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Resolving the phylogenetic history of the short-necked turtles, genera *Elseya* and *Myuchelys* (Testudines: Chelidae) from Australia and New Guinea

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ABSTRACT

Phylogenetic relationships and taxonomy of the short-necked turtles of the genera *Elseya*, *Myuchelys*, and *Emydura* in Australia and New Guinea have long been debated as a result of conflicting hypotheses supported by different data sets and phylogenetic analyses. To resolve this contentious issue, we analyzed sequences from two mitochondrial genes (*cytochrome b* and *ND4*) and one nuclear intron gene (*R35*) from all species of the genera *Elseya*, *Myuchelys*, *Emydura*, and their relatives. Phylogenetic analyses using three methods (maximum parsimony, maximum likelihood, and Bayesian inference) produce a single, well resolved, and strongly corroborated hypothesis, which provides support for the three genera, with the exception that the genus *Myuchelys* is paraphyletic – *Myuchelys purvisi* is the sister taxon to the remaining *Elseya*, *Myuchelys* and *Emydura*. A new genus is proposed for the species *Myuchelys purvisi* to address this paraphyletic relationship. Time-calibration analysis suggests that diversification of the group in Australia coincides with periods of aridification in the late Eocene and between the mid-Miocene and early Pliocene. Other speciation events occurred during the faunal exchange between Australia and the island of New Guinea during the late Miocene and early Pliocene. Lineages distributed in New Guinea are likely influenced by the complex geologic history of the island, and include cryptic species diversity.

1. Introduction

Turtles of the genera *Elseya* and *Myuchelys* are widely distributed in eastern and northern Australia and New Guinea where they live in sympatry with other short-necked species in the genera *Elusor*, *Emydura*, and *Rheodytes* (Georges and Thomson, 2010). They altogether belong to the family Chelidae, which was once widely distributed in the Gondwana, but today has relict distributions in South America, New Guinea, Indonesia, and Australia. Chelid turtles are conservative morphologically, and, as a result, they have a complicated and often confused taxonomic history (Thomson and Georges, 2009). Although the species boundaries for Australasian taxa are well established (Georges and Adams, 1996; Georges et al., 2002), the taxonomy of the genera *Elseya*, *Myuchelys* and *Emydura* and assignment of species to them has been remarkably dynamic because of conflicting phylogenies.

The genus *Elseya* has been particularly problematic. It was initially erected for *Elseya dentata* and *Elseya latisternum* (Gray, 1867) with *E. dentata* (Gray, 1863) later designated as the type species (Lindholm, 1929). Boulenger (1889) redefined the genus as being characterized by the alveolar ridge, a longitudinal ridge on the maxillary triturating surface, present only in *E. dentata*. *Elseya latisternum* and *E. novaeguineae* were placed in the genus *Emydura*. In the decades that followed, species of *Elseya* were included in and excluded from the genus *Emydura*, because of morphological similarity and lack of consensus on what constitutes synapomorphies of the group (Boulenger, 1889; Goode, 1967; Gaffney, 1977; McDowell, 1983). Early molecular work based on an unweighted consensus of 54 nuclear markers (allozymes) split *Elseya* into two major clades, one of which (*Elseya dentata* and related taxa) was the sister group to *Emydura* (Georges and Adams, 1992, Fig. 1a). This paraphyly was also supported by the analysis of morphological data (45 morphological characters, 24 cranial and 21 postcranial), and

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the new genus *Myuchelys* was erected (Thomson and Georges, 2009) for the clade comprising *Elseya latisternum*, *E. georgesi*, *E. Belli*, and *E. purvisi* to resolve the paraphyletic relationships. The genera as currently defined are *Elseya* (6 species), *Myuchelys* (4 species) and *Emydura* (4 species) (Georges and Thomson, 2010; van Dijk et al., 2011). We retain *Elseya novaeguineae* in the genus *Elseya*.

Many problems remain. First, Georges and Thomson (2010) tentatively placed *Elseya novaeguineae* (Meyer, 1874) in *Myuchelys* based on morphological features, while acknowledging that allozyme evidence was to the contrary (Georges and Adams, 1992). The position of this species within a chelid phylogeny remains unresolved. Second, data from three mitochondrial genes and one nuclear gene (Georges et al., 1998) do not support the monophyly of species now in *Myuchelys*, a result recently confirmed with additional taxa and mitochondrial sequences (Fielder et al., 2012). Both studies revealed *Myuchelys purvisi* to be the sister taxon to the remaining *Myuchelys* and *Emydura*, despite being so similar in external morphology to *M. georgesi* that the two were regarded as a cryptic species pair (Georges and Thomson, 2010; Fielder, in press). Third, the phylogenies including *Elseya*, *Myuchelys* and *Emydura* based on morphological data (Megirian and Murray, 1999; Thomson and Georges, 2009) differ in substantial respects from those recovered from molecular data (Georges and Adams, 1992; Georges et al., 1998; Fielder et al., 2012). Other uncertainty surrounds the placement of the monotypic short-necked genera *Rheodytes* and *Elusor*.

To stabilize the taxonomy of the genera, a well-resolved and strongly supported phylogeny is critically needed. To date, the study with best taxonomic sampling (Thomson and Georges, 2009) only included morphological characters, which might be subject to a high level of homoplasy, especially at the deep nodes, as demonstrated in earlier studies of other turtle groups (Hirayama, 1984; Yasukawa et al., 2001; Joyce and Bell, 2004; Le, 2006). For example, on morphological ground, Hirayama (1984) and Le (2006) showed that the turtle family Geoemydidae is paraphyletic with the tortoise family, Testudinidae, although virtually all comprehensive molecular analyses supported the monophylies of both groups (Le, 2006; Le and McCord, 2008; Barley et al., 2010). A potential problem associated with skull morphology, which has been used extensively in phylogenetic analyses of morphological characters in turtles, derives from adaptations to food types. These adaptations include expansion of the triturating surface, which in turn exerts substantial changes to other skull characters, e.g., vomer, pterygoid, and parietal contacts, presumably due to the limitation of morphological space in turtle skulls (Le et al., 2006).

To assess the phylogenetic relationships of the genus and its current taxonomy, we sequenced three genetic markers, including two mitochondrial protein-coding genes, *cytochrome b* (*cytb*) and *NADH dehydrogenase subunit 4* (*ND4*), and one nuclear intron of G protein-coupled receptor R35 gene (*R35*). We included all currently recognized species in the genera *Elseya* and *Muychelys* and related genera, *Emydura*, *Elusor*, and *Rheodytes* in the current study. We also calibrated temporal divergences using the Bayesian relaxed clock approach to elucidate the diversification patterns and biogeography of these poorly known turtles.

2. Materials and methods

2.1. Taxonomic sampling

Since the species boundaries of all taxa represented here, except for *Elseya novaeguineae*, have been well established in a previous comprehensive study based on allozymic data (Georges and Adams, 1996), a minimal sampling scheme was employed in this study for all species except *E. novaeguineae*. As a result, we sequenced DNA from 30 individuals: 2 samples for 1 species of *Rheodytes*, 2 samples for 1 species of *Elusor*, 7 samples for 5 species of *Emydura*, and 4 samples for 4 species of *Myuchelys*, and 15 samples for 6 species of *Elseya*. This included eight samples of *Elseya novaeguineae* representing the major taxa (Georges et al., unpublished data). We sequenced all species of *Elseya*, with the single exception of *M. latisternum* (however, this species was included in our phylogenetic analysis, based on sequences available on GenBank, see Table 1). *Rheodytes* was used as the outgroup (separate analyses using *Chelodina longicollis* as the outgroup recovered the same topology but with slightly lower support values in some nodes).

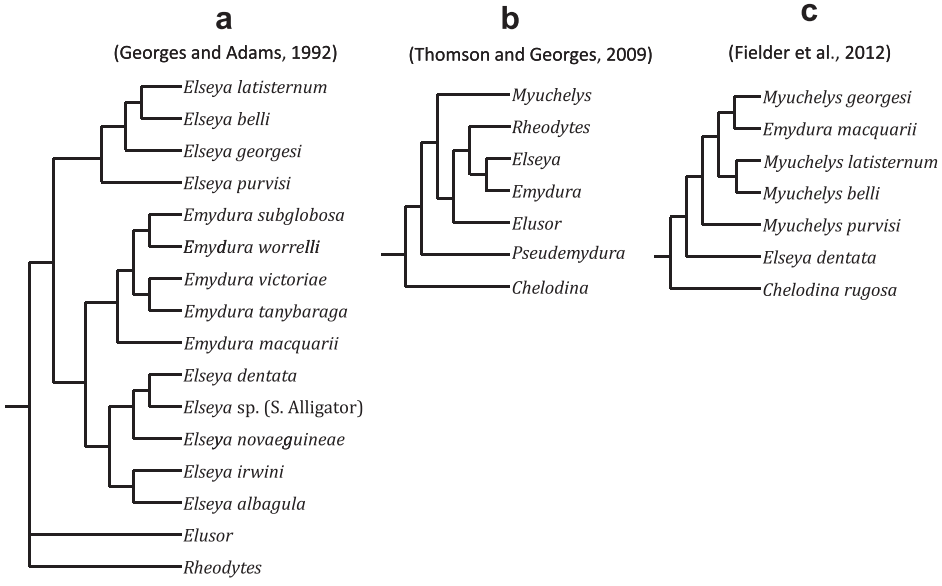

Fig. 1. Previously supported hypotheses for the relationships of Elseya, Emydura, and their relatives. (a) The phylogenetic relationships based on 54 allozyme loci from Georges and Adams (1992). (b) The relationships based on morphological data from Thomson and Georges (2009). (c) The relationships based on *ND4* and Control Region from Fielder et al. (2012, Fig. 2A).

Table 1
GenBank accession numbers, and associated voucher specimens/tissues that were used in this study. All sequences generated by this study have accession numbers: KC755109–KC755195.

Species names	GenBank no. (*ND4*)	GenBank no. (*R35*)	GenBank no. (*cytb*)	Voucher numbers for this study
Elseya albagula	KC755109	KC755139	KC755168	AGF-055
Elseya branderhorsti	KC755110	KC755140	KC755169	AMNH FS-27450
Elseya branderhorsti	KC755111	KC755141	KC755170	AMNH FS-27451
Elseya dentata	KC755112	KC755142	KC755171	AMNH FS-27452
Elseya dentata	KC755113	KC755143	KC755172	AMNH FS-27453
Elseya irwini	KC755114	KC755144	KC755173	AG-135
Elseya lavarackorum	KC755115	KC755145	KC755174	AGF-010
Elseya novaeguinea	KC755116	KC755146	KC755175	AMNH FS-27454
Elseya novaeguinea	KC755117	KC755147	KC755176	AMNH FS-27455
Elseya novaeguinea	KC755118	KC755148	KC755177	AMNH FS-27456
Elseya novaeguinea	KC755119	KC755149	KC755178	AMNH FS-27457
Elseya novaeguinea	KC755120	KC755150	KC755179	AMNH FS-27458
Elseya novaeguinea	KC755121	KC755151	KC755180	AMNH FS-27459
Elseya novaeguinea	KC755122	KC755152	KC755181	AMNH FS-27460
Elseya novaeguinea	KC755123	KC755153	KC755182	AMNH FS-27461
Elusor macrurus	KC755124	KC755154	–	AMNH FS-27462
Elusor macrurus	KC755125	KC755155	–	AMNH FS-27463
Emydura macquarii	KC755126	KC755156	KC755183	AMNH FS-27464
Emydura subglobosa	KC755127	KC755157	KC755184	AMNH FS-27465
Emydura subglobosa	KC755128	KC755158	KC755185	AMNH FS-27466
Emydura tanybaraga	KC755129	KC755159	KC755186	AMNH FS-27467
Emydura tanybaraga	KC755130	KC755160	KC755187	AMNH FS-27468
Emydura victoriae	KC755131	KC755161	KC755188	AMNH FS-27469
Emydura victoriae	KC755132	KC755162	KC755189	AMNH FS-27470
Emydura worrelli	KC755133	KC755163	KC755190	AGF-004
Myuchelys belli	KC755134	KC755164	KC755191	AGF-064
Myuchelys georgesi	KC755135	KC755165	KC755192	AGF-059
Myuchelys latisternum[a]	–	AY339643	U81354	–
Myuchelys purvisi	KC755136	KC755166	KC755193	AGF-054
Rheodytes leukops	KC755137	KC755167	KC755194	AMNH FS-27471
Rheodytes leukops	KC755138	–	KC755195	AMNH FS-27472

[a] Genbank sequences only.

2.2. Molecular data

Two mitochondrial genes, *NADH dehydrogenase subunit 4* (*ND4*) and *cytochrome b*, and one nuclear intron, *R35*, were employed to address the phylogenetic relationships of the target taxa. The utility of these markers in resolving relationships among turtles have been well demonstrated in earlier studies (Engstrom et al., 2004; Fujita et al., 2004; Stuart and Parham, 2004; Le et al., 2006; Naro-Maciel et al., 2008). For primers, we used EX1 and EX2 (Fujita et al., 2004) for *R35*, GLUDGE (Palumbi et al., 1991) and mt-E-Rev2 (Barth et al., 2004) for *cytb*, and ND4 and Leu (Arevalo et al., 1994) for *ND4*.

Total genomic DNA was extracted from blood or tissue samples using a commercially available DNeasy Tissue Kit following manufacturer's instructions (QIAGEN Inc., Valencia, CA, USA). PCR was performed using PuRe Taq PCR beads (GE Healthcare, Piscataway, NJ, USA) to amplify an 839-bp fragment of the mitochondrial cytochrome b (cytb) gene (primers GLUDGE, Palumbi et al., 1991; mt-E-Rev2, Barth et al., 2004), an 868 bp fragment of the *nicotinamide dehydrogenase 4* (*ND4*) gene (868 bp, primers ND4/Leu, Arevalo et al., 1994), and approximately 1.2 Kbp of the nuclear RNA fingerprint protein 35 (*R35*) gene intron 1 (primers EX1 and EX2, Fujita et al., 2004). The standard PCR conditions used to amplify *ND4* and *R35* were: 95° C for 5′, 35 cycles of [95° for 45″, 50° for 45″, 72° for 45″], and 72° for 6′. The standard PCR conditions used to amplify *cytb* were: 95° for 5′, 35 cycles of [95° for 45″, 52° for 45″, 72° for 45″], and 72° for 6′. All PCR products were visualized on a gel before sequencing. For several gene/species combinations, a second band of unexpected size was produced when standard conditions were used (*Elseya albagula* for cytb; *M. purvisi* for ND4; and *E.albagula*, *E.irwini*, *Emyduraworrelli*, *Elusor macrurus*, and some specimens of *Elseya novaeguineae* for R35). In each of these cases, raising the annealing temperature by 2 °C yielded a single product of the proper size. For several low-concentration samples (from *Myuchelysbellii*, *M. georgesi*, and *M. purvisi*) a hot-start PCR program (95° for 15′, 35 cycles of [95° for 30″, 52° for 30″, 72° for 1′], and 72° for 6′) in conjunction with HotStar Taq (Qiagen, Valencia, CA, USA) was required for proper amplification. The cytb gene failed to amplify for *Elusor macrurus* under all conditions reported here.

PCR products (50 μl of each sample) were cleaned on a BIOMEK automated apparatus using the Ampure system (Beckman-Coulter Inc., Danvers, MA, USA). Cleaned PCR products were cycle-sequenced at the American Museum of Natural History's Sackler Center for Comparative Genomics using BigDye reagents (Perkin Elmer, Waltham, MA, USA), after which cycle sequencing products were ethanol-precipitated and run on an ABI3770 automated sequencer (Applied Biosystems, Foster City, CA, USA). *Cytb* and *R35* sequences generated from Shaffer et al. (1997) and Fujita et al. (2004) for *Myuchelys latisternum* were downloaded from GenBank (Table 1). Sequences were edited, aligned, and trimmed using Geneious Pro 5.3.3 (BioMatters Inc.).

2.3. Phylogenetic analyses

We aligned sequence data using ClustalX v2.0 (Thompson et al., 1997) with default settings. Data were analyzed using maximum parsimony (MP) and maximum likelihood (ML) using PAUP∗4.0b10 (Swofford, 2001) and Bayesian analysis using MrBayes v3.2 (Huelsenbeck and Ronquist, 2001). For maximum parsimony analysis, we ran heuristic analyses with 100 random taxon-addition replicates using the tree-bisection and reconnection (TBR) branch swapping algorithm in PAUP, with no upper limit set for the maximum number of trees saved. Bootstrap support (BP) (Felsenstein, 1985) was assessed using 1000 pseudoreplicates and 100 random taxon-addition replicates. All characters were equally weighted

and unordered. Gaps in sequence alignments were treated as a fifth character state (Giribert and Wheeler, 1999).

For maximum likelihood analysis the optimal model for nucleotide evolution was determined using Modeltest v3.7 (Posada and Crandall, 1998). Analyses used a randomly selected starting tree and heuristic searches with simple taxon addition and the TBR branch-swapping algorithm. Support for the likelihood hypothesis was assessed by bootstrap analysis with 1000 replications and simple taxon addition. We consider bootstrap values of $\geqslant$70% as potentially strong support and bootstrap values of <70% as weak support (Hillis and Bull, 1993).

For Bayesian analyses we used the optimal model selected by Modeltest with parameters estimated by MrBayes Version 3.2. Analyses were conducted with a random starting tree and run for 1×10^7 generations. Four Markov chains, one cold and three heated (utilizing default heating values), were sampled every 1000 generations. Log-likelihood scores of sample points were plotted against generation time to detect stationarity of the Markov chains. Trees generated prior to stationarity were removed from the final analyses using the burn-in function. Two independent analyses were started simultaneously. The posterior probability values (PP) for all clades in the final majority-rule consensus tree are reported. We ran analyses on both combined and partitioned datasets to examine the robustness of the tree topology (Brandley et al., 2005; Nylander et al., 2004). In the partitioned analyses, we divided the data into seven separate partitions, including *R35*, and the other six based on gene codon positions (first, second, and third) in the two mitochondrial markers, *cytb* and *ND4*. Optimal models of molecular evolution for each partition were selected using Modeltest and then assigned to these partitions in MrBayes 3.2 using the command APPLYTO. Model parameters were estimated independently for each data partition using the UNLINK command.

2.4. Divergence-time analysis

Divergence times were calculated using a relaxed-clock model (Drummond et al., 2006) as implemented in the computer program BEAST v.1.6.2 (Drummond and Rambaut, 2007). The program BEAUti v.1.6.2 was used to set criteria for the analysis. We used four calibration points to calibrate the phylogeny. For the first one, all species of the genera *Elseya*, *Myuchelys*, and *Emydura* were considered to form a clade, and this node was constrained to 55 million years ago (MYA) with a 95% confidence interval from 50 to 60 Myr based on the fossil, *Emydura* s.l.s.p., found in Redbank Plains and dated back to the Eocene (Lapparent de Broin and Molnar, 2001). The second calibration point, a clade of three species, *Elseya dentata*, *E. irwini*, and *E. lavarackorum*, was constrained to 3.6 MYA with the confidence interval from 3.2 to 4.0 MYA according to the fossil related to *E. irwini* from the early Pliocene Bluff Downs (Thomson and Mackness, 1999; Mackness et al., 2000). Two other calibration points were derived from recent work on the *E. novaeguineae* species complex (Georges et al., unpublished data) based on vicariance events on New Guinea. Specifically, *E. novaeguineae* as a whole was set to 5.2 MYA, consistent with the emergence of the Birds Head region at the end of the Miocene, with a confidence interval from 4.7 to 5.7 MYA and the other three mutually exclusive clades within this species complex were constrained to 3.5 MYA, coinciding with the uplifting of the Central Ranges in the Pliocene, with confidence interval from 3.1 to 3.9 MYA .

A GTR model using gamma + invariant sites with four gamma categories was used along with the assumption of a relaxed molecular clock. As for the priors, we used all default settings, except for the Tree Prior category that was set to Yule Process, as this setting is recommended for a species-level phylogeny by the program manual. The combined and non-partitioned dataset was used for

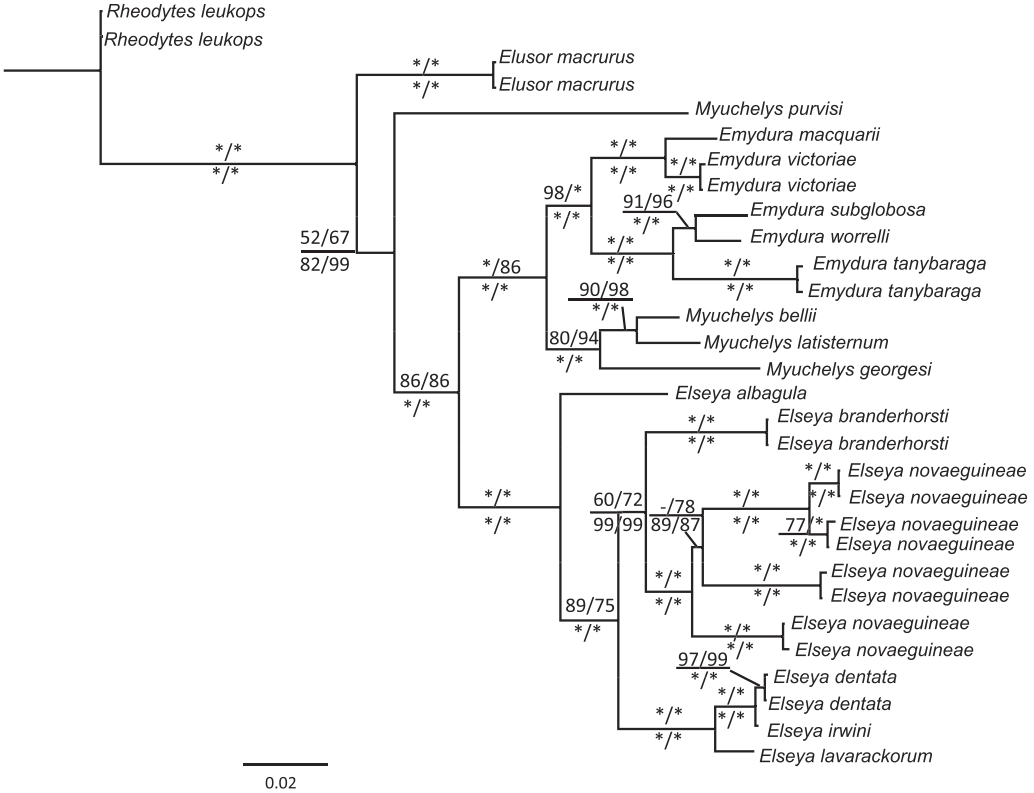

Fig. 2. The single tree generated from MP, ML, and Bayesian analyses of combined mitochondrial and nuclear genes with branch length estimated by the Bayesian analyses. Numbers above branches are MP and ML bootstrap values, respectively. Numbers below branches are Bayesian single-model posterior probability and mixed-model posterior probability values, respectively. Asterisk indicates 100% value.

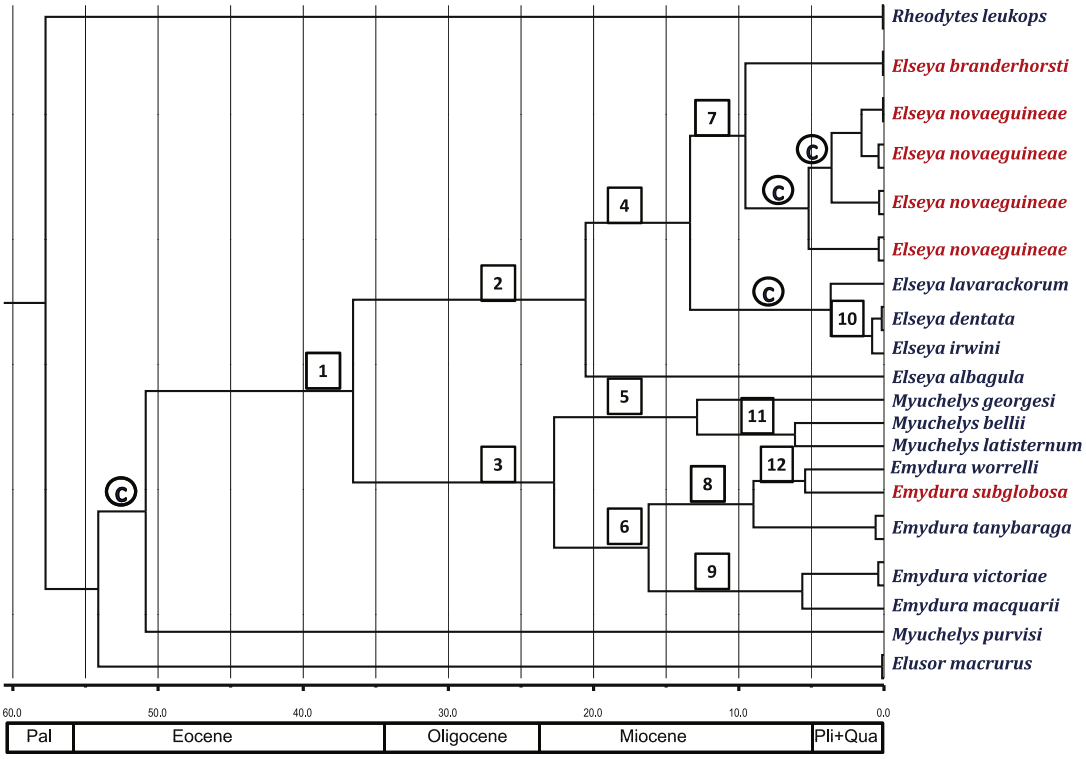

Fig. 3. Time calibration using the program BEAST. The 95% confidence interval values for each numbered node are presented in Table 2. Red color denotes taxa distributed in New Guinea, and blue denotes taxa in Australia. C: calibration point. Pal: Paleocene. Pli + Qua: Pliocene + Quaternary. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

a single run. In addition, a random tree was employed as a starting tree. For this analysis, the length chain was set to 5×10^6, and the Markov chain was sampled every 1000 generations. After the dataset with the above settings was analyzed in BEAST, the resulting likelihood profile was then examined by the program Tracer v1.5 to determine the burn-in cutoff point. The final tree with calibration estimates was computed using the program TreeAnnotator v1.6.2 as recommended in the BEAST program manual.

3. Results

3.1. Phylogenetic analyses

The final data matrix contained 30 terminals and 2832 aligned characters (*ND4*: 868 characters; *cytb*: 850 characters; *R35*: 1114 characters). Two species had missing data, as we were unable to sequence *cytb* for *Elusor*, and *ND4* was unavailable for *Myuchelys latisternum* (the only species for which we did not have tissue available).

Using MP, we analyzed the data three ways: mitochondrial only, nuclear only, and both combined. MP analysis of the *R35* intron included 1114 aligned characters, of which 1037 were constant, and 50 were parsimony informative. The number of trees retained was 3712 with the tree length (TL) of 83, consistency index (CI) of 0.95, and retention index (RI) of 0.98. The consensus topology based on trees retained was very poorly resolved. MP analysis of the mitochondrial genes contained 1718 aligned characters, of which 1151 were constant, and 487 were parsimony informative. The single tree was generated with TL of 1265, CI of 0.56, and RI of 0.79. The combined analysis of all data produced one tree with TL of 1371, CI of 0.58, and RI of 0.79.

The topology of the mitochondrial tree was very similar to the tree generated by combining the nuclear and mitochondrial data (see below, Fig. 2), except for three differences: *Myuchelys purvisi* is the sister taxon to all other taxa exclusive of the *Rheodytes*, *M. georgesi* is the sister taxon to *Emydura*, and minor differences in rearrangements of terminals among the clades within the *E. novaeguineae* species complex. In general, many nodes, especially the basal ones, received lower BP in the mitochondrial compared to the combined tree. Based on the poorly resolved and weakly supported phylogenetic hypotheses in the partitioned analyses of nuclear and mitochondrial genes, respectively, we consider our tree based on the combined data to be the optimal hypothesis.

The MP analysis of the combined data generated a single tree (Fig. 2) with approximately 90% of its nodes receiving strong support (BP > 70%). The three nodes with low bootstrap values are: the placement of *Myuchelys purvisi* (BP = 52), the sister–taxon relationship between *Elseya branderhorsti* and the *E. novaeguineae* complex (BP = 60), and one of the nodes within the *E. novaeguineae* species group (BP < 50). The phylogenetic results indicate that the genus *Myuchelys*, as defined by Georges and Thomson (2010), is paraphyletic. Of the three major clades identified for *Elseya* and *Myuchelys*, the first clade consists of six species, *Elseya albagula*, *E. branderhorsti*, *E. dentata*, *E. irwini*, *E. lavarackorum*, and *E. novaeguineae*. The second clade, containing three species, *M. bellii*, *M. georgesi*, and *M. latisternum*, is strongly supported as the sister group to the genus *Emydura*. The third clade consists only of *Myuchelys purvisi*, the sister taxon to *Elseya*, the remaining *Myuchelys*, and *Emydura*. *Elusor macrurus* is the sister lineage to all species of *Elseya*, *Myuchelys*, and *Emydura*.

We ran the maximum likelihood and single-model Bayesian analyses based on combined matrix using the TIM + I + G model of molecular evolution as selected by the ModelTest. The parameters calculated by the AIC criterion were: Base frequency A = 0.3275, C = 0.2490, G = 0.1494, T = 0.2741; ML $-\ln L$ = 10528.5469; rate matrix: A–C: 1.0000, A–G: 5.8442, A–T: 0.4377, C–G: 0.4377, C–T: 8.0794, G–T: 1.0000; proportion of invariable

Table 2
Time calibration for important nodes in the phylongeny. Node numbers are defined in Fig. 3.

Nodes	Age estimate (MYA)	95% CI (MYA)
1	36.6	25.1–49.7
2	20.6	13.1–32.8
3	22.7	14.3–32.7
4	13.4	8.55–20.8
5	12.9	5.4–21.6
6	16.21	8.3–23.4
7	9.5	5.8–14.6
8	9.0	4.7–15.2
9	5.62	1.8–11.2
10	0.8	0.22–1.9
11	6.1	1.8–12.6
12	5.4	1.8–10.4

sites (I) = 0.6427; gamma distribution shape parameter (G) = 1.0255. For the ML analysis, a single tree was produced with the total number of attempted rearrangements of 7958, and the score of the best tree recovered was 10519.468. All nodes have potentially strong support (BP > 70%), except for the position of *Myuchelys purvisi* (BP = 67) (Fig. 2). In the single-model Bayesian analysis, ln$L$ scores reached equilibrium after 12,000 generations, while in the mixed-model Bayesian analysis ln$L$ attained stationarity after 17,000 generations in both runs. Except for the node within the *E. novaeguineae* species group, where both Bayesian analyses gave low support (PP < 95%), all other nodes in the mixed-model analysis receive strong support, while the position of *M. purvisi* has a low PP support value of 82 in the single-model analysis. The topologies of MP, ML and the Bayesian consensus trees, both single and mixed model, were completely resolved and identical (Fig. 2).

3.2. Divergence-time analysis

After 500 initial trees were discarded from the analysis as suggested by the program Tracer v1.5, final divergence times were generated using the program TreeAnnotator v1.6.2. The topology inferred by the program BEAST (Fig. 3) is identical to the one supported by the phylogenetic analyses (Fig. 2). Values of effective sample size (ESS) are all higher than 350 for the likelihood and calibrated nodes. Age estimates and 95% confidence intervals for important nodes are shown in Table 2. According to the results, *Myuchelys purvisi* diverged around 51 MYA. The other lineage leading to all other species started to diversify around 37 MYA, producing major clades. The seven most recent speciation events occurred within the last 10 MYA (Fig. 3).

4. Discussion

4.1. Phylogenetic relationships

Using both mitochondrial and nuclear markers, we resolve the phylogenetic relationships of the genera *Elseya*, *Myuchelys*, and *Emydura*. The single tree generated by three types of phylogenetic analyses has very high statistical support at almost all nodes, except for the position of *M. purvisi* and the relationships within the *E. novaeguineae* species complex. Nevertheless, even these nodes receive good support from the Bayesian mixed-model analysis (PP = 87–99%), while the latter also obtains high bootstrap value (BP = 78%) from the maximum likelihood analysis.

Our phylogenetic results show that three species, i.e., *M. bellii*, *M. georgesi*, and *M. latisternum*, form a monophyletic group with strong support (Fig. 2). This set of relationships to the exclusion of *M. purvisi* was recovered in Georges and Adams's (1992) allozyme study, but not corroborated in Fielder, in press) molecular analysis (Fig. 1a and c). Similarly, the sister-group relationship between these three species of *Myuchelys* + *Emydura* and the group consisting of *E. dentata* and all other species of the genus (excluding *M. purvisi*) as hypothesized in this study was not recovered by any previous study (Fig. 1). The relationships within *Emydura* are well resolved and robust, but the positions of *E. macquarri* and *E. tanybaraga* are substantially different from those proposed by Georges and Adams (1992). Within *Elseya*, the *E. dentata* species group is not shown as the sister taxon to *E. novaeguineae*, and *E. albagula* is not the sister taxon to *E. irwini* as indicated in Georges and Adams (1992). Instead, *E. novaeguineae* along with *E. branderhorsti* forms a distinct clade with *E. dentata* and *E. irwini* being sister species, and *E. albagula* is recovered as the sister taxon to all other species (Fig. 2).

Our study supports the hypothesis that *Elseya*, *Myuchelys*, and *Emydura* form a clade to the exclusion of *Rheodytes* and *Elusor* as indicated by Georges and Adams's (1992) study (Fig. 1a). Georges et al. (1998, Fig. 4 therein) hypothesized that the genera *Myuchelys* (excluding *M. purvisi*) and *Emydura* formed a clade, to the exclusion of *Rheodytes* and *Elusor*, but greater resolution was not possible. Thomson and Georges's (2009) morphological results show *Rheodytes* as the sister taxon to *Elseya* + *Emydura*, with *Elusor* as the closest relative of the clade. *Myuchelys* is recovered as the sister taxon to the entire clade (Fig. 1b). Even though Fielder et al. (2012) support *Emydura* + *Myuchelys* + *Elseya* as a clade, their study did not include *Rheodytes* and *Elusor* (Fig. 1b).

It is also important to note that while molecular sequence analyses (Georges et al., 1998; Fielder et al., 2012; this study) support the sister–taxon relationship between *Myuchelys* and *Emydura*, the allozyme and morphological analyses (Georges and Adams, 1992; Thomson and Georges, 2009) group *Emydura* and *Elseya* as sister taxa. In particular, this set of relationships is strongly supported by morphological data (BP = 98) (Thomson and Georges, 2009). This suggests a potentially high level of morphological homoplasy in this group of side-necked turtles. The position of *M. purvisi* recovered by this study is novel, as previous studies make it the sister taxon to either the remaining *Myuchelys* (Georges and Adams, 1992, Fig. 1a), to the remaining *Myuchelys* + *Elseya* + *Emydura* + *Elusor* + *Rheodytes* (Georges et al., 1998) or to the remaining *Myuchelys* + *Emydura* to the exclusion of *Elseya* (Fielder et al., 2012, Fig. 1c).

4.2. Biogeography

Fossil records of Australia are still poorly understood, as only fragmentary materials have been discovered (Gaffney et al., 1989; Lapparent de Broin and Molnar, 2001; Smith, 2010). The earliest fossils, which can be assigned to *Elseya* + *Emydura*, occur in the early Eocene (Lapparent de Broin and Molnar, 2001), and demonstrate that this group was established by this time in present-day northeastern Australia. Our time-calibrated molecular results reveal that the two major groups of the short-necked turtles did not evolve until the end of the Eocene (Fig. 3). This event coincides with the transition of the paleoclimate in Australia, from mesic conditions during the Eocene to the increasingly arid environment in the Oligocene (Alley, 1998; Clarke, 1998; Martin, 2006). Another extensive period of aridification occurred between the mid and late Miocene (Martin, 2006; Dawson and Dawson, 2006), which coincides with other four lineage-diversification events of the short-necked turtles. This suggests that paleoclimate, especially aridification, plays an important role in shaping the evolution of the turtles by increasing the speciation rate, as also demonstrated in other vertebrate groups (Dawson and Dawson, 2006; Dubey and Shine, 2010; Fujita et al., 2010).

Faunal exchange between Australia and New Guinea appears to have provided another means for diversification within this turtle group. Australia clearly forms an ancestral origin of the group, as many basal divergences are inferred to occur in the continent. In addition, the group's fossil record in Australia dates back to the early Eocene (Lapparent de Broin and Molnar, 2001), during which New Guinea was still a series of island arcs (DiCaprio et al., 2011; Baldwin et al., 2012). Our phylogenetic results reveal that the group twice dispersed out of Australia to the island of New Guinea. One dispersal is dated to around 9.5 MYA (node 7), and the other to around 5.4 MYA (node 12) (Table 2). The events are consistent with those reported in mammals (Alpin et al., 1993; Malekian et al., 2010), birds (Norman et al., 2007), and snakes (Wüster et al., 2005), which reached New Guinea from Australia multiple times during these two periods. Growing evidence strongly supports landbridges forming between the two landmasses during the late Miocene and the early Pliocene. Subsequent divergences of the turtle lineages in New Guinea appear to have been strongly influenced by the geological history of the island, including the uplift of the Central Range and the isolation of the Birds Head during the Pleistocene (Georges et al., unpublished data).

4.3. Taxonomic issues

Our phylogenetic results support the retention of *Myuchelys* for three species *M. bellii*, *M. georgesi*, and *M. latisternum* – Type species *Myuchelys latisternum* (Gray, 1867) – and support the restriction of six species, *E. albagula*, *E. branderhorsti*, *E. dentata*, *E. irwini*, *E. lavarackorum*, and *E. novaeguineae* to the genus *Elseya* – Type species *Elseya dentata* (Gray, 1863).

Owing to the distinct position of *Myuchelys purvisi*, we propose the following new genus:

Family Chelidae Gray 1831.

Flaviemys gen. nov.

Type species: *Myuchelys purvisi* (Wells and Wellington, 1985) [=*Flaviemys purvisi*].

Diagnosis – A genus of short-necked turtles with the following character combination: (1) broad cervical scute; (2) bright yellow coloration on the ventral marginal and the plastron; (3) bright yellow stripe on the ventral aspects of legs, running from the plastron to the distal of the first toes; (4) three bright yellow stripes on the tail, with one mid-ventral and the others lateral; (5) bright yellow marking on the ventral distal tip of the tail; (6) neural bones present.

Content: One species, *Flaviemys purvisi* (Wells and Wellington, 1985).

Distribution: Northeastern Australia in the Manning River system.

Etymology: The generic name "*Flaviemys*" is based on a distinctive yellow color pattern on the plastron of the species. From the Greek, *flavus* (yellow) and *emys* (turtle).

5. Conclusion

Using a broad sampling scheme and inclusion of both mitochondrial and nuclear markers, we provide a well resolved and robust phylogenetic hypothesis for the genera *Elseya*, *Myuchelys*, and *Emydura*. The results help to clarify many long-standing taxonomic issues extending over 100 years of the genus history with high confidence levels. Nonetheless, some outstanding problems remain, in particular, with regard to the nomenclature of the lineages within the *Elseya novaeguineae* species group, which we suspect to represent a New Guinean complex of at least three species. Although these distinct evolutionary units have been demonstrated to have long evolved independently (Georges et al., unpublished data and this study), morphological characters to diagnose these clades are currently lacking. Future research describing the morphological variation within this complex can be expected to provide insights into the taxonomy of the lineages.

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