys* and *Podocnemis* is strongly supported by bootstrapping of the ML tree (72%) but receives less support from the unweighted MP analysis (62%) and the weighted MP analysis (65%).

The unweighted parsimony analysis of *CO1* produced eight equally parsimonious trees of length 565 steps. The weighted analysis produced a single most parsimonious tree. Only two nodes received unanimous bootstrap support across the two MP analyses and the ML analysis, namely a clade comprising *Pelusios* and *Pelomedusa* (94/99/100%) and a clade comprising *Phrynops (Mesoclemmys)* and *P. (Batrachemys)* (100%). Neither of these nodes were in conflict with the topology established by the

TABLE 2. Comparisons of five published hypotheses of chelid relationships with the unweighted MP hypothesis to emerge from this study using mt ribosomal genes only. The single MP tree had a length of 823 steps. Statistical comparisons were made with Wilcoxon Sign-Rank tests (Templeton, 1983) as implemented in PAUP*. *N* is the sample size, Z is the test statistic, *P* is the two-tailed probability of obtaining the observed result by chance, the two values in the column headed steps are the number of sites at which each tree provides more parsimonious explanation (our tree listed first)

Hypothesis	Tree	Length	*N*	Z	*P*	Steps
Gaffney (*Pseudemydura*)	1	827	24	0.71	0.47	14/10
	2	827	22	0.74	0.45	13/9
	3	827	18	0.82	0.40	11/7
	4	827	26	0.68	0.49	15/11
	5	827	18	0.82	0.40	11/7
Burbidge (*Pseudemydura*)	1	831	20	1.56	0.11	14/6
Legler (*Pseudemydura*)	1	855	52	3.86	0.0001*	42/10
Gaffney (Long-neck)	1	840	50	1.97	0.04*	33/17
	2	840	43	2.15	0.03*	30/13
Gaffney (Full)	1	850	55	2.95	0.003*	40/15
	2	850	51	3.05	0.002*	38/13

analysis of *12S* and *16S rRNA*. A further three nodes received significant support in at least one of the analyses—a clade comprising the Australasian short-necks (excluding Pseudemydura) (64/71/56%), a *Chelodina* clade (65/91/69%), and a clade uniting *Chelus* and *Acanthochelys* (56/87/74%). Only the last is in conflict with the topology established by the analysis of *12S* and *16S rRNA*. The *CO1* gene was uninformative on the questions of reciprocal monophyly of the South American and Australasian chelids, the monophyly of *Chelodina* and *Hydromedusa* and indeed, the reciprocal monophyly of the pelomedusids and chelids.

We compared the tree lengths of constrained trees under five hypotheses derived from the literature: (1) Gaffney's (1977) placement of *Pseudemydura* as sister to all other chelids; (2) Burbidge *et al.*'s (1974) placement of *Pseudemydura* as sister to the remaining Australasian chelids; (3) Legler's (1981) suggested close relationship between *Pseudemydura* and *Platemys*; (4) Gaffney's hypothesis of the monophyly of the long-necked chelids; and (5) Gaffney's fully specified phylogeny. We obtained the unweighted most parsimonious tree subject to the constraints dictated by each of the five hypotheses using the combined data for *12S rRNA* and *16S rRNA* only. We then compared the resultant constrained MP trees with the most parsimonious solution to emerge from our unweighted analyses of the same genes, using Templeton tests (Templeton, 1983) (Table 2). We were unable to reject either Gaffney's (1977) hypothesis on the placement of *Pseudemydura* as the sister to all other chelids or the hypothesis derived from the study of Burbidge *et al.* (1974) which places *Pseudemydura* as the sister to the other Australian chelids. This reflects the low bootstrap values among the basal nodes of the chelid phylogeny. However, Legler's (1981) suggested close relationship between *Pseudemydura* and *Platemys* is soundly rejected by our data ($P<0.0001$). Gaffney's hypothesis of a monophyletic long-necked chelid lineage (incorporating *Chelodina, Chelus* and *Hydromedusa*) is rejected by our analysis ($P<0.04$). Similarly, Gaffney's almost fully specified model is firmly rejected by our analysis ($P<0.003$).

We used MacClade (Maddison & Maddison, 1992) to reconstruct the most parsimonious distribution of the character states 'short-neck' and 'long-neck' on the combined ribosomal RNA unweighted MP tree. This character changes its state

ambiguously on the three deepest branches within the chelid radiation, that is, the common ancestor of chelids is equally likely to have had a long or a short neck. Either way, three character state changes are required to explain the current distribution of long and short-necked forms across our phylogeny.

Separate analyses for South American chelids, pelomedusids, and Australasian chelids (mitochondrial data only)

Our intention was to analyse separately the relationships within each of three major lineages, the South American chelids, the Australasian short-necked chelids and the pelomedusids using the combined data from all genes if possible. Combinability tests involving all mitochondrial genes revealed significant heterogeneity for South American chelid lineage (using *Hydromedusa* as the outgroup) when *CO1* was included ($P<0.01$). As separate analyses have already been conducted for *CO1* and the ribosomal genes, we did not consider the question of relationships among South American chelids any further.

For the pelomedusids, with *Chelodina rugosa* and *Phrynops* (*Phrynops*) *geoffroanus* as the outgroup, combinability tests revealed no heterogeneity among the data for all three genes ($P=0.52$), so the data for *12S rRNA*, *16S rRNA* and *CO1* were pooled. Unweighted and weighted branch and bound analyses each produced single most parsimonious trees. There was very strong support for a sister relationship between *Pelomedusa* and *Pelusios* (100%) and for a clade comprising *Peltocephalus*, *Podocnemis* and *Erymnochelys* (63/75/80%), but unlike previous analyses, there was no supported arrangement within this later clade. Hence, these analyses failed to provide support for any arrangements additional to those supported in the previous analyses of all taxa.

For the Australasian short-necked chelids, with the three species of *Chelodina* as outgroup, combinability tests revealed no heterogeneity among the data for all three genes ($P=1.0$), so again the data for *12S rRNA*, *16s rRNA* and *CO1* were pooled. Unweighted and weighted branch and bound analyses each produced single most parsimonious trees. The two MP trees and the ML tree differed in the relationships among the short-necks (excluding *Pseudemydura*) but none of these differences survived bootstrap analysis. The results of the bootstrapping are shown on the ML tree in Figure 3. Bootstrapping provided support for a sister relationship between *Chelodina oblonga* and *C. longicollis* (85/91/86%), and strong support for a monophyletic assemblage of all short-necked species excluding *Pseudemydura* (95/97/98%), as do all previous analyses. However, it also provided support for an *Emydura*/*Elseya latisternum*/*Elseya georgesi* clade (72/76/83%), leading to the conclusion that, like *Phrynops*, the genus *Elseya* is paraphyletic (Fig. 3).

Combined allozyme and DNA data

Reanalysis of the allozyme data set of Georges and Adams (1992), with taxa restricted to those for which we had DNA sequences, yielded four equally parsimonious trees each of length 188 steps. A consistent feature of all four trees was the grouping of *Elseya latisternum*, *E. georgesi* and *E. purvisi* (80% bootstrap value), with *E. latisternum* and *E. georgesi* as sister taxa (76%). This is in contrast with the outcome of the mtDNA analyses, which had *Emydura macquarii*, *Elseya latisternum* and *Elseya georgesi* in a strongly supported clade (72/76/83%) to the exclusion of *Elseya purvisi*. This conflict in topologies eliminated significant support for any topological structure

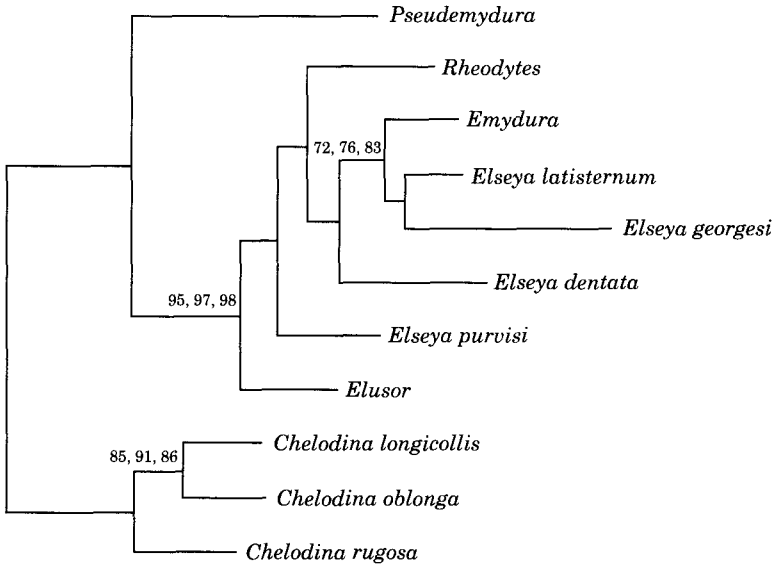

Figure 3. A phylogeny for the Australasian chelid turtles generated from partial sequences of mitochondrial *CO1*, *12S rRNA* and *16S rRNA* using the criterion of maximum likelihood. The three values shown on each of several nodes are the bootstrap percentages for maximum parsimony analysis (unweighted), maximum parsimony analysis (weighted) and maximum likelihood analysis respectively. Bootstrap values are shown only for nodes with 70% or greater bootstrap support in one or more analyses.

within the short-necked Australasian species (excluding *Pseudemydura*) when data for the three mitochondrial genes and the allozymes were combined (combinability test $P=0.13$). There were three equally parsimonious trees (583 steps) with no clear consensus on the arrangement of the short-necked taxa.

Consensus of all analyses

A consensus of the results of all the analyses excluding *CO1*, with 70% or greater bootstrap support, is shown in Figure 4. Relationships among the pelomedusids and South American chelids are fully resolved, establishing the previously uncertain affinities of *Hydromedusa* as sister to the remaining South American chelids and the paraphyly of *Phrynops*. Reciprocal monophyly of the Australasian and South American chelids could not be unequivocally established, though it was a feature of the majority of analyses, so the Australasian short-necked chelids, the long-necked *Chelodina* and the South American chelids form an unresolved trichotomy in the final consensus tree. *Pseudemydura* is shown as the sister to the remaining Australian short-necked species, a consistent topological feature of the MP and ML analyses, though support for this result is tenuous, resting upon a single substantive bootstrap value (77%, ML). The genus *Elseya* is paraphyletic, as their closest common ancestor includes *Emydura* among its descendants. No other relationships could be established among the short-necked Australian forms (excluding *Pseudemydura*), and they collectively form an unresolved polytomy. Results to emerge with bootstrap support from the

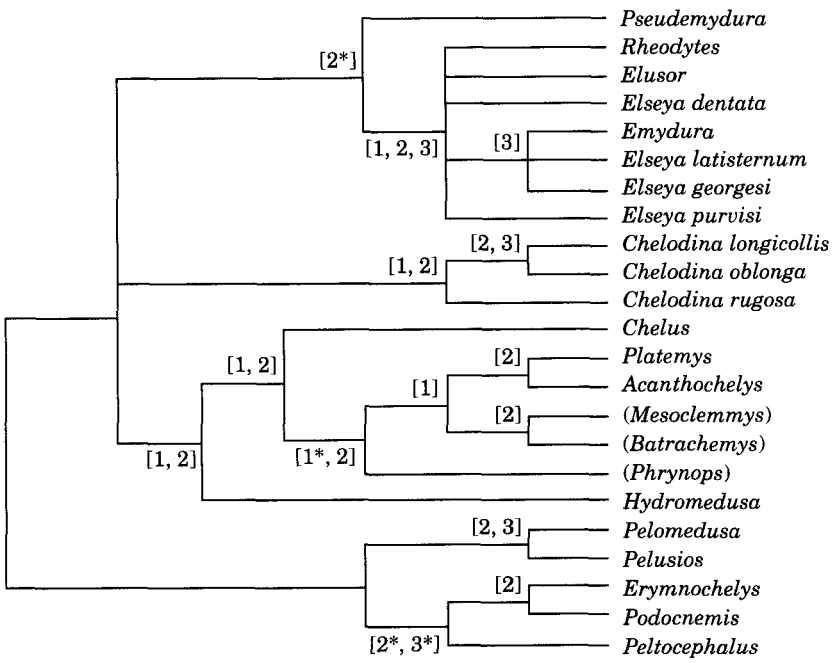

Figure 4. A consensus of nodes in the pleurodiran phylogeny to receive 70% or greater bootstrap support in one or more of the maximum parsimony (weighted and unweighted) or maximum likelihood sub-analyses. Numbers shown in parentheses give the analyses that provide the support: 1, restricted taxon set using *12S rRNA, 16S rRNA* and c-*mos*; 2, all taxa using *12S rRNA* and *16S rRNA* only; 3, Australasian chelids using *12S rRNA, 16S rRNA* and *CO1*. Numbers bearing an asterisk indicate that bootstrap support was equal to or greater than 70% only in some of the three sub-analyses.

separate analysis of the *CO1* gene are in conflict with this consensus topology only in placing *Acanthochelys* as the sister taxon to *Chelus*.

DISCUSSION

It seems likely that the Australasian and South American chelid turtles are reciprocally monophyletic, this being the case in all but one of our analyses, and in the analysis of sequence variation in the cytochrome *b* gene by Shaffer *et al.* (1997). Certainly, we have demonstrated the monophyly of the South American chelids, including *Hydromedusa*. This was the main new result to emerge from the analysis involving the restricted taxon set and both mitochondrial and nuclear genes. Furthermore, all published hypotheses in conflict with the notion of reciprocal monophyly (Gaffney, 1977; Legler, 1981) were rejected by our analyses, whereas hypotheses of reciprocal monophyly (Burbidge *et al.*, 1974; Seddon *et al.*, 1997; Shaffer *et al.* 1997) were consistent with the present larger molecular dataset. However, we cannot be definitive on this point because there is only weak bootstrap support for the monophyly of the Australasian chelids in the analyses of Seddon *et al.* (1997) (64%), Shaffer *et al.* (1997) (58–69%) and in the present study (52–66%).

Strictly speaking, there is now an unresolved trichotomy comprised of the South American chelids, *Chelodina*, and the Australasian short-necks, including *Pseudemydura*. Similarly, *Elseya*/*Emydura*, *Rheodytes* and *Elusor* remain as an unresolved polytomy, despite extensive investigation involving allozyme electrophoresis and analyses of sequences from three mitochondrial genes.

There are three possibilities to consider in explaining these unresolved polytomies, the first two representing one end and the third representing the other end of a continuum of possibilities. The first possibility is that the groups involved represent a true polytomy, having evolved from three allopatric drainages of their parent species. The sequence of isolation of these parent populations may have been sequential, but happened in such rapid succession that no differential accumulation of mutations occurred to reflect that sequence of events. In this case, synapomorphies available to support a dichotomous branching structure simply may not exist, and no further addition of data will resolve the issue. Evidence that would appear to resolve the polytomy would have either arisen through homoplasy or through the coincident retention of polymorphisms present in the ancestral species. The polytomy is a real feature of the evolutionary history of the organisms.

The second possibility is that the species arose from a series of dichotomous speciation events, but that they happened in rapid succession (rapid cladogenesis). If the three groups radiated rapidly, the short time available for molecular or morphological character evolution between speciation events would provide little evidence for deducing their sequential pattern of cladogenesis. Furthermore, what evidence there was could be obscured by convergent evolution among the lineages since their divergence (Kraus & Miyamoto, 1991). Under this scenario, our ability to extract what evidence survives to the present is severely limited, requiring large suites of characters to ensure reasonable representation of those that evolved along the short ancestral branches connecting the taxa. Without such extensive sampling, we are unlikely to resolve the polytomy. *16S rRNA* and c-*mos* provided the additional data needed to resolve the relationship of *Hydromedusa* among the Chelidae, thus breaking what was previously an unresolved polytomy (Seddon *et al.*, 1997). However, we must also admit that in other cases, as a result of homoplasy, the information required to resolve a polytomy may simply no longer exist.

The third possibility in the continuum is that the radiation of the three groups was not particularly rapid, but that the divergence of the three is very deep. Under this scenario, any evidence in the form of synapomorphies that could be used to reconstruct the branching structure has been overwritten by homoplasy in the substantial time that elapsed since the last group emerged. Good molecular evidence for the branching structure of relationships among the groups may have once existed in abundance, but because much of the genome used in phylogenetic studies evolves in roughly clocklike fashion, unequivocal evidence no longer exists. We could look for conservative sequence characters that have persisted to the present day, such as the relatively conservative exons from nuclear genes (Shaffer *et al.*, 1997), but such characters are conservative because they change infrequently. If they change so infrequently as to survive the long periods that followed divergences deep in a phylogeny, they probably accumulate too infrequently to occur often enough during the *relatively* brief period that contained the branching events of interest. So again, we are unlikely to resolve the trichotomy through the addition of further gene sequence data. However, morphological evidence (or for that matter, isozyme characters [Buth & Rainboth, 1999] may be superior to gene sequences under these

circumstances, because a morphological innovation can permanently make its presence known by locking the organism into a particular evolutionary trajectory. Such morphological innovation may indeed be a partial cause of the rapid radiation, and the refinement of the characters involved in the innovation may accompany the radiation. The search for such morphological evidence to confirm or refute the phylogenetic relationships of *Hydromedusa* and *Pseudemydura* among the chelids in general, to establish the relationships of the *Chelodina* and the short-necked Australasian chelids, and to establish the relationships of *Elusor* and *Rheodytes* among the short-necked Australian chelids, is currently under way (Thomson and Georges, unpubl. data)

Strongly conflicting phylogenies for the same taxa derived independently from morphological and molecular data sets are rare (Goodman, Miyamoto & Czelusniak, 1987; Hillis, 1987; Shaffer, 1991; Shaffer *et al.*, 1997). Nevertheless, chelid phylogenies derived from morphological analyses (Gaffney, 1977; Shaffer *et al.*, 1997) differ dramatically from those derived from molecular analyses in this paper and elsewhere (Seddon *et al.*, 1997; Shaffer *et al.*, 1997). In the preferred phylogeny of Gaffney and in two other trees broadly consistent with his data (Gaffney, 1977), the long-necked chelid turtles of Australasia and South America form a single clade, an outcome confirmed by a morphological analysis of the same data by Shaffer *et al.* (1997). Molecular analyses prior to the present study have failed to resolve the relationship of *Hydromedusa* within the Chelidae. When bootstrap support is taken into account, Seddon *et al.* (1997) were unable to resolve a polytomy between *Hydromedusa*, *Chelodina*, the Australasian short-necked turtles, and the remaining South American chelids. Hence they were not able to unequivocally reject the monophyly of long-necked forms across continental boundaries because they were unable to establish unequivocally the nature of the relationship between *Chelodina* and *Hydromedusa*. In contrast, our analyses firmly reject any notion of close relationships among any combination of the three long-necked lineages, even between the South American taxa.

These two phylogenetic arrangements of long-necked chelids, that of Gaffney (1977) and that of the present study, are irreconcilable, insofar as both cannot be a reflection of the true pattern of ancestry and descent among these turtles. Congruence between morphological and molecular studies is strong evidence that the underlying phylogenetic history has been uncovered; conflict may indicate theoretical or procedural problems in one or both analyses (Hillis, 1987). We believe that the way in which the above morphological analyses have been conducted, though common practice, is fundamentally flawed.

The common evolutionary descent of species imposes a nested hierarchical pattern of evidence for groups within groups on their morphological and molecular variation (Larson, 1994). Correlation among characters arises because character states are jointly inherited down a bifurcating branch of phylogeny. Hierarchical correlation among characters is thus interpreted as evidence of common ancestry (Jones, Kluge & Wolf, 1993) and phylogenetic analysis aims to recover the historical pattern of ancestry by identifying congruent hierarchical patterns of variation in morphological and molecular characters. (Larson, 1994). The more characters that are correlated in support of a synapomorphy, the greater is the support for the associated node in the phylogeny. However, there are many causes of correlation among characters in addition to common ancestry (Kluge, 1989). In particular, correlation among characters may arise because of a functional relationship among them, such as when the occurrence of one (shape of the octogossal in salamanders) depends in part on

occurrence of another (junction of octogossal with the radial, in Jones *et al.*, 1993). Another example might be the possible convergent evolution of the biochemical, physiological, morphological and behavioural traits functionally correlated with homeothermy in birds and mammals (Chippindale & Weins, 1994).

In the absence of homoplasy, the topology of a tree will be unaffected by functional relationships among the characters used to support it (Jones *et al.*, 1993); however, the weight of evidence in support of some nodes will be inflated (Shaffer, 1991). Scoring two functionally correlated characters as independent inflates the contribution made by information they jointly contain. In an extreme case of 100% correlation (vomer contacting the pterygoids vs palatines entirely separated by vomer of turtles, for example), the contribution that each character makes to the phylogenetic analysis is held in common. Giving equal weighting to each character effectively doubles the weight given to their common contribution to recovering the phylogeny.

In the presence of homoplasy, both the topology and the support for particular nodes can be affected by functional correlation among characters. The inflated contribution of functionally correlated characters in support of a false synapomorphy (the result of convergence) could potentially overwhelm evidence to the contrary. It is the combination of homoplasy and lack of independence of characters, not homoplasy on its own, that results in potential distortion of the resulting phylogeny.

In the studies of Gaffney (1977) and Shaffer *et al.* (1997), morphological characters and states are identified and scored across all taxa, then subjected to a parsimony analysis. In order to maintain objectivity, no differential weightings are assigned to characters, the implication being that all characters are equally weighted. However, if the characters are functionally correlated, their contribution to resolving the phylogeny is inadvertently differentially weighted (Donoghue & Sanderson, 1992). A long neck, attenuated strike-and-gape mode of feeding and piscivorous habits necessarily involve a suite of correlated character changes (such as those suggested by Pritchard, 1984; Pritchard & Trebbau, 1984) and there are a finite number of options for achieving this condition. In those forms that have been examined, the cervical vertebrae (2–8 in pleurodires; 2–7 in cryptodires) are elongated with attendant modifications associated with the mechanical consequences of this elongation. The atlas-axis complex is fused and elongated, presumably to contribute to elongation of the neck and provide mechanical stability to the head during the strike action. The longissimus dorsi muscles which drive the strike are enlarged and accommodated by lateral expansion of the rib heads. Neural bones, present as subsurface elements in most chelids (Thomson & Georges, 1996), are expanded, perhaps as a consequence of the laterally expanded rib heads below or to provide greater stability to the mid shell sutures and counter the torsion that results from an asymmetric strike action of side-necked turtles (Thomson & Georges, 1996). The anterior of the shell must be expanded to provide protection for the greater bulk of the head and neck, with a series of attendant changes to the anterior scutes and plates of carapace and plastron (Pritchard, 1984). The skull is depressed to reduce its resistance to rapid passage through water and the position of the orbits is modified to facilitate binocular vision. While there is some scope for achieving these adaptive changes by altering characters in fundamentally different ways, development of a long neck will require a suite of related changes to cranial and post-cranial characters. Scoring each separately may have greatly inflated the contribution a long neck and associated characters make to establishing a long-neck clade in the resulting phylogeny. If the long necks of the South American and Australasian chelids are a result of convergence,

this greatly inflated contribution may have overwhelmed evidence in the data set to that contradicts their presumed close relationship.

Of course, molecular characters are not entirely independent. Compensatory changes in paired nucleotides of the stem regions of ribosomal RNA (Wheeler & Honeycutt, 1988) and concerted evolution of multiple copies of nuclear rRNA genes (Hillis & Dixon, 1991) are examples. In *18S rRNA* of vertebrates, both avian and mammalian lineages have a higher GC content than non-homeotherms which may have constrained the pattern of substitution which will artificially enhance relatedness of birds and mammals in comparison with other vertebrates (Hedges, Moberg & Maxson, 1990; Huelsenbeck *et al.*, 1996). However, correlated sequence change is not a widespread phenomenon, especially across multiple genes, nor is it predicted to be from current knowledge of the causes and functional consequences of gene sequence variation. We regard the potentially distorting combination of homoplasy and lack of independence of characters to be much less prevalent in molecular data sets than in morphological data sets.

We have used multiple genes including three samples of the mitochondrial genome and an independent sample of the nuclear genome and regard the phylogeny most strongly supported by our molecular data to be the best working hypothesis. The evolution of morphological traits, such as the long neck, can then be interpreted in the context of this phylogeny, an approach recommended by Hedges and Maxson (1996). If the more pluralistic view of phylogenetic reconstruction is to prevail, where molecules and morphology are to work as equal partners (Lee, 1997), then the morphological analyses of Gaffney (1977) should be revisited with a view to in some way eliminating the functional overlap among characters. Few share Pritchard's optimism that this can be achieved without descending into the realms of rampant speculation. Functional correlations among characters are notoriously difficult to demonstrate, requiring solid developmental or functional evidence, which is usually lacking. This is not to say that functionally related morphological character complexes are rare, or that in combination with convergent structures, they rarely influence the phylogenies that are recovered. Regardless of the practical difficulties, we need be reasonably sure that both the molecular and morphological approaches are providing unbiased estimates of the true phylogeny, or that they are at least statistically congruent (Bull *et al.*, 1993; de Queiroz, 1993), before we combine data sets, whether by consensus (Mickevich, 1978) or pooling (= total evidence) (Miyamoto, 1985; Kluge, 1989).

The most parsimonious reconstructions are ambiguous about the ancestral state (long or short neck) in chelids, there being three equally parsimonious scenarios. As the pelomedusids have short necks, one possibility is that the long neck evolved somewhere along the stem branch leading to the chelids. If so, two reversals to the short-necked state would have had to have occurred along the branches leading to the Australasian short-necks and the South American short-necks respectively (three steps in total). However, Pritchard (1984) has argued convincingly for independent origins of the long-necked state of *Chelodina*, *Chelus* and *Hydromedusa*. Indeed, if a long neck is the ancestral state, and the ancestor had associated modifications of the shell to accommodate its greater bulk when withdrawn, it is difficult to explain how *Chelodina* and *Hydromedusa* could have achieved such modifications in such fundamentally different ways without a reversal to a short neck then again to a long neck in at least one lineage (then four steps in total). This, we believe, eliminates the option of a long-necked chelid ancestor from the set of three most parsimonious

scenarios. A second and now favoured possibility is that the ancestral state for the chelids is a short neck, having been retained in the Australasian short-necked chelids and secondarily derived in the South American short-necked chelids (three steps). The long necks of the *Chelodina* would have been independently derived, with the long necks of *Chelus* and *Hydromedusa* inherited from their common ancestor. A third possibility is that the ancestral state for the chelids is a short neck, with independent origins for the long necks of each of *Chelodina*, *Chelus* and *Hydromedusa* (three steps), the option most favoured by Pritchard (1984)). The highly specialized body form and feeding habits of *Chelus* make it difficult to distinguish between these latter two possibilities.

The genus *Phrynops* has a confusing taxonomic history, and is not particularly well defined, having been a receptacle for those South American chelids that do not have some overt morphological peculiarity (Pritchard, 1984). Earlier this century, species that are now in the genus were grouped into three genera: *Phrynops* for *P. geoffroanus* and related forms (Wagler, 1830), *Mesoclemmys* for *P. gibbus* (Gray, 1873) and *Batrachemys* for *P. nasutus* and related forms (Stejneger, 1909). These genera were reduced to subgeneric rank by Zangerl and Medem (1958), an arrangement that is widely accepted today, though at least one author has recommended that the subgenera be abandoned (Bour, 1973).

In Gaffney's (1977) cladistic analysis of skull characters, he was unable to find unique derived characters to establish monophyly of *Phrynops*, but nor could be demonstrate that it was paraphyletic. The phylogenies of Seddon *et al.* (1997) and those presented here are congruent in putting *Acanthochelys* as the sister to *Platemys* (except for *CO1*), and *Phrynops* (*Batrachemys*) as the sister to *Phrynops* (*Mesoclemmys*), but they differ in the relative placement of *Phrynops* (*Phrynops*). Seddon *et al.* reported that the genus *Phrynops* is paraphyletic, with *Chelus* as the sister taxon to *Phrynops* (*Phrynops*), though bootstrap support for this conclusion was only 60%. In contrast, our analyses place *Chelus* as the sister to the other South America genera (*Hydromedusa* excluded) with 98–100% bootstrap support and 77–85% support for a clade containing *Phrynops* (*Phrynops*) to the exclusion of *Chelus*. The analysis involving *12S rRNA*, *16S rRNA* and c-*mos* provided strong support for a sister relationship between *Phrynops* (*Mesoclemmys*) and *Acanthochelys* to the exclusion of *Phrynops* (*Phrynops*) (89–93% bootstrap support). We regard this as conclusive evidence that the genus *Phrynops* is paraphyletic, there being no strong evidence to the contrary in our other analyses.

Assuming the paraphyly of *Phrynops* is unacceptable as a taxonomic arrangement, it can be resolved by elevating the sub-genera to generic status, in which case we will have come full circle. Alternatively, given the well-supported sister relationship between *Phrynops* (*Batrachemys*) and *Phrynops* (*Mesoclemmys*), these two taxa could be regarded as comprising a single genus (*Mesoclemmys* has precedence), with the genus *Phrynops* retained for forms in the subgenus *Phrynops* (*Phrynops*). We have no strong views on this, but given that the three sub-genera are well recognised and accepted, we believe that taxonomic stability is best served by elevating these three sub-genera to genera. This is our recommendation. Further work on the relationships between species within the new *Mesoclemmys* and *Batrachemys* will decide whether their separate generic identity should stand.

Parsimony analyses of allozyme data (Georges & Adams, 1992) and *12S rRNA* sequence data (Seddon *et al.*, 1997) indicate that, like *Phrynops*, the Australian genus *Elseya* is paraphyletic. The common ancestor of species of *Elseya* has *Emydura* and possibly *Rheodytes* and *Elusor* among its descendants. Georges and Adams, (1992)

recommended that the genus *Elseya* be redefined to contain *Elseya dentata, Elseya novaeguineae* and their close but undescribed relatives (Georges & Adams 1996). Following from their analysis, a new genus would need to be erected to contain the monophyletic assemblage of species closely aligned with *Elseya latisternum* (Georges & Adams, 1996), namely *E. georgesi, E. purvisi* and an undescribed form from the inland tributaries of the Murray–Darling drainage. The results of the present study provide support for this recommendation in the form of 72–83% bootstrap support for a clade including *Emydura, Elseya latisternum* and *Elseya georgesi* to the exclusion of *Elseya dentata.* However, a new genus defined as suggested by Georges and Adams (1992) would remain paraphyletic, as the supported clade containing *Emydura, Elseya latisternum* and *Elseya georgesi* established in the present study excludes *Elseya purvisi.* The differences in topological arrangement of the sibling species among the other Australian short-necks, as established by allozyme electrophoresis and mitochondrial gene sequences respectively, are difficult to reconcile. *Elseya georgesi* and *Elseya purvisi* are a sibling pair, formerly cryptic. External differences between them are negligible, and involve subtle differences in coloration, although there are substantial internal morphological differences (Thomson & Georges, 1996). Until the 20% fixed differences between them were established using allozyme electrophoresis, they were widely regarded as the same species. Their separation in the mtDNA trees is greater than unexpected, and is in stark contrast to the robust results of the allozyme analyses (Georges & Adams, 1992). This may well be a case of a mitochondrial gene tree, as reflected in the mtDNA sequences, differing from the species tree, as reflected in the multi-locus allozyme analysis (54 loci).

There are two additional areas in which the phylogeny presented in this paper is in conflict with other published arrangements. Molecular data are unanimous in their very strong support for a sister relationship between *Chelodina oblonga* and *C. longicollis* (Georges & Adams, 1992; Seddon *et al.*, 1997) to the exclusion of *C. rugosa. C. oblonga* is superficially more similar in morphology and more similar in habits to species in the *C. rugosa* group and so its affinities are often regarded as lying with *C. rugosa* (Goode, 1967; Legler, 1981; Legler & Georges, 1993). A detailed morphological analysis of the relationships among these forms has not been published, and is needed to resolve the issue. A second point of conflict occurs in the relationship between *Peltocephalus, Podocnemis* and *Erymnochelys. Peltocephalus* and *Podocnemis* have well-developed saddle-shaped cervical articulations, regarded by Gaffney (1988) as a shared derived character that unites the two to the exclusion of *Erymnochelys.* Meylan (1996) has them as an unresolved polytomy in his analysis of 35 morphological characters. Pritchard and Trebbau (1984:38) list a range of other similarities between *Erymnochelys* and *Peltocephalus*, but consider it likely that these arose as a result of parallelism rather than an indication of a close relationship. Instead, they cite chromosomal and serological data (Frair, Mittermeier & Rhodin, 1978) and the existence of fossil forms from Kenya that share characters of both *Erymnochelys* and *Podocnemis* (Wood, 1971) as evidence of a close relationship between *Erymnochelys* and *Podocnemis.* Our data support this latter arrangement.

Finally, there have been several efforts to establish sub-familial relationships among pleurodiran genera. However, support for separation of South American and Australasian chelid genera in particular, has been equivocal, despite their long separation (Pritchard, 1984). Insofar as a classification should reflect phylogeny, the substantial alterations to the currently accepted phylogeny as a result of the injection of molecular data require corresponding adjustments to Pleurodire classification.

Below is a reclassification of the extant pleurodiran genera consistent with our phylogeny and the analysis of Shaffer *et al.* (1997). Figure 4 represents the well-supported outcomes of the present paper, yet fails to resolve a trichotomy comprising the South American chelids, *Chelodina*, and the remaining Australasian chelids. However, all but one of our analyses had the *Chelodina* as sister to the remaining Australasian chelids, as the most parsimonious solution. Reciprocal monophyly of the South American and Australasian chelids was also the most parsimonious outcome of the analysis of sequence variation in an additional gene, cytochrome *b*, by Shaffer *et al.* (1997), though with bootstrap support of only 68%. The following classification of extant forms is tendered with the caveat that further work is required to better establish the sister clade to the *Chelodina*. Note that the genus *Elseya* is paraphyletic. Some authors have suggested that the family Pelomedusidae be restricted to the African genera *Pelusios* and *Pelomedusa* and that a new family, the Podocnemidae, be erected for the South American *Podocnemis* and *Peltocephalus* and the Madagascan *Erymnochelys* (de Broin 1988), contained within the overarching Hyperfamily Pelomedusoides. Our data show these two groups to be monophyletic, but we retain the traditional arrangement of Pelomedusidae recognized at family level, with the proposed families of de Broin recognized at sub-family level.

Sub-order Pleurodira Cope, 1868a
  Family Pelomedusidae Cope, 1868b
    Sub-Family Pelomedusinae de Broin, 1988 (new rank)
      Pelusios, Pelomedusa
    Sub-Family Podocneminae de Broin, 1988 (new rank)
      Podocnemis Peltocephalus, Erymnochelys
  Family Chelidae Gray, 1831
    Sub-Family Chelodininae (new)
      Chelodina, Elseya, Emydura
      Pseudemydura, Rheodytes, Elusor
    Sub-Family Chelidinae Gray, 1825
      Chelus, Phrynops, Platemys
      Acanthochelys, Mesoclemmys (new rank),
      Batrachemys (new rank)
    Sub-Family Hydromedusinae, Gaffney 1977 (new rank)
      Hydromedusa

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APPENDIX

Alignment of *12S rRNA* and *16S rRNA* gene sequences from 18 chelid and five pelomedusid taxa. Taxon names are abbreviated to the first two letters of the genus name followed by the first three letters of the species name. Other abbreviations are: (.), base same as for first taxon; (−), alignment gap; (?), nucleotide unknown. Sequences are numbered from the first base in the reference sequence. Regions of questionable homology removed prior to analysis are indicated by asterisks. The unaligned sequences for *12S rRNA*, *16S rRNA*, *CO1* and c-*mos* are available from GenBank. GenBank accession numbers are: c-*mos* AF109200-9; *12S* AF095893-4; *16S* AF113620-43; *CO1* AF113644-67.

12S rRNA

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                      *****  **********  **********  **********  *
Psumb  CCTTAAACC-  TAGATTTTTT  T----ATATA  T-AAAAATTT  ACC-AGAGAA  -CT-ACAAAC
Rhleu  ..C.......  ..........  A..TTT...-  -........G  G.........  A.........
Elmac  ..C...T...  ..........  A..AA.C.C-  -.......C.  G.........  ..........
Emmac  ..C...T...  ..A.......  A..AG...C-  -.....T..G  G.........  ..........
Ellat  ..C.......  ..A.....G.  A.....?...  C..G?.....  G.........  ..........
Elden  ..C.......  ..A?......  A....--...  .A.......G  G.......T.  ...T......
Elpur  ..C.......  ..........  ....A.....  C.......C.  G.........  ..........
Elgeo  T??.......  ..A.....C.  C...A.?...  C.???.T..?  G........?  ........?.
Chlon  ..........  ...TA..-CC  ACAGCCC.C.  .A.GG...CC  G.........  ..........
Chobl  ..C.....A.  ......--CC  ACAACCC.C.  .A.GG...CC  G.........  .......G?.
Chrug  ..C.....T.  C.....--CC  ACAACCC.C.  .A..G...CC  G.........  ..........
Chfim  ..CC..TTA.  C........C  .CCA.--.C.  ........CC  G.........  ......C...
Plpla  ..C...TAA.  .........C  .CAA.-..C.  ......T.CC  G.......C.  A?....T.TT
Acpal  ..C.....A.  .G.......C  .?TACG..C.  ........CC  G.........  AT..TTT..T
Phgeo  ..C.....A.  ..T.......C  -CCA.-..C.  ........CC  G.......G.  AT..T.C...
Phgib  ..C.....A.  ..........  -CCA.-..C.  A.....T.CC  G.......TT  ....TTT?..
Phnas  ..C.....A.  ..........  -CAA.-?.A.  A.....-.CC  G..C....T-  ....?TTT..
Hytec  ..C.......  A........A  ACA..-..C.  .A..GG-.CC  G.........  A.....C..T
Pesub  ..C...?...  AT...A...A  ACA..-..AC  AC...T..CC  G...C.....  .....G.G.
Ermad  ..C...TT.T  C....A...A  -C.T.-..C.  ..G..T..CC  G.........  ........G.
Pelsin ..C....T..  AT...A..CC  ACAT.-A.C.  .....T..CC  G...C.....  ......G.G.
Podexp ..C.......  C....A--CC  CACT.-..C.  A..G.T..CC  G.........  ........G.
Pedum  ..C.....A.  .T...A.--C  .ATT.-..C.  ...G.C..CC  G.........  ......G.G.

Psumb  TAAACGTTTA  AAATTCAAAG  GACTTGGCGG  -TACCTCAA-  ACCCACCTAG  AGGAGCCTGT
Rhleu  C...T...GG  ...C....G.  ..........  C.G..C....  ..........  ..........
Elmac  C...A....G  ...C......  ..........  ..G..C....  ..........  ..........
Emmac  C...T...GG  ...C....G.  ..........  ..G..C....  ..........  ..........
Ellat  C.........  ?..C....G.  ..........  ..GT.C....  ..........  ..........
Elden  C...T....G  ...C....G.  ..........  ..G.......  ..........  ..........
Elpur  ....A....G  ...C......  ..........  ..G..C....  ..........  ..........
Elgeo  C.........  ...C......  ..........  ..G..C....  ..........  ..........
Chlon  C.........  ...C....G.  ..........  ..G.T.....  ..........  ..........
Chobl  C.?.......  ?.......G.  ....G..G..  ..GTTC....  ..........  ..........
Chrug  C.........  ...C......  ..........  ..G.T?....  ..........  ..........
Chfim  CCTGAA..AG  ...C......  ......A...  ..GT......  .....A....  ..........
Plpla  C.T..A..?G  ..GC...GG.  .TT.?..T..  ..TT......  C....A....  ....?.....
Acpal  C.C.AA....  ..TC...GG.  .TT...CG..  ..GT......  .T...T....  G.......T.
Phgeo  C.CGAA..GG  ...C....G.  ..T....G..  ..GTT.....  .....A....  ..........
Phgib  A...AA..GG  G..?....G.  .G?.......  .GGT......  .....AT.T.  G..G....T.
Phnas  CC..AA...G  G..C......  .G........  .GG?......  ....CA..?.  .....?....
Hytec  C....A...G  ...C....G.  ......A...  ..GT......  -.........  ..........
Pesub  CT.CA.C...  ...C.T..G.  ..........  ..GT.C..T.  .....A....  ..........
Ermad  A...T.C.CG  ...C....G.  ......A...  ..GT......  ....C.....  ..........
Pelsin .T.TA.C...  ...C......  ..........  ..GT.C..T?  ....TA....  ..........
Podexp C...T.C...  ...C....G.  ......A...  ..G.......  ..........  ..........
Pedum  -.CC..C..G  ...C......  ..........  ..GT..T...  .....T....  ..........
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Psumb  TCTATAATCG ATAACCCACG ATTAACCTCA CCACCCCTTG CCCT--CAGC CTATATACCT
Rhleu  .......... ....T..... ...C.....G .....T..C. ...A..... ..........
Elmac  .......... ....T..... ...T.....G .....T..C. ...A..... ..........
Emmac  ...?..... ....T..... ..?C..... .....T..C. ...A..... ..........
Ellat  .......... ....T.?... ...C..... .....T..C. ...A..... ..........
Elden  .......... ....T..... .?CT..... .....T..C. ...A..... ..........
Elpur  .......... ....T..... ...C..... .....T..C. ...AC.... ..........
Elgeo  .......... ....T..... ...C..... .....T..A. ...AC.AGC. TATATAC.TC
Chlon  .......... ....T..... ..AC..... ...T.T..A. ...A..... ..........
Chobl  .......C.. ....T..... ...TG.... ...T.T..A. ...A..... ..........
Chrug  .......... ....T..... ..CT..... ...T.T..A. ...A..... ..........
Chfim  C.C.A..C.. ....T..... ..AC....A. ....TT..A. ...A..... ..........
Plpla  ......T... ...CT..... ..CT..... ......A.A? ...AT?...T A.....?...
Acpal  .......... ...CT..... ..CTC..... ......A.G. ...?C..... ......C..C
Phgeo  ...TC..C.. ..T.T..... ...T..... .........A. ..AAT..... ..........
Phgib  ...T.T.... G..CT..... ...T..... ......T.A. G..A.....G ..T.T.T..C
Phnas  .......... ....T..... ...T..... ......T.A. ...C..... ......?...
Hytec  .......... ....T..... ...C..... ...T.T.... .......... ..........
Pesub  .......G.. ..T.T..... ..C.....T. ......T... ..A.C..... .........G
Ermad  .......G.. ....T..... ..A.....T. .....T.... ...AT..... .........G
Pelsin ....C..G.. ....T..... ..A.....T. ......T... ..A.?..... .........G
Podexp .......G.. ....T..... ..A.....T. ...T.TT... ...CC..... .........G
Pedum  .......G.. .C...A.... ..A.....T. .....T..C. ..A..... .........G

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Psumb  CCGTCTACAG CTCACTCTAC AA?GAGAT-A AAAGTAAGCC AA?CAATC-- TACATAATTA
Rhleu  .....C.... ..T..C..G. T.A.GA.... .........A C.AT.G.T.. C.T?C..C..
Elmac  .....C.... ..T..C.... T.G.G..C.. .........A T.AT.G.T.. ..T.C..C..
Emmac  ...?.C.... ..T..C.... T.A.GA.?.. .........A C.A..G.... .?..C..C..
Ellat  .....C.... ..T..C.... C.C.GAGA.. .........A C.AT.G.... ....C..C..
Elden  .....C.... ..T..C.... T.A.GA.C.. .........A C.AT.G.T.. ....C..C..
Elpur  .....C.... ..T..C..G. T.A.GA.... .........A C.AT.G.T.. ..T.C..C..
Elgeo  .GTC.ACAGC T.AC.CTACT ..G..A-... .........A C.AT.G.T.. C...C..C..
Chlon  .......... ..T..C..GT G.A.GA..T. .........A T.A..G.TA. -...C.GC..
Chobl  ...?..C... ..T..C...T G.A.GA.C.. ....C....A T.C..G.TA. -T..C.GC..
Chrug  .......... ..T..C...T G.A.G..... .........A T.AT.G.TA. -...C..C..
Chfim  ......C... .CT..CT..T G.AAGT.C.. ......G..A C.A..G.TAA C---C..C..
Plpla  ..T...C... .CTT....TT GGG?G...A. T.......GA CCA..GC.A? C..---.C.C
Acpal  ..C...T... .CT..CT..T G.A.GTT... ..G...G.?A T.AT?GC.AA C.AGC.-C..
Phgeo  ......T... .CT..CT..T G.AAG-.C.. ......G..A C.AT.G..AC A.A.AC.C..
Phgib  ..C...T... .CTC.C..GG G.AAGAT..T ..G..GG..A C.AT.G..AC C.A-GC.C..
Phnas  ......T... .CT..C..GT G.AAG....T ......G..A C.AT.G..AA C.A-GC.C..
Hytec  .......... T.T..CT..T G.AAGT.... .......A.A C.AT.G.TAA C...A..C..
Pesub  .......A.. .....CT..T G.AAGCTA.. .........A ..AT.GCT.. ACT.C.GC..
Ermad  ......CT.. .CT..CT.GT G.AAGATC.. .....T...A C.AT.G..C. ACT.C..C..
Pelsin .......... ..T..CT..T G.AAGCTA.. .........A ..AT.GC.C. ATT.C.GC..
Podexp ......C... .CT..CT.GT G.GAG..A.. G........A C.AT.G..C. A-T.C..C..
Pedum  ...C.GC... .CT..CT.GT G.AAGC.C.. T........G ..AT.G.AT. A-A.CC.C..
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Psumb  ATAAGTCAGG TCAAGGTGTA GCCAA-TGGG GTGGAAGAAA TGGGCTACA- --TTTTCTAA
Rhleu  ?C........ .......... .....C..A. .......... .......... .........C
Elmac  .C........ .......... .....C..A. A......... .......... .........C
Emmac  .C........ .......... .....C..A. .......... .......... .........C
Ellat  .C........ .......... T....C..A. .......... .......... .........C
Elden  .......... ......?... .....C.... ....?..... ...C...... .........C
Elpur  .......... .......... .....C..A. .C........ .......... .........C
Elgeo  .......... .......... .....C..A. .......... .......... .........C
Chlon  .C........ .......... ....?C..A. A......... .......... .........T
Chobl  GC........ .......... .....C..A. A......... .......... .........T
Chrug  .C........ .......... .....C..AA A......... .......... .........T
Chfim  .C........ .......... ........AA A...TG..G. .......... .........C
Plpla  .C....G..C .........? C..C?..... ....?....G .....?.... .........CC
Acpal  .C........ ...G...... ...C...... .?........ .C.?...... .........CC
Phgeo  .C..A..... .....T.... ?...T..?A. .......... .......... .........TC
Phgib  TC........ .......... .......TA. C...G..... .......... .........C
Phnas  .C........ .......... T......TA. .......... .......... .........T
Hytec  .......... .......... ...T.T..A. A......... .......... .........C
Pesub  GA........ ..C....... .......A.. .....T.T.. .......... ..........
Ermad  .A........ ..C....... ........A. .......... .......... ..........
Pelsin GA........ ..C....... .......A.. ....GT.C.. .......... ..........

Podexp GA.C...... ..C...C... ........A. A......... .......... ..........
Pedum  .......... ..C....... ........A. ....C..... .......... ..........

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Psumb  ATTAGAAATA -ACTAA---- -CGGAAAGAA CCTTGAAAT- -ATGGGTCT- AAAAATAGGA
Rhleu  .C........ .TTC.-.... .........G .......... .....AC... ....G.....
Elmac  CCC...... .TTC.-.... .........G ..C....... .....AC..T ....G.....
Emmac  .C........ .TTC.-.... .........G ..C....... .....AC... ....G.....
Ellat  .C........ .TTC.-.... .........G .TC....... ...A.AC... ....GC....
Elden  .C........ .TTA.-.... .......... ..C....... .....ACT.. ....G.....
Elpur  .C........ .TT..C.... .......... .......... ......C... ....G.....
Elgeo  .C......C. .TTC.-.... .........G ..C....... .....ACT.. ....G.....
Chlon  .C.....T.. TTT....... .........G ........A. .CATT.CTCT ....GC....
Chobl  .......C.. CTTC.-.... .........G ........A. .CATT.CTCT T...GC....
Chrug  .......T.. ACTC--.... A........G ..C.....A. .CATT.CTCT ....GC....
Chfim  .A......C. TCT..CACTA A.A...C.G. TT........ ..AACCCGA. ....GA....
Plpla  CA........ C...TTA... A.....G.G. .........-. .CAA.AC.CA .C..GC....
Acpal  CAA..G..AT CT.CTTA... A.A.G.G.GG ..C..G.... .TA..AC.CA T...GC....
Phgeo  CA...TC... CC.CCTA... A....TT.G. T.A.....C. .CAATCC.CA ....GC....
Phgib  .A........ .TTCTT.... A....GG.C. .T......-. .CA..AC.CA .T..GC....
Phnas  .A........ .TTCTT.... A....GG.C. .T......-. .CA..AC.CA .T..GC....
Hytec  TA........ TTT-.-.... A......ACC .........T T......T.A T...GC....
Pesub  .A........ .TTC.-.... .....C...C .........-. .CA...A..A .T..G.T...
Ermad  CA....T... .GTC.-.... .......... ..C.....-. .CAA.....A .G..GCT.A.
Pelsin .A.......? .TTC.-.... ...A.C...C .......... .TA......T .G..G.....
Podexp TA........ .GTC.-.... .....G...G ..C....-.. ..AA..C..C ....GC..A.
Pedum  .A........ .TT..-.... A....G...C T.C.....-. .TAA.A...C T...GC....
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Psumb  TTTAGCAGTA ATGTGTGAAC AGAGAGCCCA CATTAACCCG GACCTGA-GA T
Rhleu  .......... .....AA.?. .....?.... .......... ..T...G..- C
Elmac  .......... .....A..G. .......... .......T.. ..T...G.AG C
Emmac  .......... .....AA.G. .......... .......T.. ..T...G... C
Ellat  .......... .....AA.G. .......... .......T.. ..T...G... C
Elden  .......... .....AA.G. .......... ........A. ..T....... C
Elpur  .......... .....AA.G. .......... .......T.? ?????????? ?
Elgeo  .......... .....AA.G. ......T... ..C....T.? ?????????? ?
Chlon  .......... ..A..A.... ...A...... T......AA. ........AG C
Chobl  .......... ..A..A.... .......T.. T......AA. ........TAG C
Chrug  ....T..... ..A..A...T .......T.. T......AA. ........CAG C
Chfim  .......... .....A...T .......TT. ........A. .......... C
Plpla  ........C. .....A.... .......... ......T.A. ..T.......
Acpal  .......... ....AA.... ........T. ......TTA. ..-....... C
Phgeo  .A..T..... ....AA...T .......TT. ......T.A. .......... C
Phgib  .......... ....AGA.G. ........T. ....C.TTA. .......... C
Phnas  .......... ....AGA.G. .......... ......TTA. .......... C
Hytec  .....T.... ....AA.... ........T. ........A. ..T....... C
Pesub  .......... ..C.AA...T ...A....T. T.....A... .C....G... C
Ermad  .......... ..T.TGA... ......T.A. A.....GTA. ..TA..G... C
Pelsin .......... ..T.AG...T ........T. T.....G... .C....G... C
Podexp .......... ..T.TG...T ..T.....A. A.....A.A. ..AA....AG C
Pedum  .......... ..T.TG...T ........A. A..G..A.A. .CAA...... C
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Psumb AGGGGTGATG CCTGCCCAGT GACATTGTTT AA?GGCCGCG GTATACTGAC CGTGCAAAGG
Rhleu ...A...... ....?..... ....C..... ..C....... ....C..... .....G....
Elmac ...A...... .......... ....C..... .?C....... ....C..... .....G....
Emmac ...A...... .......... ....C..... ..C..T.... ....C..... .....G....
Ellat ...A...... .......... ....C...?C ..C....... ....C..... .....G....
Elden ...A...... .......... ....C..... ..C....... ....C..... .....G....
Elpur ...A...... ....G..... ....A..... ..C..?.... ...CC..... .....G....
Elgeo ?????????? ?????????? ?????????? ??.???..T. .G?.C..... ....GG....
Chlon .......... .......... ....C..... ..C....... ....C..... .....G....
Chobl .......... .......... ....C..... ..C....... ....C..... .....G....
Chrug .......... .......... ....C..... ..C....... ....C..... .....G....
Chfim .A........ .......... .....A..?C ..C....... ....C..... .....G....
Plpla ?????????? ?????????? ?????????? ??.??????. ....T..... .....G....
Acpal ?????????? ?????????. ..?.-..?.C .TC..?..T. ....T..... .....G.?..
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Phgeo ?????????? ?????????? ?????????? ??C..?..?. ....T..... .....G.?..
Phgib .......... ..G....... .........C .TC....... ....C..... .....G.?..
Phnas .......... ..?....... ....C....C ..C....... ....T..... .....G....
Hytec ?????????? ?????????? ??..A.?..A ..T....... ....C..... .....G..A.
Pesub ?????????? ?????????? ?????????? ??.??????? ??????.... ..?..G.?..
Ermad ?????????? ?????????? ???????... ..C..T.... ....C..... .....G....
Pesin ..A...A?.. ....T....? ..T-CCA..A ..C..T..?. ...ACA.... ..C..G....
Poexp ?????????? ?????????? ????????.. ..C..?.... ....C..... .....G....
Pedur ?????????? ?????????? ????????.A ..C....... ...CC?.... .....G....
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Psumb TAGCATAATC ACTTGTCTTT TAAATAAAGA CTAGAATGAA TGGCTAAACG AGGTTCTATC
Rhleu ....G..... ...C...... .......... .......... .......... ..........
Elmac ....G..... ...C...... .......... .......... .......... ...C......
Emmac ....G..... ...C...... .......... .......... .......... ..........
Ellat ....G....A ...C...... .......... .......... .......... ..........
Elden ....G..... ...C...... .......... .......... .......... .A........
Elpur ....G..... ...C...... .......... .......... .......... ...C......
Elgeo ...?G..... ...C...... ...?...... .......... .......... ..........
Chlon .......... ...C...... .......... .......... .......... ...C....C.
Chobl .......... ...C...... .......... .......... .......... ...C....C.
Chrug .......... ...C...... .......... .......... .......... ...C....C.
Chfim ....G..... G..C...... .......... ..G....... .......... ...A......
Plpla ....G..... .......... .......... ..G....... ......?... ...C.T..C.
Acpal ....G..... .......... .......... ..G....... .......... ...C.?....
Phgeo ....G..... .......... ......G... ..G....... ..A....... ...C......
Phgib .......... .......... .......... ..G....... .......... ...C......
Phnas .......... .......... .......... ..G....... .......... ...C......
Hytec ....G..... ...C...... .......... .......... .......... ....C...C.
Pesub ?......?.. .......... ...G..G... .......... ......C... ..........
Ermad .......... .......... .......... .......... .......... .......CC.
Pesin .......... .A........ ....C.G... ..G....... ....C.T... ..........
Poexp .......... G......... .......... .......... .......... ..........
Pedur .......... .........C .......... .......... A......... ........C.

Psumb TGTCTCTTAC AAACAATCAG TGAAATTGAT CTTCTCGTGC AAAAGCGAGA ATAACACCAT
Rhleu .......... ...T...... .......... .CC....... .......... ....TT...C
Elmac .......... ...T...... .......... ..C?...... .......... ..?......C
Emmac .......... .......... .......... .CC.C..... .........G .....C....
Ellat .......... ...T...... .......... .CC.C..... .......... ....TC....
Elden .......... .......... .......... ..C.C..... .......G.. .....C....
Elpur .......... ...T...... .......... .CC....... .......... .........C
Elgeo .......... .......... .......... .CC.C..... .......... ..:.T.....
Chlon .......... ....T..... .......... A.C....... .......... ....TC....
Chobl .......... ...TT..... .......... A.C....... .......... ....TC....
Chrug .......... ...TT..... .......... ..C....... .......... ...T..A...
Chfim .......... .GGT.....A .......... ..A....... .......... ..G.T.A...
Plpla ........?. GGGT...... .......... ..C?...... .........? ..?.T.A...
Acpal .......... GGGA...... .......... ..C....... .......... ....T.A...
Phgeo ......C... .GG.G..... .......... ..C?....?. .......... ..GGT.A...
Phgib .......... .GGA..C... .......... .CC....... .......?.G ....T.A...
Phnas .......... .GGA..C... .......... .CC....... .......G.. ....T.A...
Hytec .......... .......... .......... .CC?.....? ?.........? ....T.A...
Pesub .......... .......... .......... ..C....... ..??...... ..?C.CA...
Ermad .......... .......... .......... T..ACT.... ......AGT. ...T.C....
Pesin .......... .......... .......... ..C....... .......... ..GT.C....
Poexp .......... .......... .......... ..A....... .........T ..TTTC....
Pedur ......C... ..C....... .......... ..G?C...?. ....?..G.C ..?..C....
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Psumb AAGACGAGAA GACCCTGTGG AACTTTAAGT -ACAAGTCGA CCATTAAAT- --------CTT
Rhleu .......... .......... .........C ..TG....A- TT.CC.--CT A.ACTAA..A
Elmac .......... .......... .......... .GT.....A- TA..?.--CT A.ACTAA..A
Emmac .......... .......... .......... ..TG....A- T..CC.--.T A.ACCAA..A
Ellat .......... .......... .......... ..T.....A- T..CC.--.T A.ACCAA.CC
Elden .......... .......... .......... ..T.G...A- .A..C.--.C A.ACCAA..A
Elpur .......... .......... .......... .GT.....A- ...CC.--CT A.ACTAA..A
Elgeo .......... .......... .......... ..T.....A- T..CC.--.T A.ACCTA..A
Chlon .......... .......... .......... ..T.....A. .T.C.....T ACAATAC.AA
Chobl .......... .......... .......... ..T.....A. .T.C....CT ACAACAC.AA
Chrug .......... .......... .......... ..T.....A. .T.CC...CT AAAACTCTAA
Chfim .......... .......... .....A..A. ..AC...TA. AGGA.G---T AAATTAC.AA
Plpla .......... .......... ?.......A. ..TG.C.AA. .A..A.G.-. AACCCAC-CA

Acpal .......... .......... .....C..A. ..TC..CTA. AG.CC...-. AACCAAC-AA
Phgeo .......... .......... ........A. ..AC..CTA. TAGCC.T.-. AACCCACTAA
Phgib .......... .......... ........T. T.A....TA. .A.C...--T AA?CCCA.AA
Phnas .......... .......... ........A. T.A....TA. .A.C...--C AAACTCA.AA
Hytec .......... .....C.... ?.T.....A. .?.GGT.TA. ...--?T.?T AAATTTAT-A
Pesub .......... .......... .....C.... ?TT..CCAAC AT.C.TC.AA GACCCAC.CG
Ermad .......... .......... ?........A ACT..TCA.C GAGCC....T TAACTAC.CC
Pesin .......... .......... .....C.... ..TT.AC.A. T-..ACTT.T ACACCTCTAC
Poexp .......... .......... .........A ACT..TCA.C ---C.TC..G TAA.TACTAC
Pedur .......... .......... ?....C...A A.T..TCA?C .A..?.GCCA CACCAAA.--

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Psumb TCCTATA--- -AACTT-TAC CCCTAA-T-T ACTCGACTAT TA-CTTTTGG TTGGGGTGAC
Rhleu CT.A.C.GAA ....AC.... TTA.--.C.C ..CT....C. A.A....C.. ......C...
Elmac CT.A.C.GAA T...AC.... TTAC..CC.. .TCT...C.A A.A....C.. ......C...
Emmac CT.A.C.GAA ....AC.... T-AC..CC.. TACT....TC ..A....C.. ......C...
Ellat CT.A.C.GAA T..TAC.... T.A...CC.C T.CT....T. C.A....C.. ......C...
Elden .T.A.C.GAA ....AC.--. .TG...AA.C ..CT....TA A.A....C.. ......C...
Elpur CT.A...GAG ....AC.C.A TTA...AC.. ..CT....CA A.A....C.. ......C...
Elgeo CT.A.C.GAA .T.ACC.... T.A...CC.. T.CT....TC C.A....C.. ......C...
Chlon CT...AT.GA ....AC.C.. ATAC.G.... ...T...CT. --A....C.. ..........
Chobl CT...CT.GA ....AC.C.. ATAA...... ...T...CT. --A....C.. ..........
Chrug CT.C.CTTGA .G..AC.... ATAC...... ...T...CT. --A....C.. ..........
Chfim AT.C.ACAGG ....AC.A.G .TAC..C-.C CA.TT.ACCC ...T...... ..........
Plpla A..G.C.CGG .CC.AC.C.A ..AC..TCT. T.ATT.GCC. ...T...C.. ..........
Acpal A..A....GG .?C.AC.C.A ..AC..TCCC TACTT.GCC. ...T...C.. ..........
Phgeo A..G....GG ..C.AC.... TA-.G.CCAC T.-TT.GCTC ...T...C.. G.........
Phgib ?-.C...GGG ..G?AC.ATA T.A.G.T?.C CACTT.GCC. ..AT...C.. ..........
Phnas A-.C...TGG ..G.AC.ATA T.A.G.T..C CACTT.GCC. ..AT...C.. ..........
Hytec C..A.GGAAA ?.TTAC.C.T TAT.G.G... ---TA..CC. --A....?.. ..........
Pesub C.TGT?GGGA A.GGAAA..A A.AC.GT?TC C.AT.G-.TA .CA...GG.. .?....C...
Ermad ..TCCCCATG GGT.CA.C.T TAAAC.T..C C.CT..-.TA .TA....C.. ......C...
Pesin C.TA.GGGAA AG.AA..A.A ATT...G.A. CTC.TGG.TA .TC..... ......C...
Poexp A.--CCCACG GG..CA.C.T .AAATTT..C C.CT..-.TA .TA....C.. ......C...
Pedur -.GCCCTACG GG..GA.C.. AAACC.CAAC CTCT..-.TA C.A....C.. ......C...
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Psumb CTCGGAGAAC AATAAACCCT CCGAAAAT-- --TTTTT--- ----ACTGAG ATACCACAAA
Rhleu .......... ......A... .......-.. ....AC.... .......A.. .C.A......
Elmac .......?.. ......A... .......AA. ...CAC.... .......A.. ...A......
Emmac .........A ..C...A... .......-.. ....AC.... .......AC. ...AT.....
Ellat .........A ......A... .......-.. ....AC.... .......A.. ...GT:....
Elden .......G.T ......A..C .......-.. ....AC.... ......CA.. ...G......
Elpur .......... ..C...A... ......--.. ....AC.... .......A.. ...GT.....
Elgeo .........A ......A... .......-.. ....AC.... .......A.. .C.AT.....
Chlon ..T....... ......A... ..A.....A. ...-A-.... ......CA.. ..TA-....C
Chobl ..T....... ......A... ..A....AA. ...-A-.... ......CA.. ..TA-...GC
Chrug ..T....... ..C...A... ..A.....A. ...-A-.... ......CA.. ..CA-...C.
Chfim .........A .GA...A... .......-.. ..C..A.... ....G.CA.. .ACA-...GG
Plpla .......?.A ..CC..A... .....--GA. .....AC... ......C?.. .A.A-.....
Acpal .....G...T ..C...A..C .....?.-.. ..CA.?.... ....?..A.. .ATA-....G
Phgeo .......?.A ..CG..A... .......-.. ..CC.A.... .......A.. .ACA-.....
Phgib .........A ..C?..A... ....?--GA. ..?.A?.... ......CA.. .ACA-...G.
Phnas .........A ..C...A... .....--GA. ..C.AA?... .......A.. .ACA-.?.G.
Hytec ?......... ..A...A... .......-.. ..C.AC.... ......CA.. ...TA.....
Pesub .........- ..C.C-T... ......?GA. ..G.ACATCT CAT...CT.. ..T--...CC
Ermad .......... ......A... .....G..AG GGC.A..TCT CCTA.T.A.. .ATG-...TT
Pesin .......T.A ..C...A... .......GA. ..G.A-.TCT CAT....A.. .ATA-...CC
Poexp .......T.. ..G....... .....G..AG GAC-AAAA.T CCAC..CA.. .AT.-...CC
Pedur .......C.. ..A....... ........AG GAC-ACCACT CCAC..AA.. ..CA-...C.

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Psumb CCTAAGTGCT TT-CGGC--A AAGCGATCCA A-AAACTTGA TCAACGAACC AAGCTACCCC
Rhleu .........C .......... ..A....... .TTT.AA... .......... ..........
Elmac .........C -.A....... ..?....... -T.T..A... .......... ..........
Emmac .........C -.A....... ..A....... .T.C...... .......... ..........
Ellat .........C -.A....... ..A....... .T.T...... .......... ..........
Elden T.......TC -.A..A.... ..A....... .T.T..A... .......... ..........
Elpur .........C .......... ..A....... .T.T..A... .......... ..........
Elgeo .........C -.A....... ..A....... .T.T...... .......... ..........
Chlon ..A......C C.AAA..... ..A....... .T.CT..... .......... ..........
Chobl ..A......C -.AA...... ..A....... .T.C.T.... .......... ..........

Chrug ..A.....?C -CAA...... ..A....... .T.T.T.... .......... ..........
Chfim ..A...CA.C A.AGATG..T ..A....... .T.T.T.... ....T....A ..........
Plpla T.A......C -CA......T ..?....... .T.C.T.... .T..T..... ..........
Acpal ..A....?.C -.A...?..T ..A....... .T.C.T.?.? .T..T..... ?.........
Phgeo ..A......C -.C......T ..?....... .T.T.?...? .T..T..... ..........
Phgib ..A.?....C -.C.?..... ..A....... .T.C.T.... .T..T..... ..........
Phnas ..A......C -.C....... ..A....... .T.?.T.... .T..T..... ..........
Hytec .........TC .....A...? ..?....... .?.T.T.... .......... ..A.......
Pesub T.A?-..... ..AAA..... ..A....... .T?T.T...? .........A ..........
Ermad T.A..A.A.C ACTA..TCCC ..AT...... .T.TTT.... .........A ..........
Pesin T.A......C ..AAA..... ..A....... .T.T.T.... ...G.....A ..........
Poexp T.A....A.. AGCAA.T.A. ..A....... .T...T.... .........A ..........
Pedur ..A.GTGCAA A.T?AA?CAG ..A....... .T........ .A.......A ..........
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Psumb AGGGATAACA GCGCAATCCC CTCTTAGAGT TCATATCAAC GACGGGGGTT TACGACCTCG
Rhleu .......... .......... .......... C........T .......... ..........
Elmac .......... .......... .......... C........T .......... ..........
Emmac .......... .......... .......... C........T ..T....... ..........
Ellat .......... .......... .......... C........T .......... ..........
Elden .......... .......... .......... C........T .......... ..........
Elpur .......... .......... .......... C........T ..T....... ..........
Elgeo .......... ..?....... .........? C......TTT ..?....CC. ??........
Chlon .......... .......... .......... C.......... ..T....... ..........
Chobl .......... .......... .......... C.......... ..T....... ..........
Chrug .......... .......... .......... C.......... ..T....... ..........
Chfim .......... .......... .......... C.C........ ..T....... ..........
Plpla .......... .......... .......... C.C........ ..T....... ..........
Acpal .......... .?........ .......... C.C........ ..?........ .?........
Phgeo .......... .?........ .......... C........? ..T.....?. .?........
Phgib .......... .......... .......... C.C........ ..T....... ..........
Phnas .......... .......... .......... C.C........ ..T....... ..........
Hytec G......... ..?....... .......... C.C....G.. ..T....... ..........
Pesub .......... .......... T...C.A... C.C...T.?T ..T....... ..........
Ermad .......... .......... .CT.C..... .......... AG-....... ..........
Pesin .......... .......... T.....A..C C.G...T... ..T....... ..........
Poexp .......... .......... ..T.C..... .......... A.-....... ..........
Pedur .......... .......... ...CC....? C.......... A.-....?.. ..........

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Psumb ATGTTGGATC AGGACATCCT AATGGTGTAG ?CGCTATTAA GGGTTC
Rhleu .......... .......... .......C.A C.....A... ......
Elmac .......... .......... .......C.? C.....A... ......
Emmac .......... .......... .......C.. C.......... ......
Ellat .......... ......C... .......C.. C.......... ......
Elden .......... ......C... .......C.A C.......... ....-.
Elpur .......... .......... .......C.? C.....?... ..?.-.
Elgeo ...A...??? ?????????? ?????????? .????????? ??????
Chlon .......... .......... .......C.. C.....A... ......
Chobl .......... .......... .......C.. C.......... ....C.
Chrug .......... .......... .......C.. C.....?... ......
Chfim .......... .......... .......C.. C.......... ......
Plpla .......... .......... .......??? .????????? ??????
Acpal .......... .......... .........? C.....???? ??????
Phgeo .......G.. .??....... .????????? .????????? ??????
Phgib .......?.. .......... .......C.. C.......... ..A.-.
Phnas .......... .......... .......C.. C.......... ....-.
Hytec .......... ....?..... .......??? .????????? ??????
Pesub .......??? ?????????? ?????????? .????????? ??????
Ermad .......... ..?....... .????????? .????????? ??????
Pesin .......... .......... .......C.? CA........ ....-.
Poexp .......... .......... .......C.T CA....A... ......
Pedur .......T.. .......... .......??? .????????? ??????
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