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A Phylogeny for Australian Chelid Turtles Based on Allozyme Electrophoresis

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Abstract

Allozyme electrophoresis was used to explore relationships among the Australian Chelidae in order to test the currently accepted phylogeny and to determine the affinities of several distinctive but undescribed species. The data set was comprehensive, being based on 54 loci for turtles from 76 populations of 22 species (excluding *Pseudemydura umbrina* and *Chelodina novaeguineae*). Analysis by both phenetic and phylogenetic methods revealed that the genus *Elseya*, as currently recognised, is paraphyletic. The closest common ancestor to species of *Elseya* has *Emydura* (and possibly *Rheodytes*) among its living descendants. Five clades among the short-necked taxa were considered distinctive enough to warrant recognition at the generic level, namely (1) *Emydura*, (2) *Elseya* (to include only *El. novaeguineae*, *El. dentata* and three related but undescribed taxa), (3) *Rheodytes*, (4) the *El. latisternum* group (to include *El. latisternum* and three related but undescribed taxa), and (5) 'short-necked alpha', an undescribed species with no clear affinities. Levels of divergence among these generic groups were similar to levels of divergence among genera of cryptodiran turtles. A phylogeny for the Australian Chelidae, incorporating the results of the allozyme analyses, is presented. Further work is required to firmly establish the affinities of *Rheodytes* and 'short-necked alpha' in a phylogeny for the Australian chelid turtles.

Introduction

Australian freshwater turtles, with the exception of *Carettochelys insculpta*, belong to a single family of side-necked turtles, the Chelidae. The current classification (Cogger *et al.* 1983) lists 15 species in five genera—6 in *Chelodina*, 2 in *Elseya*, 5 in *Emydura*, *Pseudemydura umbrina* and *Rheodytes leukops*. Chelid turtles occur in Australia, New Guinea and South America, and as no fossil material has been found outside their present range (Pritchard 1979) the family is considered to be of Gondwanan origin (Burbidge *et al.* 1974). Whether the chelid turtles of the Australian region and those of South America represent two monophyletic assemblages is not clear. Certain morphological features align *Pseudemydura umbrina* most closely with the South American *Platemys* (Legler 1981) and there is debate as to whether the specialised long necks and other skeletal features of some Australian and South American species are independently derived (Gaffney 1977; Pritchard 1984).

Burbidge *et al.* (1974) made the first attempt to develop a phylogeny for the Australian Chelidae. They undertook a phenetic analysis of morphological and serological data and concluded that three divergent groups, equally related to each other, exist within the Australian Chelidae—the species of *Chelodina*, the species of *Emydura/Elseya* [to which Legler and Cann (1980) later added *Rheodytes leukops*], and *Pseudemydura umbrina*. This trichotomy is supported by other studies of morphology (Goode 1967; Gaffney 1977; Legler and Cann 1980), total-protein electrophoresis (Frair 1980), karyotypes (Bull and Legler 1980)

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and behaviour (Webb 1978). The phylogeny of Gaffney (1977), which incorporated South American forms and was based on a cladistic analysis, does not conflict in any important respects with that of Burbidge *et al.* (1974). Gaffney would have the divergence of *Pseudemydura* predate that of the divergence of *Emydura* and *Elseya* from *Chelodina*. The phylogeny shown in Fig. 1 combines the views of Burbidge *et al.* (1974), Gaffney (1977) and Legler and Cann (1980). For the purposes of this paper it is considered to be the currently accepted phylogeny for the Australian chelid turtles.

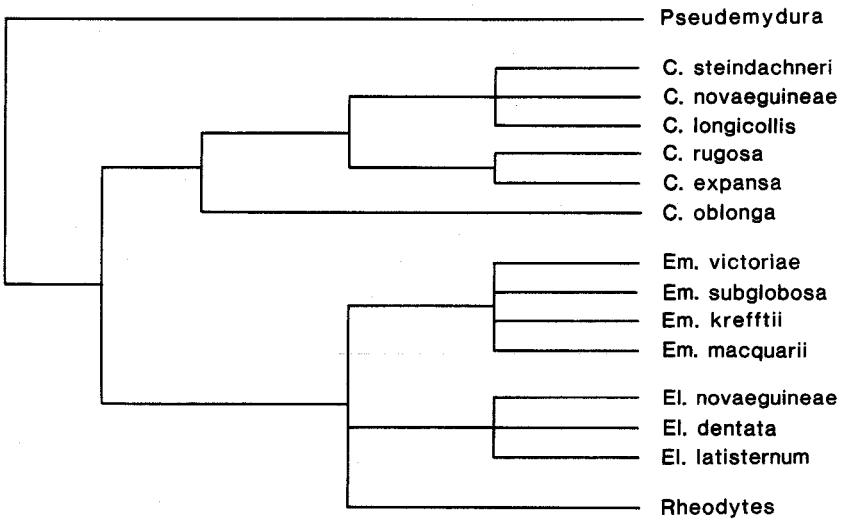

Fig. 1. The current phylogeny for Australian chelid turtles, including *El. novaeguineae* from New Guinea, based on that produced by Burbidge *et al.* (1974), but incorporating the modifications of Gaffney (1977) and Legler and Cann (1980), and the nomenclatural changes of Cogger *et al.* (1983). The *Elseya/Emydura/Rheodytes* trichotomy is unresolved. 'Short-necked alpha' is unplaced, but its affinities lie with the *Elseya/Emydura/Rheodytes* group.

Attempts to have this phylogeny reflected in the generic-level classification have led to considerable debate on the status of the genus *Elseya*. The genus was initially erected for *Elseya dentata* (the type species) and *El. latisternum*. It was characterised by the presence of a horny shield on the dorsal surface of the head, prominent tubercles on the dorsal surface of the neck, a pair of tubercles on the chin and the usual absence of a cervical scute (Gray 1867, 1872). Boulenger (1889) redefined the genus as being typified by the alveolar ridge, a longitudinal ridge on the maxillary triturating surface, present only in *El. dentata*. *El. latisternum* and *El. novaeguineae* were placed in the genus *Emydura*. Subsequently, Goode (1967) expressed little faith in the alveolar ridge as a taxonomic feature at the generic level, citing cases of variation in this feature among species of well recognised cryptodiran turtle genera, and transferred *El. latisternum* and *El. novaeguineae* back to *Elseya*.

Authors who agree with Goode's rejection of the importance placed on the alveolar ridge by Boulenger (1889) have nevertheless argued that the species of *Elseya* are insufficiently distinct to warrant separate recognition at the generic level. Gaffney (1977) was unable to differentiate the two genera consistently using cranial characters and Frair (1980) could not differentiate them using total serum protein electrophoresis. Species within the two genera have indistinguishable karyotypes (Bull and Legler 1980), and the level of divergence of

Elseya and *Emydura* in serological comparisons is comparable only to that of species groups within *Chelodina* (Burbidge *et al.* 1974). Gaffney (1979) chose to include the species of *Elseya* in *Emydura*, and Frair (1980) suggested on the basis of his studies that *El. novaeguineae*, *El. latisternum*, *Em. signata* and *Em. subglobosa* should be placed in the one genus. More recently, McDowell (1983) interpreted a wide range of morphological characters as indicating that the closest relative of *El. dentata* is *Em. australis* (including *Em. krefftii* and *Em. subglobosa*) and not *El. latisternum*. He concluded that 'the generic recognition of *Elseya* seems unwarranted' and synonymised *Elseya* and *Emydura*.

In contrast to the views of McDowell, Gaffney and Frair, Legler and Cann (1980) considered that morphological similarities between species in the two genera and the new genus *Rheodytes* are sufficient to indicate a common ancestry, but not to warrant lumping of any of the three genera. The move to combine *Elseya* and *Emydura* has not gained wide acceptance (Cogger *et al.* 1983).

Chelid turtles are conservative in many morphological features, and their present classification is poorly founded and in drastic need of review (Cogger 1983). Allozyme electrophoresis provides an alternative to traditional morphological approaches to systematics (Avise 1975; Buth 1984; Richardson *et al.* 1986; Hillis 1987) and is particularly suited to systematic studies of groups with the appropriate level of genetic divergence, typically congeneric species or closely related genera. It provides a large number of qualitative characters that are genetically determined. Sufficient is known of the enzyme systems in use to ensure that these characters are independent of each other and any morphological characters used, and that they are generally expressed in all individuals regardless of age or sex. Moreover, when compared with morphological studies, fewer individuals may need be sampled per population in order to identify diagnostic character states, and within-species variation is readily distinguishable from between-species differences. Lastly, biochemical characters tend to diverge at a more constant rate than morphological characters and hence may give useful information about the relative age of lineages (Avise 1983).

In this paper, we use allozyme electrophoresis to explore relationships among the Australian Chelidae and test them for consistency with the currently accepted phylogeny. This phlogeny is extended to include several new species, including an enigmatic form colloquially known as 'short-necked alpha'. The implications of suggested modifications to the chelid phylogeny for chelid classification are discussed, particularly with regard to the genus *Elseya*.

Materials and Methods

Specimen Collection and Identification

Fifteen species of Australian chelid turtle are recognised by Cogger *et al.* (1983) (Table 1), and to this we add the 10 forms listed in Table 2. These latter forms are considered to be species because of electrophoretic evidence to be presented elsewhere (Georges and Adams, unpublished data). Many also have the support of preliminary morphological analyses undertaken by Legler (1981). However, we have been unable to distinguish between *Em. macquarii*, *Em. krefftii* (including the Fraser I. and Cooper Ck forms: Goode 1967; Georges 1982) and *Em. signata* on the basis of allozyme characters so, for the purposes of developing the phylogeny, we have considered them to be the one taxon, *Em. macquarii*. Thus, for the purposes of phylogenetic reconstruction, we recognised 23 Australian species. Of these, only *Pseudemydura umbrina* and *Chelodina novaeguineae* were unavailable for study.

A total of 277 specimens of chelid turtle from 76 populations of 21 Australian species and one endemic New Guinean species were collected from drainages in the five Australian mainland states, the Northern Territory and Papua New Guinea (Fig. 2, Tables 1 and 2). The sampling strategy involved obtaining a minimum of five turtles (not always achieved) from each of several populations of species in the genera *Elseya*, *Emydura* and *Rheodytes*, and at least five turtles from each species of *Chelodina*. Samples for *Chelodina expansa*, *C. longicollis*, *Em. macquarii* and *Emydura* sp. (Cooper Creek, S.A.) were supplemented from the tissue collection of the South Australian Museum. *Elseya novaeguineae* (Meyer), endemic to New Guinea, was obtained from Baitetta (5°00'S.,145°44'E.) and Warabruk

Table 1. Currently recognised species of Australian chelid turtle, drainages from which they were collected, and sample sizes

Elseya novaeguineae, also included in the present study, is endemic to New Guinea. Classification follows that of Cogger *et al.* (1983). Location numbers correspond to those used in Fig. 2

Species	Locations sampled	*n*
Chelodina expansa Gray, 1857	6, 7, 10, 25	8
Chelodina longicollis (Shaw, 1794)	14, 19, 21, 22, 23, 24, 25	34
Chelodina novaeguineae Boulenger, 1888	Unavailable	0
Chelodina oblonga Gray, 1841	28	5
Chelodina rugosa Ogilby, 1890	31	5
Chelodina steindachneri Siebenrock, 1914	27	1
Elseya dentata (Gray, 1863)	29, 30	9
Elseya latisternum Gray, 1867	1, 2, 7, 9, 10, 12, 13, 31, 32	29
Elseya novaeguineae (Meyer, 1874)	34, 35	5
Emydura krefftii (Gray, 1871)	5, 6, 7, 8($n=4$), 9, 26($n=12$)	35
Emydura macquarii (Gray, 1830)	20, 22, 23, 25	23
Emydura signata Ahl, 1932	11, 12, 13, 14, 16, 17	35
Emydura subglobosa (Krefft, 1876)	36	3
Emydura victoriae Gray, 1842	29, 30	14
Pseudemydura umbrina Siebenrock, 1901	Unavailable	0
Rheodytes leukops Legler & Cann, 1980	6	1

Table 2. Distinctive forms of Australian chelid turtles considered to be species for the purposes of the phylogenetic analyses

Elseya (Bellingen) and *Elseya* (Manning) are sibling species. *Emydura* (Sleisbeck) and *Emydura* (Daly Mission) were *Em. australis* (Gray, 1841) before the holotype bearing this name was found to have come from the Macquarie River in New South Wales (Cogger *et al.* 1983). Photographs of many of these distinct forms appear in Cann (1978)

Species	Current designation	Locations sampled	*n*
Chelodina (Mann)	*Chelodina rugosa*	32	1
Elseya (Sth Alligator)	*Elseya dentata*	31	5
Elseya (Burnett)	*Elseya dentata*	6, 7	13
Elseya (Johnstone)	*Elseya dentata*	4	6
Elseya (Bellingen)	None	15	5
Elseya (Manning)	None	18	5
Elseya (Gwydir)	*Elseya latisternum*	21	10
Emydura (Daly Mission)	*Emydura 'australis'*	3, 30, 31	10
Emydura (Sleisbeck)	*Emydura 'australis'*	1, 30, 33	9
'Short-necked alpha'	None	Probably 9	5

(5°23'S.,145°05'E.) in the Madang Province (Fig. 2). *Emydura subglobosa* has been reported from the northern tip of Cape York Peninsula, Queensland, but specimens used in this study were collected from near Port Moresby in New Guinea.

All turtles representing described species were identified with the aid of keys provided by Cogger (1983). Distinctive populations and undescribed species were assigned to genera by means of these keys and were associated with literature references on the basis of locality and comparisons with published photographs or descriptions. One exception, a form colloquially known as 'short-necked alpha', could not be assigned to any known genus on morphological grounds and its natural distribution is unknown.

'Short-necked alpha' is known to science only from pet-shops in Victoria. With few exceptions, the specimens were lodged with Australian museums and cross-referenced to tissue samples for future reference.

Specimens were returned alive to the laboratory, where they were killed by intra-cranial injection of sodium pentabarbitone (Nembutal). Samples of liver, heart, muscle, kidney, whole blood and plasma were removed, immediately frozen by immersion in liquid nitrogen and stored at −70°C prior to use.

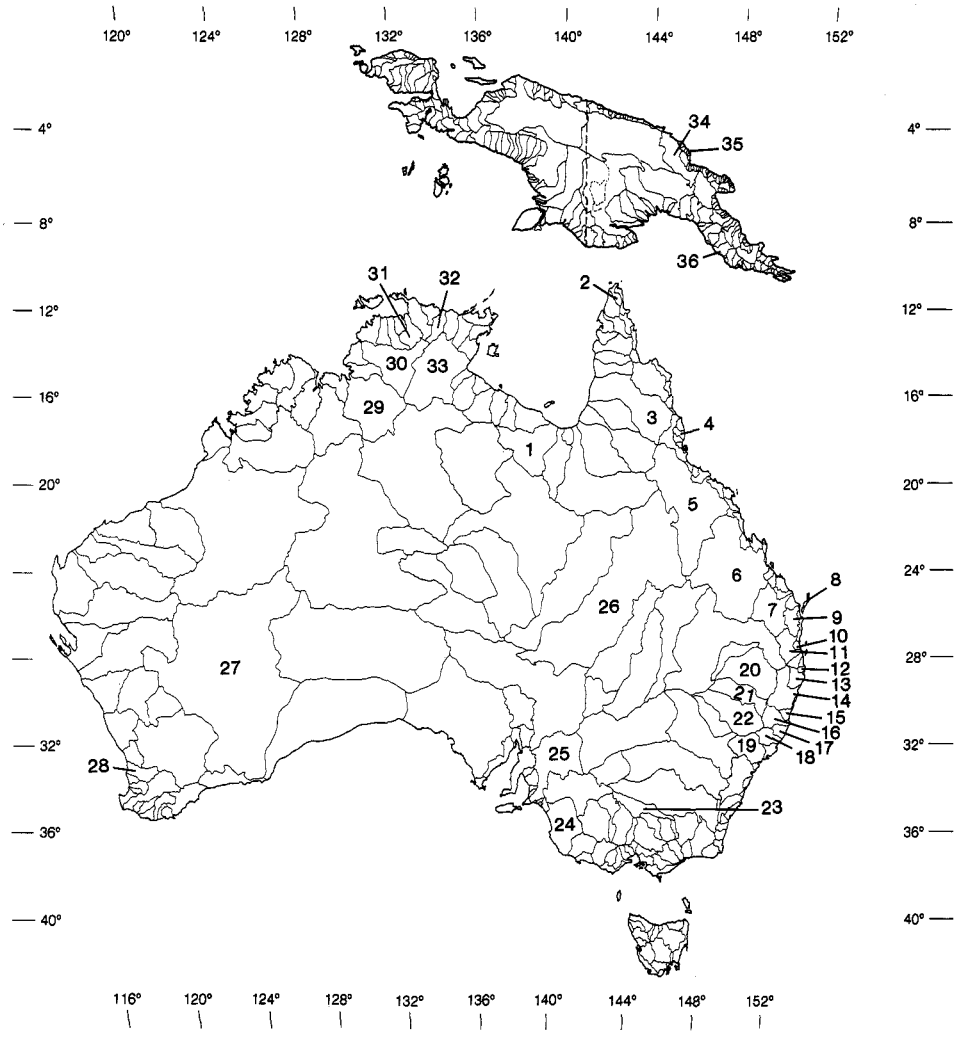

Fig. 2. Australian and Papua New Guinean drainage basins, showing the 36 drainages from which samples were collected. Drainage basins are numbered as follows. *Queensland*: 1, Nicholson R.; 2, Jardine R.; 3, Mitchell R.; 4, Johnstone R.; 5, Burdekin R.; 6, Fitzroy R.; 7, Burnett R.; 8, Fraser I.; 9, Mary R.; 10, Pine R.; 11, Brisbane R. *New South Wales*: 12, Tweed R.; 13, Richmond R.; 14, Clarence R.; 15, Bellingen R.; 16, Macleay R.; 17, Hastings R. (including Nambucca R.); 18, Manning R.; 19, Hunter R.; 20, Border Rivers (Severn R., Dumeresque R.); 21, Gwydir R.; 22, Namoi R. *Victoria*: 23, Murray Riverina Basin. *South Australia*: 24, Millicent Coast; 25, Lower Murray R.; 26, Cooper Ck. *Western Australia*: 27, Salt Lake Basin (Wiluna); 28, Murray R. *Northern Territory*: 29, Victoria R.; 30, Daly R.; 31, South Alligator R.; 32, Liverpool R. (including Mann R.); 33, Roper R. *Papua New Guinea*: 34, Ramu R.; 35, un-named drainage, Madang Province; 36, Lokoti R., Port Moresby.

Electrophoresis

A pilot study identified liver and muscle as the most suitable tissues for electrophoretic analysis. Liver and muscle homogenates were stored and prepared as detailed in Adams *et al.* (1987) and were screened electrophoretically on 'Cellogel' (Chemetron, Milan) using techniques described previously (Richardson *et al.* 1986). The principles used to assign mobility states, conduct allozymic interpretations and confirm electromorph identity are also detailed in Richardson *et al.* (1986). The enzyme products of 54 presumptive loci gave staining of sufficient intensity and resolution to be scored. The abbreviations for the enzymes used in this paper (see Table 3) follow Harris and Hopkinson (1976) and Richardson *et al.* (1986).

Phenetic Analysis

A descriptive summary of genetic similarities among species was obtained using a phenetic approach based on principal co-ordinates analysis (PCoA: Pielou 1984) as implemented in the program PATN (Pattern Analysis Package: Belbin 1987). Genetic distances D, calculated as the complement of Rogers' S (Rogers 1972), were obtained for all possible pairwise comparisons of populations and the results were assembled in a 76×76 matrix. Rogers' distance was chosen instead of Nei D or per cent fixed differences (argued as being more appropriate for systematic studies by Richardson *et al.* 1986) because, unlike the other measures, Rogers' distance is metric and therefore suitable for analysis by PCoA and distance-Wagner procedures. In PCoA, not to be confused with principal components analysis (PCA), the distances between species are mapped in a multi-dimensional space which is then reduced to a workable dimension by ordination (PATN option PCA preceded by a Gower transformation). A three-dimensional visual summary of genetic relationships resulted and was displayed using SAS/GRAF (SAS Institute Inc. 1987). It is now generally recognised that purely phenetic methods are inappropriate for phylogenetic analysis, so adjacency of taxa in Fig. 3 should not necessarily be interpreted as indicating monophyly. Adjacency may also belie hidden distance in deeper dimensions, but this possibility was routinely checked by examination of eigenvalues (Pielou 1984) and analyses were repeated for each emergent grouping of taxa where necessary.

Phylogenetic Analysis

A variety of methods are available for phylogenetic reconstruction. Character-state data can be analysed manually according to strict Hennigian principles whereby plesiomorphic states are identified by comparing outgroup and ingroup species, and clades are defined on the basis of synapomorphies (Baverstock *et al.* 1979; Patton and Avise 1983; Richardson *et al.* 1986). Alternatively, algorithms employing a range of parsimony criteria are available for constructing cladograms directly from the character-state data (Felsenstein 1982; Swofford 1985). Distance data can also be analysed directly, with each method differing in the philosophy of how best to construct the tree. The distance-Wagner procedure (Farris 1972) is designed to find the tree of the shortest total length, equivalent to the least number of evolutionary changes. The Fitch and Margoliash (1967) approach aims to minimise the distortion between the input distances and the distances between species on the resulting tree. None of the above approaches to analysis of electrophoretic data is universally accepted (Farris 1981; Mickevich and Mitter 1981; Felsenstein 1982, 1983; Buth 1984; Richardson *et al.* 1986; Swofford and Berlocher 1987).

The first analysis of the present study was undertaken using the distance-Wagner procedure (Farris 1972; Swofford 1981) based on Rogers' D with the species of *Chelodina* as a composite outgroup for the remaining species. The program WAGPROC (Version 3.3) was implemented on an IBM PC using Lahey Computer Systems implementation of FORTRAN 77. Options for randomly shuffling the species before analysis and to begin tree building at a random branch were added to the program. The heuristic approach of repeated runs of WAGPROC (with shuffling and random-branch starts) in search of the shortest tree was adopted. On each iteration of WAGPROC, the length of the resulting tree was compared with the previously found shortest tree and only a set of the shortest trees (tolerance 2%) was retained. All options available in the WAGPROC program (Swofford 1981) were used in an effort to find the shortest tree. Approximately 25 000 trees were compared.

A second approach to the distance analysis was achieved by constructing a Fitch–Margoliash tree from the Rogers' distances using the FITCH program of the PHYLIP 2.31 package (kindly supplied by J. Felsenstein). Global branch swapping was used under the default options of the program and the shortest of 1698 trees were selected.

While distance analyses have their limitations, the presence of polymorphism within species creates a problem for character-based methods that has yet to be satisfactorily solved (Swofford and Olsen 1990). Our approach to character-based analysis was to undertake a parsimony analysis with loci as characters and allozyme mobilities as character states using the program PAUP (Swofford 1985). PAUP was not designed to analyse electrophoretic data, and does not cater for polymorphism. Swofford and Berlocher (1987, p. 317) recommend it 'in the absence of polymorphism in the terminal taxa', as an alternative to their FREQPARS procedure 'which extends naturally to accommodate polymorphism ...'. Rearrangements of electrophoretic data to conform with the requirements of PAUP, for example, by treating alleles as characters and their presence or absence in a taxon as the character state, have been shown to be invalid (Richardson *et al.* 1986). We chose to eliminate polymorphisms from the data, prior to analysis by PAUP, in the following way.

1. Rare allozymes ($P<0\cdot1$) were deleted. This was designed to minimise the effect of spurious synapomorphies arising because of low sampling intensity (Mickevich and Mitter 1981; Swofford and Berlocher 1987) and to eliminate polymorphism arising solely from the presence of a rare allele.
2. Any remaining allozymes contributing to polymorphism but not shared across taxa (e.g. allozyme *h* at *Acon-1*) were deleted. Autapomorphic allozymes contribute no phylogenetic information.
3. The highly polymorphic loci *Gpt* and *Np* were excluded.
4. Polymorphisms involving allozymes shared across taxa were declared missing (e.g. polymorphisms *bc* and *bd* at *Adh*).
5. All remaining uninformative loci, that is, those for which only one allozyme was observed in more than one taxon, including the outgroup taxa (e.g. *Acp* and *Adh*), were excluded.

The PAUP analyses were run with character states declared unordered and the options swap = global, hold = 10, mulpars, maxtree = 100, confile, root = outgroup (*Chelodina*). In the first and most conservative PAUP analysis, only those loci with no polymorphism following steps 1 and 2 above (18 loci; Group A of Table 5), were used. Uninformative loci were discarded before analysis. In the second PAUP analysis, all loci surviving the five preparatory steps were used (43 loci; Groups A and B of Table 5). For each analysis, a consensus tree was constructed from all equally parsimonious trees with the CONTREE program included on the PAUP distribution diskettes.

These analyses, and particularly the first, are clearly wasteful of data. In addition, declaring polymorphisms missing means that species that are more intensively sampled across a wider range of allopatric populations (e.g. *Emydura macquarii*) will be more severely pruned and therefore less reliably placed in the resulting phylogeny. For these reasons, the results of the PAUP analyses, although considered by many to be superior to distance analyses for phylogenetic analysis generally, were not given the same weight as those obtained from the distance analyses. Rather, they were used to test the robustness of major features of the tree topologies resulting from the distance analyses.

A qualitative Hennigian analysis (Baverstock *et al.* 1979; Patton and Avise 1983; Richardson *et al.* 1986) of phylogenetic relationships among species of *Elseya*, *Emydura*, *Rheodytes* and 'short-necked alpha' was attempted by using *Chelodina* as a composite outgroup for identifying plesiomorphic states.

The programs PHYLAL (Rogers 1984) and FREQPARS (Swofford and Berlocher 1987) use allele frequencies directly and promise a possible solution to the difficulties of analysing allozyme characters, but unfortunately the algorithims in these programs, modified from the Wagner procedure (Farris 1970), are not particularly efficient in finding the shortest tree (Swofford 1988). They can be used, however, to judge between topologies (Swofford 1988), so we used FREQPARS to compare the relative fits of the distance-Wagner, the Fitch-Margoliash and the six equally parsimonious trees of the second PAUP analyses with the allele frequencies. There was very little variation among the trees (lengths 481·42–487·3 units) and this, together with computational difficulties of the large data set, led us to pursue the technique no further. For comparison, a random topology had a length of 867·0 units and the tree produced by FREQPARS itself had a length of 492·58 units.

Enzymes Examined

ACON, aconitate hydratase (Enzyme Commission No. 4.2.1.3); ACP, acid phosphatase (No. 3.1.3.2); ADA, adenosine deaminase (No. 3.5.4.4); ADH, alcohol dehydrogenase (No. 1.1.1.1); AK, adenylate kinase (No. 2.7.4.3); CA, carbonate dehydratase (No. 4.2.1.1); CK, creatine kinase (No. 2.7.3.2); CS, citrate synthase (No. 4.1.3.7); DIA, diaphorase (No. 1.6.*.*); ENOL, enolase (No. 4.2.1.11);

EST, esterases (No. 3.1.1.1); FDP, fructose-1,6-diphosphatase (No. 3.1.3.11); FUM, fumarate hydratase (No. 4.2.1.2); GAPD, glyceraldehyde-3-phosphate dehydrogenase (No. 1.2.1.12); GLO, lactoylglutathione lyase (No. 4.4.1.5); GOT, aspartate aminotransferase (No. 2.6.1.1); GPD, glycerol-3-phosphate dehydrogenase (No. 1.1.1.8); GPI, glucose-phosphate isomerase (No. 5.3.1.9); GPT, alanine aminotransferase (No. 2.6.1.2); GSR, glutathione reductase (No. 1.6.4.2); HBDH, β-hydroxybutyrate dehydrogenase (No. 1.1.1.30); IDH, isocitrate dehydrogenase (No. 1.1.1.42); LAP, leucine aminopeptidase (No. 3.4.11.1); LDH, lactate dehydrogenase (No. 1.1.1.27); MDH, malate dehydrogenase (No. 1.1.1.37); ME, malic enzyme (No. 1.1.1.40); MPI, mannose-phosphate isomerase (No. 5.3.1.8); NP, purine nucleoside phosphorylase (No. 2.4.2.1); PEP-A, dipeptidase (No. 3.4.13.11); PEP-B, tripeptidase (No. 3.4.11.4); PEP-D, prolidase (No. 3.4.13.9); 6PGD, 6-phosphogluconate dehydrogenase (No. 1.1.1.44); PGK, phosphoglycerate kinase (No. 2.7.2.3); PGM, phosphoglucomutase (No. 2.7.5.1); PK, pyruvate kinase (No. 2.7.1.40); SOD, superoxide dimutase (No. 1.15.1.1); SORDH, l-iditol dehydrogenase (No. 1.1.1.14); TPI, triose-phosphate isomerase (No. 5.3.1.1); UGPP, uradine diphosphate-glucose pyrophosphorylase (No. 2.7.7.9); XO, xanthine oxidase (No. 1.2.3.2).

Details of the number of loci examined for each enzyme system are shown in Table 3. Conventions for naming loci and allozymes follow that of Adams *et al.* (1987).

Specimens Examined

Chelodina expansa (AM R125066; QM J48005/14/15/18/20/32; SAM R21228, *Chelodina longicollis* (AM R123050–65; QM J47994, J48043/49; SAM R18997, R19005/6, R20602–4, R21232, R26898/9, R29960–2, *Chelodina oblonga* (AM R125476–80), *Chelodina rugosa* (NTM R13430/4/5/7/9), *Chelodina steindachneri* (ANC R5058), *Chelodina* sp. (Mann, aff. *rugosa*) (NTM R13525), *Elseya dentata* (NTM R12231, R13317–20, R13436, R13510/21/23), *Elseya latisternum* (AM R120997–8, R123032–9, R125474–5; NTM R13516/17/24; QM J47988/90/95, J48001/6/11/17/21/22/24/54/55/63/66), *Elseya novaeguineae* (AM R124696–8, R124702, R124793), *Elseya* sp. (Burnett, aff. *dentata*) (AM R123067, R128007; QM J47987/98, J48002/10/12/26/27/29/39/46/52), *Elseya* sp. (Johnstone, aff. *dentata*) (AM R125468, QM J48059/62/64/65/68), *Elseya* sp. (Magela, aff. *dentata*) (AM R128001–4; NTM R13512), *Elseya* sp. (Gwydir, aff. *latisternum*) (AM R123027–31; QM J48023/28/30/38/57), *Elseya* sp. (Bellingen) (AM R120965, R123043–6), *Elseya* sp. (Manning) (AM R120966–7, R123040–2), *Emydura krefftii* (AM R125473, R125486–9; QM J47993/6/7/9, J48003/4/7/9/13/16/19/25/42/51), *Emydura macquarii* (AM R120953–9, R123047–9; QM J48033–7, J48040/44/45/47/50; SAM R21229–31), *Emydura signata* (AM R120971/78/89/93/94, R123001–11, R123013–26; QM J48031/41/48/53/56), *Emydura subglobosa* ($n = 3$), *Emydura victoriae* (AM R125490/95/97; NTM R13438, R13511, R13513–15, R13518–20, R13522), *Emydura* sp. (Cooper, aff. *krefftii*) (SAM R18427/29/32/37, R20598, R31125–31), *Emydura* sp. (Fraser, aff. *krefftii*) (QM J47989, J47991–2, J48008), *Emydura* sp. (Sleisbeck, aff. *subglobosa*) (AM R128005; NTM R13428–9, R13431–3; QM J48058/61/67), *Emydura* sp. (Daly Mission, aff. *victoriae*) (AM R125469–72, R125491–4, R125498–9), *Rheodytes leukops* (AM R125481), 'short-necked alpha' (AM R125482–5, R128006).

Museum Abbreviations

AM, Australian Museum, Sydney; ANC, Australian National Wildlife Collection, CSIRO Gungahlin, Canberra; QM, Queensland Museum, Brisbane; NTM; Museums and Art Galleries of the Northern Territory, Darwin; SAM, South Australian Museum, Adelaide.

Results

Levels of Divergence

The allozyme profiles of the 22 species at 54 loci are presented in Table 3, and the genetic distances between taxa (Rogers' D and per cent fixed differences) are shown in Table 4. Average levels of divergence among species within each of the polytypic genera varied quite markedly (Table 4). The five species of *Emydura* were very similar ($\bar{D} = 0{\cdot}12$, range $= 0{\cdot}09$–$0{\cdot}17$) in comparison with the levels of divergence for the six species of *Chelodina* ($\bar{D} = 0{\cdot}32$, range $= 0{\cdot}11$–$0{\cdot}41$) and the nine species of *Elseya* ($\bar{D} = 0{\cdot}39$, range $= 0{\cdot}13$–$0{\cdot}57$). Comparisons among genera revealed that the species of *Chelodina* were on average approximately equidistant from those of *Emydura* ($\bar{D} = 0{\cdot}69$), those of *Elseya*

($\bar{D}=0\cdot73$), *Rheodytes* ($\bar{D}=0\cdot71$) and 'short-necked alpha' ($\bar{D}=0\cdot71$). These high levels of divergence, taken together with their similar magnitudes, are consistent with the notion that *Chelodina* is a reliable outgroup for the remaining species.

Levels of divergence between species of *Elseya* and *Emydura* varied between 0·31 and 0·50, a range that lay entirely within the range of genetic distances between species of *Elseya* alone. Thus, some species of *Elseya* are electrophoretically more similar to species of *Emydura* than they are to other species of *Elseya*. For example, *El. dentata* is genetically more similar to the species of *Emydura* ($\bar{D}=0\cdot36$, range = 0·34–0·41) than to *El. latisternum* ($\bar{D}=0\cdot42$). These phenetic comparisons suggest that *Elseya* is paraphyletic, though this assumes that rates of evolution have been relatively constant.

Phenetic Analysis

The first three dimensions of the PCoA explained 63·3% of variation among populations, and revealed five distinct groups of species (Fig. 3). The groups are *Emydura*, the *El. latisternum* group (including the three new forms from the Gwydir, Bellingen and Manning drainages: Table 2), the *El. dentata* group (including the three new forms from the South Alligator, Johnstone and Burnett/Fitzroy drainages: Table 2), and two separate clusters for the outgroup *Chelodina*. The *C. expansa* group comprised *C. expansa*, *C. rugosa* and *Chelodina* sp. (Mann) and the *C. longicollis* group comprised *C. longicollis* and *C. steindachneri*. The adjacency of *Rheodytes*, 'short-necked alpha' and *E. novaeguineae* to the *El. dentata* group in the three-dimensional plot belied the high levels of divergence between these taxa evident in deeper dimensions of the ordination. The levels of divergence of the *El. dentata* group from *Rheodytes* ($\bar{D}=0\cdot38$), 'short-necked alpha' ($\bar{D}=0\cdot46$) and *El. novaeguineae* ($\bar{D}=0\cdot35$) were roughly equivalent to the divergence between the major groupings of short-necked species evident in the three dimensions of Fig. 3. Similarly, while *C. oblonga* was adjacent to the *C. expansa* group in Fig. 3, its affinities among *Chelodina* species could not be determined from the distance data.

Phylogenetic Analysis

Analysis of genetic distances using the distance-Wagner and Fitch–Margoliash procedures yielded the two trees shown in Fig. 4. Several features were common to both analyses, namely:

1. The genus *Elseya* as currently recognised is paraphyletic, for its closest common ancestor has *Emydura* among its descendants.
2. The species of *Emydura* form a monophyletic group, with the branching patterns among species invariant.
3. The four species in the *El. latisternum* group form a monophyletic assemblage, with the branching patterns among species invariant.
4. *Elseya novaeguineae* clusters with the two northern species in the *El. dentata* group, and *El. dentata* as currently recognised is paraphyletic.
5. The six species of *Chelodina* form a monophyletic assemblage, with the branching patterns among species invariant. *Chelodina longicollis* and *C. steindachneri* are sister species, as are *C. rugosa* and *Chelodina* sp. a. *Chelodina oblonga* clusters with *C. longicollis*/*C. steindachneri*.

Parsimony analysis using PAUP (Fig. 5) supported the major features of the two analyses based on the genetic distances. Again, there was evidence that the genus *Elseya* as currently recognised is paraphyletic, and the species of *Emydura*, the four species of the *Elseya latisternum* group, and the species of *Chelodina* are each monophyletic assemblages. Only the placement of *Rheodytes* and 'short-necked alpha' and the arrangement of species within the monophyletic groups mentioned above changed, depending on the dataset used.

Table 3. Allozyme profiles of each of 22 species at 54 enzyme loci

Sample sizes for each species are presented in parentheses. Rare allozymes ($P<0\cdot1$) are not shown. Three loci (*Enol-1*, *Gapd*, and *Ldh-2*) were invariant. The conventions for designating allozymes and multiple loci follow Adams *et al.* (1987); in particular, alleles are designated alphabetically according to increasing mobility. Species abbreviations: C*a*, *Chelodina* (Mann); CE, *C. expansa*; CL, *C. longicollis*; CO, *C. oblonga*; CR, *C. rugosa*; CS, *C. steindachneri*; EL*a*, *Elseya* (Sth Alligator); EL*b*, *Elseya* (Johnstone); EL*c*, *Elseya* (Burnett); ELD, *El. dentata*; EL*d*, *Elseya* (Gwydir); EL*e*, *Elseya* (Bellingen); EL*f*, *Elseya* (Manning); ELL, *El. latisternum*; ELN, *El. novaeguineae*; EM*a*, *Emydura* (Daly Mission); EM*b*, *Emydura* (Sleisbeck); EMM, *Em. macquarii*; EMS, *Em. subglobosa*; EMV, *Em. victoriae*; RHL, *Rheodytes leukops*; SNA, 'Short-necked alpha'. Details of undescribed species are given in Table 2

Locus	*Emydura*					*Elseya*											*Chelodina*					
	EMV (14)	EM*a* (10)	EM*b* (9)	EMS (3)	EMM (93)	ELL (29)	EL*d* (10)	EL*e* (5)	EL*f* (5)	ELD (9)	EL*a* (5)	EL*b* (6)	EL*c* (13)	ELN (5)	SNA (5)	RHL (1)	CE (8)	C*a* (1)	CR (5)	CL (34)	CS (1)	CO (5)
Acon-1	d	d	dh	d	d	ab	b	b	b	d	g	d	d	d	b	d	a	a	a	a	a	a
Acon-2	d	d	d	d	df	b	b	e	b	df	f	d	d	ab	f	f	ij	h	gh	gh	h	h
Acp	a	a	a	a	a	a	a	a	a	a	a	a	a	a	b	a	a	a	a	a	a	a
Ada	de	e	e	e	e	f	f	f	f	h	h	g	g	j	i	k	d	d	d	b	b	c
Adh	b	b	bc	bc	b	b	b	b	c	b	b	e	f	–	–	d	b	b	b	b	bd	b
Ak-1	a	a	a	a	a	b	b	b	b	a	a	a	a	a	a	a	a	a	a	a	a	a
Ak-2	e	e	e	ae	e	a	a	a	a	a	a	a	ac	a	a	e	a	a	a	a	d	d
Ald	a	c	ac	c	a	c	c	c	c	c	ac	c	c	–	c	c	b	d	b	b	b	b
Ca-1	f	f	f	f	f	h	b	b	b	gi	i	f	f	d	d	d	ac	c	c	c	c	e
Ca-2	c	c	c	c	c	c	c	c	c	c	c	c	c	b	c	c	d	c	c	ac	d	f
Ca-3	h	h	g	g	dg	d	d	d	d	gh	g	b	g	fg	g	e	c	c	c	a	a	c
Ck	b	b	b	b	b	a	a	a	b	b	b	b	b	–	b	b	c	c	c	c	c	c
Cs	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	ab	a	b
Dia	d	d	d	d	d	b	c	b	c	b	b	b	b	b	b	b	b	b	b	a	a	a
Enol-2	a	a	a	a	a	a	a	a	a	a	b	a	a	–	a	a	c	c	c	c	c	c
Est	c	ac	c	c	c	c	d	c	c	c	c	c	c	–	c	c	c	c	c	c	c	c
Fdp-1	c	c	c	c	c	e	e	e	e	c	c	c	c	c	c	c	bd	b	b	ab	a	b
Fdp-2	c	cf	c	c	c	c	cd	c	c	c	c	c	c	–	c	c	c	c	c	c	e	c
Fum	b	b	b	b	bc	ab	b	b	b	b	b	b	bc	d	b	b	b	b	b	b	b	b

Glo-1	a	a	a	a	a	a	a	a	a	a	a	a	a	a	b	b	b	b	b	b	b	b
Glo-2	a	a	a	a	a	a	a	a	a	a	a	c	c	a	a	a	b	b	b	b	b	b
Got-1	c	c	c	c	c	c	c	c	c	c	ce	c	c	c	e	e	bd	b	b	b	b	a
Got-2	bj	bfj	j	j	kl	j	j	j	j	h	j	j	j	j	g	j	de	ad	d	ad	d	i
Gpd-1	b	b	b	b	b	b	b	b	b	a	a	a	a	a	b	a	c	c	c	c	c	c
Gpd-2	c	c	c	c	c	c	c	c	c	c	c	c	c	ac	b	c	d	d	d	d	d	de
Gpi	a	a	ac	ac	a	b	b	b	b	b	b	a	a	a	af	e	g	g	g	g	g	h
Gpt	hjk	j	j	hj	j	e	c	g	e	ik	k	b	beh	eil	i	a	d	–	f	–	d	df
Gsr	e	e	e	e	eg	e	e	d	e	e	e	e	e	e	f	e	b	b	ab	b	b	b
Hbdh	c	c	c	c	cf	d	c	d	d	c	c	c	c	d	f	c	d	d	d	d	d	f
Idh-1	b	b	b	b	b	e	e	b	e	e	e	f	e	c	e	d	d	d	d	e	e	f
Idh-2	a	a	ac	ac	a	a	a	d	a	a	a	a	a	a	a	a	e	b	be	b	be	b
Lap	b	b	b	b	ab	b	b	b	b	b	b	b	b	b	c	b	b	b	b	a	a	a
Ldh-1	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	ac	c	c	b	d	ad
Mdh-1	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	c	d	d	c	c	c
Mdh-2	d	d	d	d	cd	c	c	c	c	d	d	d	d	d	d	d	b	b	b	b	b	b
Me-1	e	ef	de	e	be	e	e	e	e	e	e	e	e	e	e	e	e	f	f	ad	cf	cf
Me-2	ac	c	ac	c	b	g	g	g	g	h	h	e	f	dg	c	f	j	j	j	i	i	j
Mpi	d	d	d	d	d	d	d	d	d	b	b	b	a	b	b	b	d	d	d	d	d	de
Np	l	l	l	lm	lm	abc	a	a	a	djo	ej	pq	h	stu	f	s	ik	i	i	g	c	c
Pep-A	b	b	b	b	b	a	a	a	a	a	a	a	a	b	a	a	b	d	e	ab	a	c
Pep-B	gj	g	gk	g	fg	ac	a	a	a	e	gi	d	bd	f	f	e	b	b	b	b	b	be
Pep-D	j	j	j	j	ik	ef	ef	e	ef	g	g	h	h	h	c	h	d	d	ad	a	a	d
6Pgd	d	d	cd	d	d	b	b	b	f	d	d	d	cd	d	hi	e	c	c	ac	c	c	c
Pgk	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	ab	b	b	b	b	b	b
Pgm	c	ac	c	c	c	a	a	a	a	d	d	c	c	c	c	c	c	c	c	bc	b	c
Pk	b	b	bc	b	b	b	b	b	d	b	b	c	c	b	c	c	d	d	d	a	d	d
Sod	c	c	c	c	cd	d	d	c	c	c	c	d	d	d	c	d	c	c	b	c	c	b
Sordh	d	d	d	d	d	a	a	a	ac	bd	d	d	d	d	d	a	d	d	d	a	a	a
Tpi	a	a	a	a	a	a	a	a	a	b	b	a	a	b	a	a	a	a	a	a	a	a
Ugpp	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	b	b	b	b	b	b
Xo	–	–	–	–	–	d	d	d	c	a	a	b	a	a	e	f	g	h	h	g	g	h

Table 4. Rogers' genetic distances (lower matrix) and Percentage Fixed Differences (upper matrix) among 21 species of Australian chelid turtle and *Elseya novaeguineae*, endemic to New Guinea

Abbreviations are as for Table 3

	EMV	EM*a*	EM*b*	EMS	EMM	ELL	EL*d*	EL*e*	EL*f*	ELD	EL*a*	EL*b*	EL*c*	ELN	SNA	RHL	CE	C*a*	CR	CL	CS	CO
EMV	–	2	2	4	6	42	43	47	45	32	34	34	32	40	48	43	64	63	64	69	77	75
EM*a*	0·09	–	2	2	8	42	40	43	43	30	36	32	30	40	46	40	64	63	62	69	75	72
EM*b*	0·12	0·14	–	0	4	40	40	43	43	32	34	30	28	38	42	40	64	62	64	65	75	75
EMS	0·09	0·09	0·09	–	6	40	40	43	42	30	32	30	26	36	42	42	64	63	64	67	77	77
EMM	0·14	0·16	0·17	0·15	–	38	38	43	43	32	32	32	30	32	38	38	64	62	62	65	72	70
ELL	0·46	0·45	0·48	0·44	0·47	–	7	13	15	43	44	44	41	42	53	48	65	62	63	58	69	72
EL*d*	0·48	0·46	0·49	0·46	0·50	0·13	–	13	15	44	44	48	46	50	57	50	74	72	70	70	76	80
EL*e*	0·47	0·46	0·48	0·45	0·50	0·16	0·18	–	20	48	48	54	54	54	57	56	69	66	65	68	74	80
EL*f*	0·46	0·46	0·46	0·44	0·49	0·19	0·20	0·23	–	46	46	50	46	50	53	52	70	68	67	66	72	80
ELD	0·35	0·34	0·41	0·35	0·39	0·42	0·46	0·47	0·45	–	7	31	30	33	42	39	69	68	67	72	78	81
EL*a*	0·38	0·40	0·38	0·36	0·40	0·45	0·48	0·49	0·47	0·15	–	35	33	35	43	41	70	68	69	72	78	83
EL*b*	0·33	0·35	0·35	0·32	0·38	0·47	0·50	0·53	0·50	0·34	0·38	–	11	33	45	33	70	68	69	74	81	80
EL*c*	0·34	0·36	0·34	0·31	0·36	0·46	0·51	0·55	0·49	0·34	0·36	0·16	–	31	43	33	67	64	65	68	76	78
ELN	0·39	0·43	0·43	0·39	0·40	0·48	0·54	0·57	0·55	0·36	0·35	0·35	0·34	–	50	42	69	70	71	77	83	85
SNA	0·49	0·49	0·48	0·45	0·49	0·54	0·59	0·56	0·52	0·44	0·45	0·46	0·46	0·53	–	42	70	67	68	69	77	79
RHL	0·44	0·46	0·45	0·42	0·47	0·50	0·54	0·56	0·54	0·39	0·44	0·34	0·35	0·44	0·42	–	67	66	63	70	74	74
CE	0·63	0·66	0·64	0·65	0·65	0·68	0·75	0·67	0·69	0·69	0·68	0·69	0·71	0·68	0·68	0·68	–	17	15	25	33	35
C*a*	0·64	0·66	0·64	0·65	0·65	0·67	0·74	0·66	0·67	0·67	0·67	0·67	0·69	0·71	0·67	0·67	0·19	–	4	28	36	36
CR	0·65	0·67	0·65	0·66	0·66	0·66	0·74	0·67	0·69	0·68	0·68	0·67	0·68	0·70	0·68	0·66	0·22	0·11	–	25	33	30
CL	0·69	0·70	0·68	0·69	0·68	0·67	0·73	0·69	0·70	0·72	0·72	0·75	0·75	0·77	0·71	0·72	0·32	0·32	0·34	–	8	30
CS	0·77	0·77	0·76	0·77	0·76	0·73	0·78	0·75	0·73	0·77	0·77	0·80	0·79	0·83	0·77	0·75	0·35	0·40	0·40	0·18	–	33
CO	0·76	0·77	0·76	0·77	0·75	0·78	0·82	0·79	0·81	0·81	0·81	0·78	0·80	0·85	0·78	0·76	0·41	0·39	0·39	0·39	0·40	–

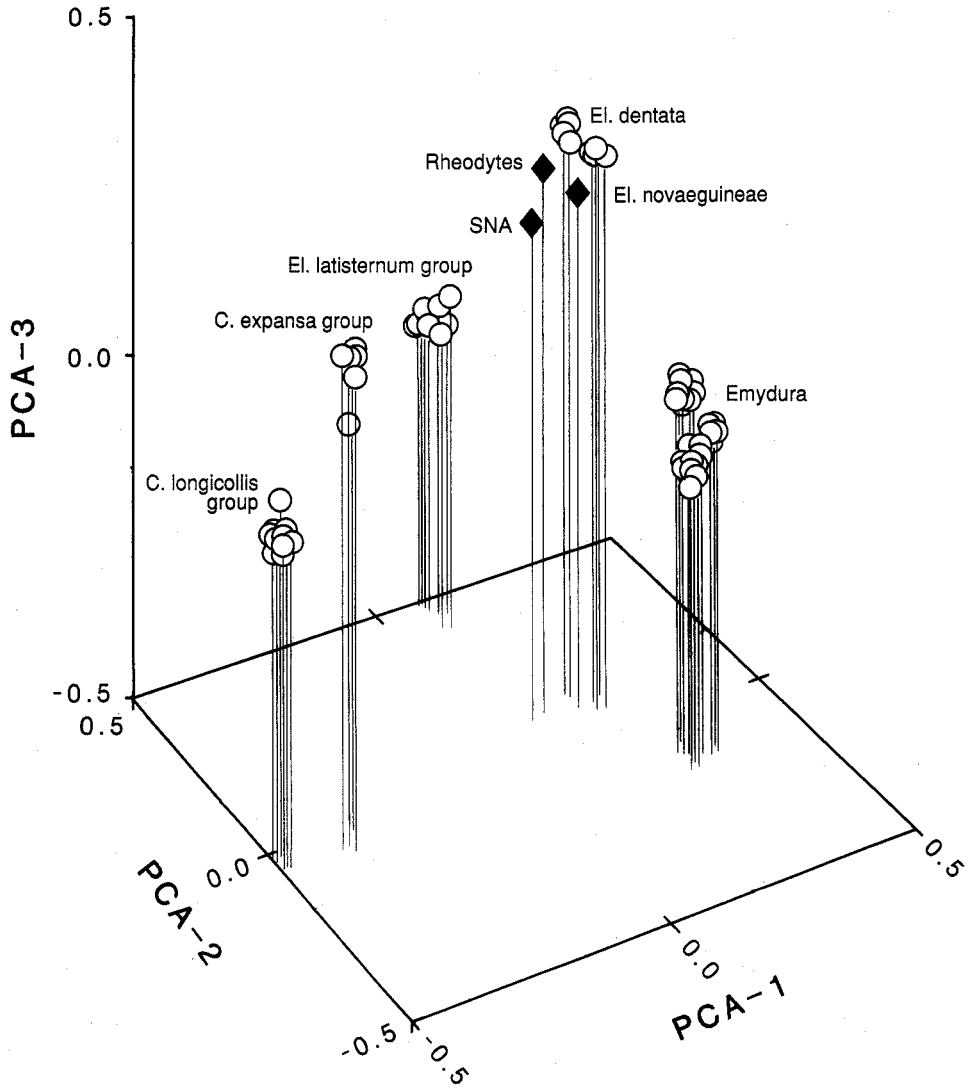

Fig. 3. Three-dimensional representation of genetic relationships among Australian chelid turtles, constructed using principal co-ordinates analysis. Five clear groups of species are evident: (1) *Emydura*, (2) *Elseya latisternum* group [including the undescribed *Elseya* (Gwydir), (Bellingen) and (Manning)], (3) *Elseya dentata* group [including the undescribed *Elseya* (Sth Alligator), (Johnstone) and (Burnett)], (4) *Chelodina expansa* group and (5) *Chelodina longicollis* group. The outlying species in the *C. longicollis* group is *C. steindachneri*, whereas the outlier in the *C. expansa* group is *C. oblonga*. The adjacency of *Rheodytes leukops* and 'short-necked alpha' (SNA) to the *El. dentata* group, and of *C. oblonga* to the *C. expansa* group, could not be sustained on examination of deeper dimensions in the ordination.

For example, *Chelodina expansa* clustered with *C. longicollis/C. steindachneri/C. oblonga* in the first of the PAUP analyses (Fig. 5*a*) and with *C. rugosa* and *Chelodina* sp. (Mann) in three of the six equally parsimonious trees resulting from the second PAUP analysis (Fig. 5*b*). Similarly, *El. novaeguineae* clustered with the northern species currently recognised as *Elseya dentata* in the first PAUP analysis, and with the southern *El. dentata* species in the second PAUP analysis.

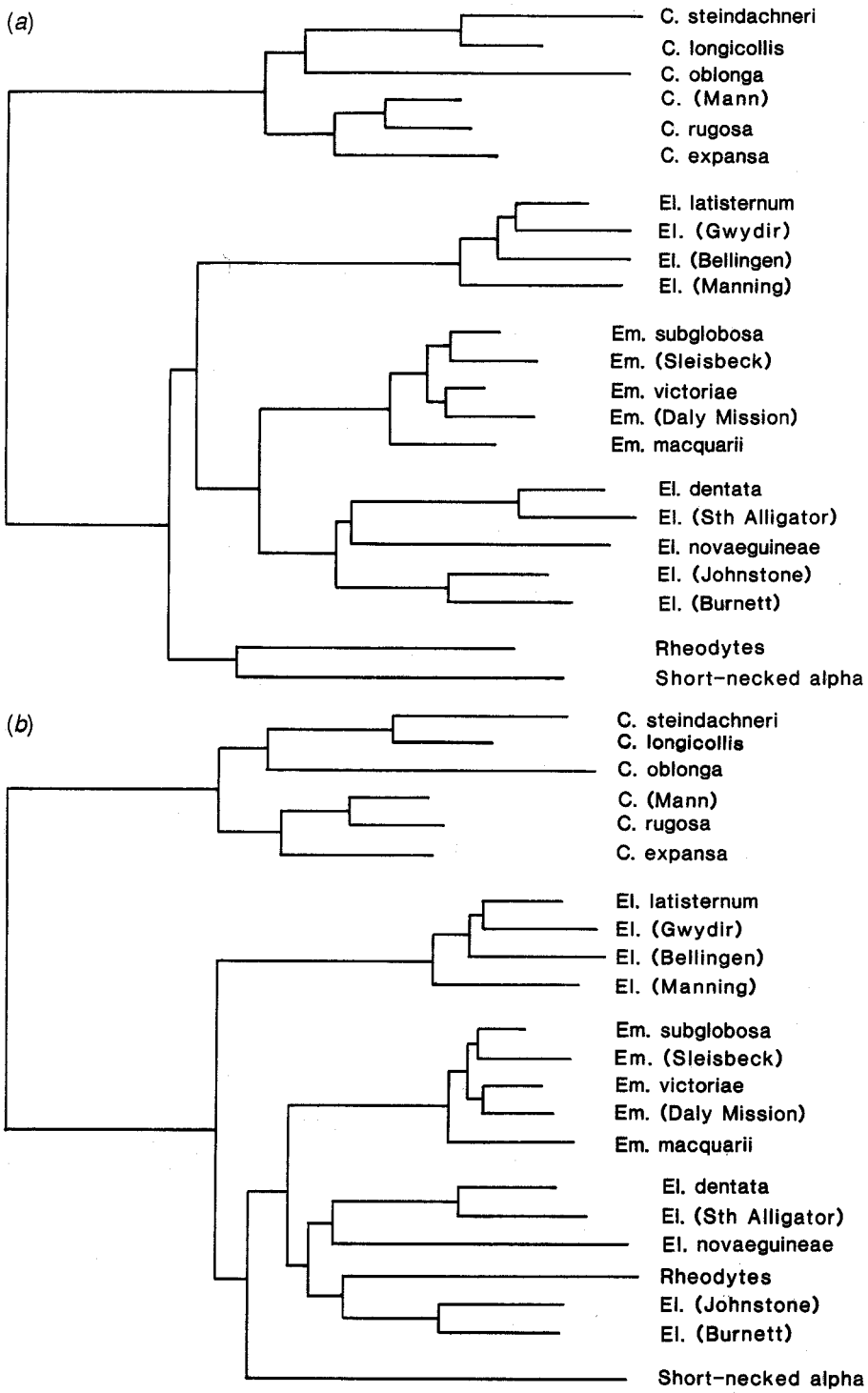

Fig. 4. Evolutionary trees for 22 species of chelid turtle based on (*a*) the distance-Wagner procedure (Farris 1972) and (*b*) the Fitch–Margoliash procedure (Fitch and Margoliash 1967). Details of undescribed species are given in Table 2.

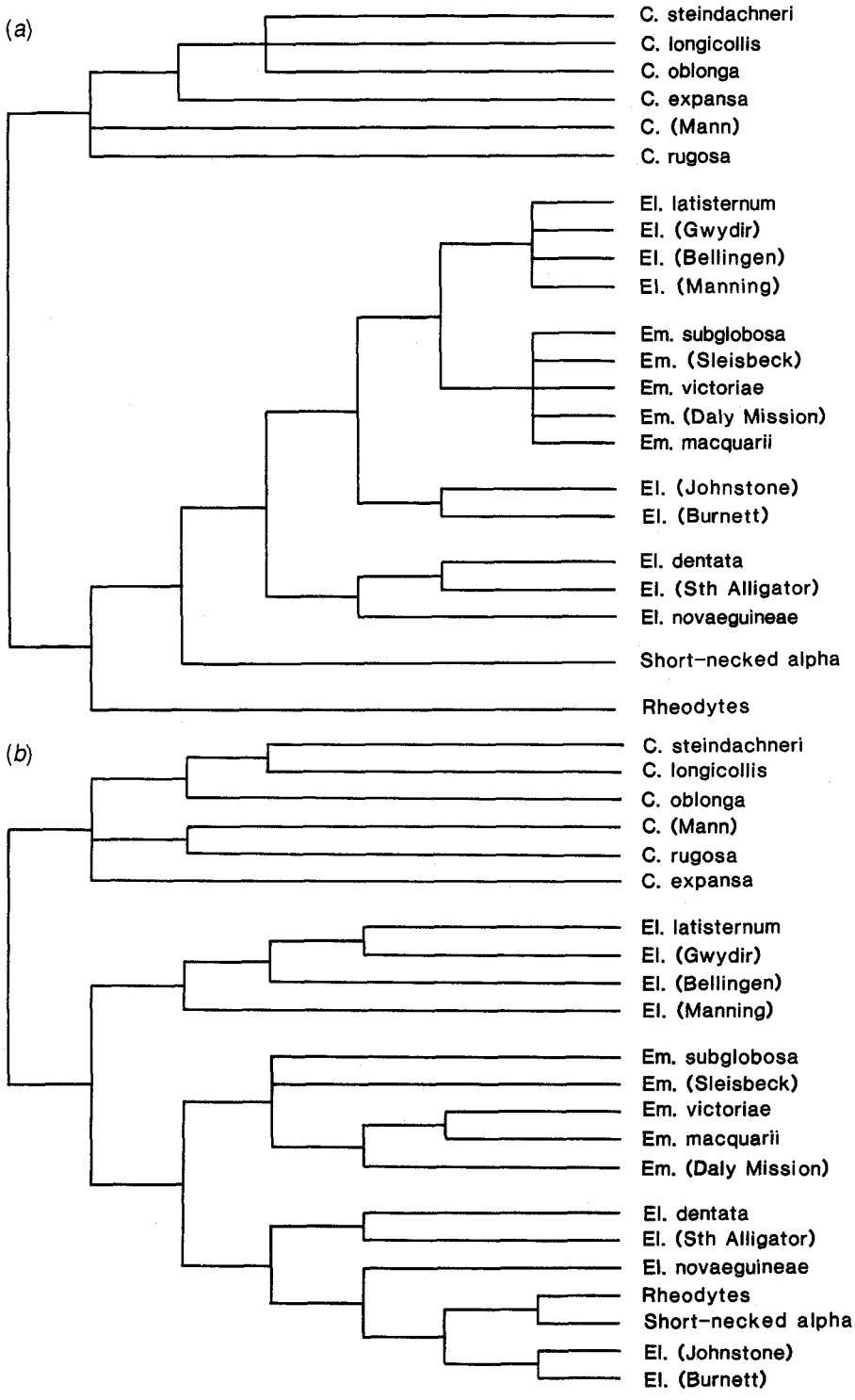

Fig. 5. Cladograms for 22 species of chelid turtle based on a strict consensus of equally parsimonious trees resulting from a character-based parsimony analysis using (*a*) Group A loci of Table 5 and (*b*) Group A and B loci of Table 5. Analyses were conducted using PAUP (Swofford 1985).

Table 5. Data remaining after preliminary analysis to remove polymorphism and uninformative loci (see text), in preparation for anlaysis by PAUP

Abbreviations and conventions are as described in Table 3. Dashes represent missing data resulting from deletion of polymorphisms; periods represent missing data as a result of inability to score gels or appropriate tissue unavailable

Locus	*Emydura*					*Elseya*											*Chelodina*					
	EMV (14)	EM*a* (10)	EM*b* (9)	EMS (3)	EMM (93)	ELL (29)	EL*d* (10)	EL*e* (5)	EL*f* (5)	ELD (9)	EL*a* (5)	EL*b* (6)	EL*c* (13)	ELN (5)	SNA (5)	RHL (1)	CE (8)	C*a* (1)	CR (5)	CL (34)	CS (1)	CO (5)
Group A loci: rare alleles and alleles unique to one taxon removed, no within-taxon polymorphisms remaining																						
Ak-1	a	a	a	a	a	b	b	b	b	a	a	a	a	a	a	a	a	a	a	a	a	a
Ca-1	f	f	f	f	f	h	b	b	b	i	i	f	f	d	d	d	c	c	c	c	c	e
Ca-2	c	c	c	c	c	c	c	c	c	c	c	c	c	b	c	c	d	c	c	c	d	f
Ck	b	b	b	b	b	a	a	a	b	b	b	b	b	·	b	b	c	c	c	c	c	c
Dia	d	d	d	d	d	b	c	b	c	b	b	b	b	b	b	b	b	b	b	a	a	a
Enol-2	a	a	a	a	a	a	a	a	a	a	b	a	a	·	a	a	c	c	c	c	c	c
Glo-1	a	a	a	a	a	a	a	a	a	a	a	a	a	a	b	b	b	b	b	b	b	b
Glo-2	a	a	a	a	a	a	a	a	a	a	a	c	c	a	a	a	b	b	b	b	b	b
Gpd-1	b	b	b	b	b	b	b	b	b	a	a	a	a	a	b	a	c	c	c	c	c	c
Gpd-2	c	c	c	c	c	c	c	c	c	c	c	c	c	c	b	c	d	d	d	d	d	d
Gsr	e	e	e	e	e	e	e	d	e	e	e	e	e	e	f	e	b	b	b	b	b	b
Idh-1	b	b	b	b	b	e	e	b	e	e	e	f	e	c	e	d	d	d	d	e	e	f
Mdh-1	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	c	d	d	c	c	c
Mpi	d	d	d	d	d	d	d	d	d	b	b	b	a	b	b	b	d	d	d	d	d	d
Srdh	d	d	d	d	d	a	a	a	a	d	d	d	d	d	d	a	d	d	d	a	a	a
Tpi	a	a	a	a	a	a	a	a	a	b	b	a	a	b	a	a	a	a	a	a	a	a
Ugpp	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	b	b	b	b	b	b
Xo	·	·	·	·	·	d	d	d	c	a	a	b	a	a	e	f	g	h	h	g	g	h

Group B loci: rare alleles and alleles unique to one taxon removed, polymorphisms declared missing

Acon-1	d	d	d	d	d	–	b	b	b	d	g	d	d	d	b	d	a	a	a	a	a	a
Acon-2	d	d	d	d	–	b	b	e	b	–	f	d	d	–	f	f	i	h	–	–	h	h
Ada	–	e	e	e	e	f	f	f	f	h	h	g	g	j	i	k	d	d	d	b	b	c
Ak-2	e	e	e	–	e	a	a	a	a	a	a	a	a	a	a	e	a	a	a	a	d	d
Ald	a	c	–	c	a	c	c	c	c	c	–	c	c	·	c	c	b	d	b	b	b	b
Ca-3	h	h	g	g	–	d	d	d	d	–	g	b	g	g	g	e	c	c	c	a	a	c
Fdp-1	c	c	c	c	c	e	e	e	e	c	c	c	c	c	c	c	b	b	b	–	a	b
Got-1	c	c	c	c	c	c	c	c	c	c	–	c	c	c	e	e	b	b	b	b	b	a
Got-2	–	–	j	j	k	j	j	j	j	h	j	j	j	j	g	j	d	–	d	–	d	i
Gpi	a	a	–	–	a	b	b	b	b	b	b	a	a	a	a	e	g	g	g	g	g	h
Hbdh	c	c	c	c	–	d	c	d	d	c	c	c	c	d	f	c	d	d	d	d	d	f
Idh-2	a	a	–	–	a	a	a	d	a	a	a	a	a	a	a	a	e	b	–	b	–	b
Lap	b	b	b	b	–	b	b	b	b	b	b	b	b	b	c	b	b	b	b	a	a	a
Ldh-1	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	–	c	c	b	d	–
Mdh-2	d	d	d	d	–	c	c	c	c	d	d	d	d	d	d	d	b	b	b	b	b	b
Me-1	e	–	–	e	e	e	e	e	e	e	e	e	e	e	e	e	e	f	f	–	–	–
Me-2	–	c	–	c	b	g	g	g	g	h	h	e	f	g	c	f	j	j	j	i	i	j
Pep-A	b	b	b	b	b	a	a	a	a	a	a	a	a	b	a	a	b	d	e	–	a	c
Pep-B	g	g	g	g	–	a	a	a	a	e	g	d	–	f	f	e	b	b	b	b	b	–
Pep-D	j	j	j	j	i	–	–	e	–	g	g	h	h	h	c	h	d	d	–	a	a	d
6Pgd	d	d	–	d	d	b	b	b	f	d	d	d	–	d	h	e	c	c	c	c	c	c
Pgk	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	–	b	b	b	b	b	b
Pgm	c	–	c	c	c	a	a	a	a	d	d	c	c	c	c	c	c	c	c	–	b	c
Pk	b	b	–	b	b	b	b	b	d	b	b	c	c	b	c	c	d	d	d	a	d	d
Sod	c	c	c	c	–	d	d	c	c	c	c	d	d	d	c	d	c	c	b	c	c	b

The main difference between the four trees resulting from the distance and parsimony analyses was in the placement of *Rheodytes* and 'short-necked alpha'. One PAUP analysis placed *Rheodytes* and 'short-necked alpha' within the *Elseya dentata* group (Fig. 5*b*) whereas other analyses variously placed one or both as sister taxa to the remaining short-necked taxa.

The qualitative Hennigian analysis with *Chelodina* as an outgroup to the remaining taxa provided little insight into relationships among the short-necked species. The ingroup and outgroup did not share alleles at 22 of the 54 loci examined, so ancestral states for these 22 loci could not be determined. Eight loci were monomorphic for the ingroup taxa and a further six loci showed only autapomorphic states. Single occurrences of what might otherwise be regarded as an ancestral state, in either the ingroup or the outgroup, were conservatively regarded as the result of convergence, eliminating a further three loci (*Acon-1*, *Ada*, *Pep-B*) from consideration. Thus only 14 loci with the potential to establish a primary dichotomy within the short-necked taxa remained (Table 6). No primary dichotomy could be clearly discerned from the pattern of synapomorphic groupings. In the absence of a primary dichotomy (Richardson *et al.* 1986), based either on the electrophoretic data or on data collected as part of other studies, the qualitative Hennigian analysis could not be carried further.

The converse situation of using the short-necked species as a composite outgroup for *Chelodina* was more successful. A clade consisting of *C. longicollis*, *C. steindachneri*, and *C. oblonga* was defined by synapomorphies at five loci (Table 6). The remaining three species were unplaced.

Discussion

In this paper, allozyme electrophoresis was used to test the arrangement of species in the currently accepted phylogeny for the Australian Chelidae (Fig. 1). The allozyme data were very comprehensive, being based on 54 loci for turtles from 76 populations of 22 species, and the resulting matrix of distances is considered to be an accurate representation of genetic similarities among the species and genera of Australian chelid turtles. The lack of *Pseudemydura umbrina* from comparisons is not considered to be a serious omission as allozyme electrophoresis loses its ability to resolve relationships once taxa differ by more than 50–70% (Richardson *et al.* 1986). *Chelodina*, while a good outgroup to the remaining species, differs from other genera at 60–85% of loci ($\bar{D}=0{\cdot}69$–$0{\cdot}73$). The view that *Pseudemydura umbrina* is outside the radiation of *Chelodina* and the remaining short-necked Australian taxa appears well accepted (Burbidge *et al.* 1974; Gaffney 1977; Legler and Cann 1980; Legler 1981). Allozyme electrophoresis would probably not be able to confirm the hypothesised placement of *Pseudemydura* shown in Fig. 1, because *Pseudemydura* and any suitable outgroup would probably differ from the remaining species to a greater extent than could be resolved by allozyme electrophoresis.

The phenetic analysis, the phylogenetic analyses based on genetic distances, and the character-based parsimony analyses concurred on several features important for inferring a phylogeny. The most striking departure from the currently accepted classification, and the phylogeny on which it is based, was that the genus *Elseya* is paraphyletic. The closest common ancestor of the species of *Elseya* has the species of *Emydura* among its descendants. This result was a consistent feature of all phenetic and phylogenetic analyses. One approach to resolving this paraphyly, assuming it is considered unacceptable, is to synonymise *Elseya* and *Emydura* (*Emydura* has precedence) as suggested by McDowell (1983). He found, in a detailed morphological analysis, that the nearest living relative to *El. dentata* was *Emydura 'australis'* (including *Em. krefftii* and *Em. subglobosa*) and not *El. latisternum*. This result is supported by the allozyme data, as the *El. dentata* group of species were genetically more similar to all species of *Emydura* than to *El. latisternum*. McDowell concluded that there was no basis for the separate recognition of *Elseya* and *Emydura*, building upon

Table 6. Allozymes present at 19 loci used as characters in the Hennigian analyses, showing the presumed ancestral state for each locus

Unambiguous synapomorphies, those not confounded by possible transformation into a more derived state, are shown in boldface capitals. Synapomorphies in the short-necked taxa are based on *Chelodina* acting as an outgroup, and vice versa. Loci for which no clear ancestral state could be identified, loci monomorphic for the ingroup of both analyses, loci showing only autapomorphic states for both analyses, and three loci where potential ancestral states were based on single presences in the ingroup or outgroup, are omitted. Abbreviations and conventions are as for Table 3

Locus	*Emydura*					*Elseya*											*Chelodina*						Ancestral
	EMV (14)	EM*a* (10)	EM*b* (9)	EMS (3)	EMM (93)	ELL (29)	EL*d* (10)	EL*e* (5)	EL*f* (5)	ELD (9)	EL*a* (5)	EL*b* (6)	EL*c* (13)	ELN (5)	SNA (5)	RHL (1)	CE (8)	C*a* (1)	CR (5)	CL (34)	CS (1)	CO (5)	
Adh	b	b	bc	bc	b	b	b	b	c	b	b	e	f	–	–	d	b	b	b	b	bd	b	b
Ak-1	a	a	a	a	a	**B**	**B**	**B**	**B**	a	a	a	a	a	a	a	a	a	a	a	a	a	a
Ak-2	e	e	e	ae	e	a	a	a	a	a	a	a	ac	a	a	e	a	a	a	a	**D**	**D**	a
Ca-2	c	c	c	c	c	c	c	c	c	c	c	c	c	b	c	c	d	c	c	ac	d	f	c
Cs	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a	a**B**	a	**B**	a
Dia	d	d	d	d	d	b	c	b	c	b	b	b	b	b	b	b	b	b	b	**A**	**A**	**A**	b
Fum	b	b	b	b	bc	ab	b	b	b	b	b	b	bc	d	b	b	b	b	b	b	b	b	b
Glo-1	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	**A**	b	b	b	b	b	b	b	b	b
Hbdh	c	c	c	c	cf	d	c	d	d	c	c	c	c	d	f	c	d	d	d	d	d	f	d
Idh-1	b	b	b	b	b	e	e	b	e	e	e	f	e	c	e	d	d	d	d	e	e	f	e
Lap	b	b	b	b	ab	b	b	b	b	b	b	b	b	b	c	b	b	b	b	**A**	**A**	**A**	b
Ldh-1	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	b	ac	c	c	b	d	ad	b
Mdh-1	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	d	**C**	d	d	**C**	**C**	**C**	d
Mpi	d	d	d	d	d	d	d	d	d	b	b	b	a	b	b	b	d	d	d	d	d	de	d
Pep-A	b	b	b	b	b	a	a	a	a	a	a	a	a	b	a	a	b	d	e	ab	a	c	a
6Pgd	d	d	cd	d	d	b	b	b	f	d	d	d	cd	d	hi	e	c	c	ac	c	c	c	c
Pgm	c	ac	c	c	c	a	a	a	a	d	d	c	c	c	c	c	c	c	c	**Bc**	**B**	c	c
Sod	c	c	c	c	c**D**	**D**	**D**	c	c	c	c	**D**	**D**	**D**	c	**D**	c	c	**B**	c	c	**B**	c
Tpi	a	a	a	a	a	a	a	a	a	**B**	**B**	a	a	**B**	a	a	a	a	a	a	a	a	a

previous support for the synonymy of the two genera from Gaffney (1977, 1979) and Frair (1980). Unfortunately, *Rheodytes* and 'short-necked alpha' were not available for study by McDowell. These two species could not be consistently placed by our analyses of the allozyme data, but the phenetic analysis and several of the phylogenetic analyses placed one or both of these species within the *El. dentata/El. novaeguineae/Emydura* clade. Synonymy of *Elseya* and *Emydura* may have to include the distinctive *Rheodytes* and 'short-necked alpha', a far less acceptable proposition when genera are largely erected on morphological criteria.

A second approach to resolving the paraphyly of *Elseya*, and the one preferred in this paper, is to erect a new genus for the *El. latisternum* group. The affinities of the redefined *Elseya* would lie closer to *Emydura* than to the new genus, in line with the findings of McDowell (1983). This is clearly the preferred position of Legler (1981), who proposed to split the genus, and of Legler and Cann (1980), who considered that morphological similarities between species in *Elseya*, *Emydura* and *Rheodytes* were sufficient to indicate a common ancestry, but not to warrant lumping of any of the three genera. The approach also receives support from a comparison of intergeneric distances for the chelid genera and proposed genera (*Elseya*, *Emydura*, *Rheodytes*, the *El. latisternum* group, 'short-necked alpha') with those calculated for 20 batagurine genera (Cryptodira: Emydidae) (Sites *et al.* 1984). The mean intergeneric distance for the five Australian chelid generic groups ($\bar{D}$ = 0·46, range = 0·37–0·55) is slightly greater than the mean distance between batagurine genera ($\bar{D}$ = 0·44, range = 0·18–0·70). Description of the proposed new genus for the *El. latisternum* group and generic recognition of 'short-necked alpha' await detailed morphological analyses currently underway (J. Legler, personal communication).

Unlike the distance methods and character-based parsimony procedures, the qualitative Hennigian analysis did not provide any significant insight into cladistic relationships among the short-necked species. More than 50 loci were available for comparisons, yet few loci yielded any cladistic information useful in discerning an initial dichotomy, and what information there was conflicted. The outgroup *Chelodina* may be too distinct genetically from the ingroup to enable ready identification of apomorphic states held by the common ancestor of the *Emydura/Elseya/Rheodytes*/'short-necked alpha' radiation. In addition, qualitative Hennigian analyses are profoundly affected by sampling error unless the survey of alleles present in each population is exhaustive (Swofford and Berlocher 1987). Our sampling regime, based on five individuals per population, is not particularly suited to a qualitative Hennigian analysis because an allele occurring with a frequency of, say, 0·1 in all populations would have been detected, on the average, in only 35% of populations sampled (according to the equations of Swofford and Berlocher 1987). The random pattern in the detection of even ubiquitous alleles will lead to many spurious apomorphies and inevitable conflicts with 'true' apomorphies. Another problem with qualitative Hennigian analysis is the risk of defining clades on the basis of synapomorphic alleles but excluding taxa that possess alleles derived from those synapomorphic alleles (Richardson *et al.* 1986; Swofford and Berlocher 1987). Without knowledge of gene phylogenies, loci displaying more than two common alleles within the ingroup are very difficult to interpret. Excluding such loci from the qualitative Hennigian analysis of relationships among the short-necked taxa left only four informative loci (Table 6).

A phylogeny for the Australian chelid turtles, based on the phylogenies of Burbidge *et al.* (1974) and Gaffney (1977), but modified in the light of the allozyme analyses is given in Fig. 6. In some respects, Fig. 6 should be considered an hypothesis to be tested by further study, as not all of the features are consistently supported by available data. For example, several of the analyses place *Rheodytes* with the *El. dentata/El. novaeguineae* group and it is genetically most similar to the coastal Queensland forms of what is currently treated as *Elseya dentata* (Table 4). However, rather than place *Rheodytes* as a sister taxon to the *Elseya dentata* group, we have conservatively chosen to place it outside the *Elseya/Emydura*

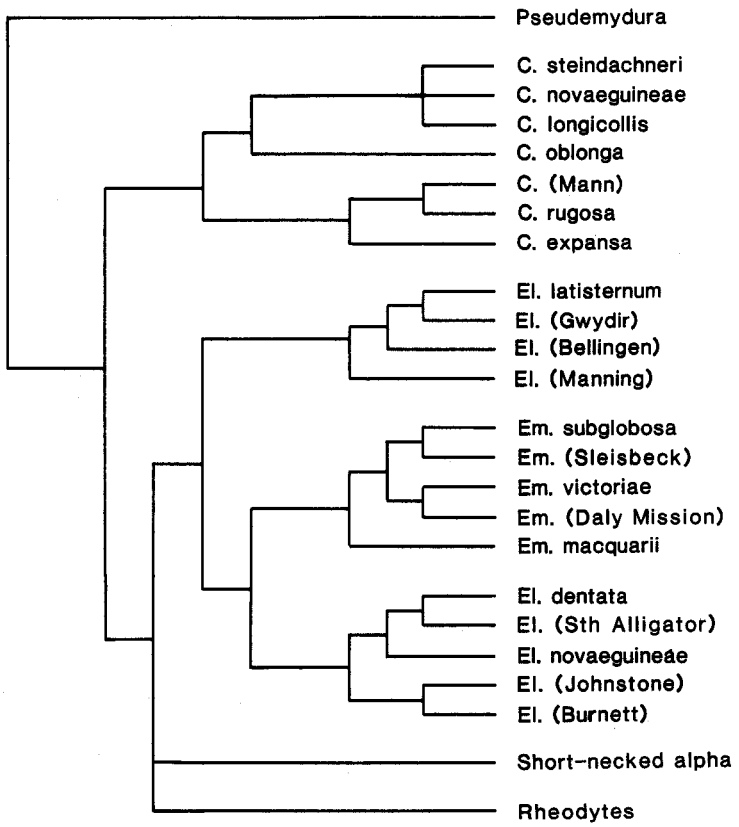

Fig. 6. A phylogeny for the Australian chelid turtles and *Elseya novaeguineae*, combining the results of the present study with the currently accepted phylogeny (Fig. 1). The phylogeny shown here should be viewed as an hypothesis for future testing, especially with regard to the affinities of 'short-necked alpha' and *Rheodytes leukops*.

radiation with the expectation that future studies will critically test this arrangement. Both *Rheodytes* and 'short-necked alpha' are monotypic genera and difficult to obtain, and 'short-necked alpha' lacks a comprehensive morphological treatment.

In testing these hypotheses, several avenues of endeavour are worthy of pursuit. An extended cladistic analysis, using all available data, would be useful to test and perhaps extend our proposed phylogeny. Both qualitative Hennigian and parsimony analyses allow a mixture of characters of different types. Such an analysis, drawing upon conservative morphological, karyotypic and electrophoretic characters would provide greater insight than any single approach. Micro-complement fixation of albumin may prove suitable for resolving the full extent of the paraphyly of the genus *Elseya* by establishing the most appropriate placement of *Rheodytes* and 'short-necked alpha'. The technique would also prove valuable for determining the affinities of more distantly related taxa such as *Pseudemydura umbrina* and South American forms, which are likely to be beyond the resolution of allozyme electrophoresis. The new DNA techniques (Hillis and Moritz 1990) can provide alternative sets of molecular characters which may also shed light on the most appropriate placement of *Rheodytes*, 'short-necked alpha' and South American forms. We are actively pursuing these latter options using our collection of frozen tissues, while the first option awaits the publication of detailed morphological data.

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