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Evolution of Afrotropical freshwater crab lineages obscured by morphological convergence

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Abstract

We use sequence data derived from six DNA gene loci to examine evolutionary and biogeographic affinities among all freshwater crab families. With an emphasis on the Afrotropical fauna that includes Africa, Madagascar, and the Seychelles, we test the proposed Gondwanan cladogenesis of the group. Phylogenetic results demonstrate that contemporary distribution patterns of freshwater crab lineages are incongruent with the expected area cladogram of continental fragmentation. Instead, our phylogenetic estimate and divergence time estimation indicate a post-Gondwanan, early Cretaceous cladogenesis for freshwater crabs implying that the acquisition of a freshwater lifestyle was achieved more recently. A dispersal hypothesis as opposed to vicariance appears to best explain the contemporary distribution pattern of this group. However, our results do not explicitly disprove a Gondwanan origin for the Afrotropical freshwater crabs. Alarmingly, these results suggest that most of the currently recognized freshwater crab families are unreliable taxonomic groupings since virtually no Afrotropical freshwater crab families formed monophyletic units thus obscuring inferred biogeographic relationships. Convergence in characters associated with the terminal segment of the mandibular palp is clearly a pervasive obstacle in the taxonomy of this group.
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1. Introduction

The distribution pattern of extinct and extant taxa associated with fragmentation and rifting of ancient continental landmasses offer an ideal opportunity with which to test biogeographic hypotheses derived from. Fragmentation of Gondwana began during the mid-Mesozoic, 158–165 million years ago (Mya), with the rifting of the Indo-Madagascar continent from Africa ending 121 Mya (Rabinowitz et al., 1983; Storey, 1995a,b). Subsequently, India and Madagascar separated (84–96 Mya), followed by the separation of the granitic Seychelles archipelago from India (68–65 Mya) while India continued drifting northeastward towards the Eurasian plate, colliding with it 55–42 Mya, during the early Eocene (Briggs, 2003). Africa's separation from South America began 106 Mya and was completed by 84 Mya, during the late Cretaceous (Goldblatt, 1993). Prolonged historic isolation of both Madagascar and the Seychelles Islands in the Indian Ocean have led to near complete endemicity in biotic assemblages that include some spectacularly diverse adaptive radiations, resulting in these areas being biodiversity hotspots. While the colonization history of Madagascar's vertebrate fauna has lately

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received significant attention recently (De Queiroz, 2005; Nagy et al., 2003; Raxworthy et al., 2002; Sparks and Smith, 2004; Vences et al., 2003; Yoder et al., 2003), however there are few studies focusing on the island's endemic invertebrate taxa. Consequently, phylogenetic and biogeographic affinities among invertebrates that also appear to have undergone a radiation on Madagascar remain enigmatic and the subject of vigorous debate (Bott, 1955, 1965; Ng et al., 1995; Ortmann, 1902; Rodriguez, 1986; Von Sternberg et al., 1999).

The distribution and phylogenetic affinities of other freshwater crustaceans, such as freshwater crayfish, support the Gondwanan origin hypothesis (Crandall et al., 2000). Nevertheless, despite constituting 15% of brachyuran diversity (Cumberlidge pers. comm.), evolutionary relationships among freshwater crab families have remained unresolved. Contemporary freshwater crabs complete their life cycles exclusively in freshwater habitats, are incapable of surviving prolonged exposure in brackish or saline environments, and are characterized by the production of a small number of yolk-rich (lecithotrophic) eggs followed by direct development and the absence of free-swimming planktonic larval phases with low assumed dispersal capacity (Von Sternberg et al., 1999). Two main hypotheses have been proposed to explain the contemporary distribution of freshwater crabs. Hypothesis one proposes an ancient Gondwanan origin for the three freshwater crab superfamilies (Potamoidea, Gecarcinucoidea, and Pseudothelphusoidea) (Ng et al., 1995; Rodriguez, 1986). Morphological similarities in diagnostic features of the terminal segment of the mandibular palp shared between African and South American freshwater crab families and between African, Madagascan, and Indian taxa have been used as evidence to support a Gondwanan origin (Rodriguez, 1986). In addition, Madagascar's freshwater crab fauna includes taxa that have long been assumed by some authors to be representative of transitional forms affected by continental fragmentation, with some of the island's taxa apparently showing evolutionary affinities with both African and Indian taxa (Bott, 1965; Ng et al., 1995; but see Cumberlidge and Von Sternberg, 2002 for an alternative view). The presence of a single freshwater crab species on the Seychelles Islands, placed in the same family present on the Indian subcontinent, would imply an ancient biogeographic link between these areas, further supporting the assumed Gondwanan origin of freshwater crabs (Ng et al., 1995). However, earlier taxonomic work undertaken by Bott (1955) placed the single Seychelles freshwater crab species as a congener of the East African *Deckenia* (as *D. alluaudi* in the family Deckeniidae), underscoring the taxonomic instability of the group. Additionally, the occurrence of freshwater crabs on continental islands (such as Madagascar, Seychelles, Socotra, and Sri Lanka) and their complete absence from oceanic islands such as the Comoros, Reunion, and Mauritius would be further evidence of an ancient origin. These biogeographic infer-

ences are derived under the assumption that the morphological characters used to unite each of the three superfamilies are synapomorphic and to suggest that freshwater crabs are monophyletic. In the absence of a phylogenetic analysis for the group, the character state remains unclear and may reflect sympleisiomorphy. Noticeably, several taxonomic authorities have questioned the validity of the superfamilies and their stability (Cumberlidge, 1999; Holthuis, 1979; Ng, 2004; Ng and Sket, 1996; Ng and Tan, 1998; Von Sternberg et al., 1999) further fueling the biogeographic debate over freshwater crab origins. This hypothesis suggests that ancient continental fragmentation from a 'centre of origin' is the main factor that explains the contemporary distribution of freshwater crabs.

Conversely, hypothesis two suggests a recent post-Gondwana (Cretaceous) evolution of freshwater crabs from a marine ancestor (Carriol and Secretan, 1992; Colosi, 1921; Glaessner, 1969; Von Sternberg et al., 1999). In addition this post-Gondwana hypothesis implies dispersal from a widespread monophyletic but polymorphic ancestral taxon to explain the contemporary distribution of the group. Furthermore, the fossil record for freshwater crabs is relatively young, with the oldest fossils being from the upper Miocence. Nevertheless, aquatic invertebrates, including freshwater crabs, have a notoriously poor paleontological record, and the absence of geologically ancient fossils cannot be unequivocally used to disprove Gondwanan origins.

In an attempt to test the two hypotheses proposed for the origin and radiation of these freshwater crabs, we collected partial sequence data from six gene loci that included four mitochondrial genes (two ribosomal loci, 16S rRNA and 12S rRNA, two protein coding loci, cytochrome *c* oxidase subunit I (COI), and cytochrome *c* oxidase subunit II (COII)), as well as two nuclear (nDNA) loci, one protein coding nuclear gene locus (histone H3), and one ribosomal locus (18S rRNA) for all three freshwater crab super families (Potamoidea, Gecarcinucoidea, and Pseudothelphusoidea). Our study includes 65 taxa representing Potamoidea (46 species), Gecarcinucoidea (18 species), and the Pseudothelphusoidea (1 species).

2. Materials and methods

2.1. Sample collection

Representatives of all the described Malagasy freshwater crab samples (excluding only *Boreas uglowi*) were obtained from the Field Museum of Natural History, Chicago (USA), from the Northern Michigan University collection or from donations to authors by individuals. The following five Afrotropical families were included: (1) four African genera in Potamonautidae (23 *Potamonautes*, 1 *Potamonemus*, 2 *Sudanonautes*, and 1 *Liberonautes*), these included sequence data from 14 southern African *Potamonautes* species from an earlier study (Daniels et al., 2002), which were downloaded from GenBank (12S rRNA, 16S rRNA, and

COI mtDNA) and included in the present analysis, and sequenced for the outstanding three gene fragments. Five Malagasy genera in Potamonautidae (1 *Madagapotamon*, 4 *Hydrothelphusa*, 1 *Marojejy*, 2 *Malagasya*, 1 *Skelosophusa*, and 1 *Foza raimundi*), the single genus in the Platythelphusidae (1 *Platythelphusa*), the single genus in Deckeniidae (1 *Deckenia*); Potamidae (1 *Socotra*—the only Afrotropical genus in the family) and Gecarcinucidae (1 *Seychellum*—the only Afrotropical genus in the family). In addition, we included representatives of the families Potamidae (1 *Potamon*, 1 *Isolapotamon*, 1 *Johara*, and 2 *Geothelphusa*), the Oriental Gecarcinucidae (4 *Barythelphusa*, 1 *Gecarcinucus*, 1 *Gubernatoriana*, 1 *Sartoriana*, and 1 *Phricothelphusa*), and the Oriental and Australasian family Parathelphusidae (1 *Ceylonthelphusa*, 2 *Parathelphusa*, 2 *Rouxana*, 1 *Sayamia*, and 1 *Oziothelphusa*) as well as a single representative of the Neotropical Pseudothelphusidae, *Guinotia dentata*. A list of the voucher specimens of the taxa sequenced in this study, together with their museum catalogue numbers and localities is provided in Table 1.

2.2. Outgroup selection

Three crabs from different families were used as outgroup taxa; these included the freshwater crab species *Dilocarcinus laevifrons* (Trichodactylidae), and two marine crabs *Pachygrapsus marmoratus* (Grapsidae) and *Carcinus maenas* (Portunidae). A number of authors (Martin and Davis, 2001; Rodriguez, 1992; Von Sternberg, 1997; Von Sternberg et al., 1999; Von Sternberg and Cumberlidge, 2003) have suggested that Trichodactylidae is sister to Portunidae while the remainder of the three freshwater super families are potentially monophyletic, hence Trichodactylidae along with two additional marine taxa were used as outgroups.

DNA was extracted from leg muscle or gill tissue using the Qiagen DNeasy tissue kit followed by polymerase chain reaction (PCR) with the following primer pairs; 16S rRNA (Cunningham et al., 1992); 12S rRNA (Kocher et al., 1989), cytochrome *c* oxidase subunit I—COI (Folmer et al., 1994), cytochrome *c* oxidase subunit II—COII (Simon et al., 1994), histone three (H3) (Colgan et al., 1998), and 18S rRNA (Whiting et al., 1997). The DNA was denatured for 94 °C for 5 min, followed by 40 cycles at 94 °C for 40 s, annealing temperatures between 48 and 42 °C for 45 s and an extension at 72 °C for 1 min, with a final extension phase at 72 °C for 10 min. PCR products were gel purified with a Qiagen PCR gel purification kit followed by direct sequencing on an ABI 3730 XL automated sequencer. DNA sequences for all six loci have the following GenBank Accession Nos., 12S rRNA (AY803490–AY803529, AY919073–AY919085), 16S rRNA (AY803530–AY803567, AY919086–AY919095), COI (AY803568–AY803593, AY919108–AY919116), COII (AY803594–AY803635, AY919117–AY919125), 18S rRNA (AY803636–AY803672, AY919096–AY919107), and H3 (AY803673–AY803723, AY919126–138).

Table 1
List of voucher specimens of freshwater crab taxa sequenced in the present study and collection localities

Species	Museum catalogue number	Locality
Johora tiomanensis	ZRC 1999.0899	Malaysia
Isolapotamon consobrinum	ZRC 2000.2215	Borneo
Geothelphusa albogilva	ZRC 1999.0202	Taiwan
Celonthelphusa rugosa	ZRC 1998.606	Sri Lanka
Parathelphusa maculata	ZRC 2000.2110	Singapore
Sayamia sexpunctata	ZRC 2001.1101	Malaysia
Phricotelphusa limula	ZRC 2005.107	Thailand
Skelosophusa eumeces	FMNH 11059	Madagascar
Hydrothelphusa bombetokensis	FMNH 6878	Madagascar
Marojejy longimerus	FMNH 4656	Madagascar
Madagapotamon humberti	FMNH 11049	Madagascar
Hydrothelphusa goudoti	FMNH 4652	Madagascar
Hydrothelphusa madagascariensis	FMNH 7591	Madagascar
Foza raimundi	FMNH 7438	Madagascar
Gecarcinus jaquemonti	NMU A28.9.2004.1	India
Barythelphusa cunicularis 1	NMU B28.9.2004.1	India
Barythelphusa jaquemonti 1	NMU 06.10.2004.1	India
Barythelphusa jaquemonti 2	NMU 19.11.2004.1	India
Barythelphusa jaquemonti 3	Unaccessioned	India
Gubernatoriana gubernatorus	NMU 20.11.2004.1	India
Barythelphusa cunicularis 2	NMU 10.4.2004.1	India
Barythelphusa sp. 1	NMU 11.10.2004.1	India
Barythelphusa sp. 2	NMU 11.10.2004.1	India
Oziothelphusa senex	NMU 07.10.2004.1	India
Sartoriana spinigera	NMU 09.09.2003.1	India
Rouxana minima	NMU A21.09.2000.1	Papua New Guinea
Rouxana ingrami	NMU B21.09.2000.1	Papua New Guinea
Parathelphusa sp.	NMU 16.08.2003.1	Malaysia
Sudanonautes floweri	NMU 03.2004.1	Gabon
Potamonautes ecorssei	NMU 07.01.2003.1	Mali
Potamonautes platynotus	NMU 23.04.2003.6	Tanzania
Guinotia dentata	NMU 04.01.2004.1	West Indies
Potamonautes odhnerhi	NMU 14.07.2004	Kenya
Potamonautes new sp.	NMU 18.10.2003	Kenya
Deckenia imitatrix	NMU 1998.1	Kenya
Sudanonautes aubryi	NMU 23.04.1984A	Nigeria
Potamonemus sachi	NMU 09.04.1983	Cameroon
Liberonautes rubigimanus	NMU 18.04. 1989	Liberia
Seychellum alluaudi	MRAC 53.653	Seychelles
Socotra pseudocardisoma	NMU 10.1998.1	Socotra
Potamonautes raybouldi	ZMA Crust. De. 204684	Tanzania
Potamonautes lirrangensis	ZMA Crust. De. 204681	DRC
Potamonautes emini	ZMA Crust. De. 204680	DRC
Potamonautes niloticus	ZMA Crust. De. 204683	Uganda
Platythelphusa armata	ZMA Crust. De. 204685	Tanzania
Carcinus maenas	BYUKC 3044	Spain
Pachygrapsus marmoratus	BYUKC 3045	Spain
Dilocarcinus laevifrons	NMU 13.12.2003.1	Peru
Potamon fluviatilis	Unaccessioned	Italy

Zoological Reference Collection of the Raffles Museum of Biodiversity Research, National University of Singapore (ZRC); Field Museum of Natural History, Chicago (FMNH); Northern Michigan University, Marquette (NMU); Zoological Museum of Amsterdam, Crustacean Department (ZMA); KC, Brigham Young University, Utah (BYU); and Musée Royal de l'Afrique Central, Tervuren (MRAC). The 14 southern African *Potamonautes* species sequenced were not deposited in a museum collection and sequences were taken from GenBank for 12S, 16S, and COI mtDNA. The abbreviation DRC stands for Democratic Republic of the Congo.

2.3. Alignment and phylogenetic analysis

The three ribosomal gene sequences 16S rRNA, 12S rRNA mtDNA, and 18S rRNA (652 bp) were aligned using ClustalX (Thompson et al., 1997) and manually adjusted where mismatches were made, while the two protein coding loci COI and COII (COI—610 bp and COII—447 bp) as well as the nuclear markers histone three (H3) (301 bp) were aligned manually. Large insertion and deletions that could not be aligned with confidence for 12S and 16S rRNA mtDNA were deleted from the phylogenetic analysis, these resulted in two short sequences that were 272 and 352 bp, respectively. Phylogenetic trees were estimated with PAUP*4.0b10 (Swofford, 2002), using parsimony (MP) and maximum likelihood (ML) and with MrBayes 3.0b4 (Ronquist and Huelsenbeck, 2003) using Bayesian inferences coupled with Markov chain Monte Carlo techniques.

For the MP and ML analysis, heuristic searches with TBR branch swapping and 100 random additions of taxa were performed. Nodal support for MP was estimated by bootstrapping analyzing 10,000 pseudo-replicates of data sets, while due to computational constraints only 100 pseudo-replicates were performed for ML. For each Bayesian analysis 10 Markov chains were run, with each chain started from a random tree and running for six million generations and sampling from the chain every 5000 trees. In the combined Bayesian analyses, data sets were partitioned and independent models were selected for each gene using unlinked parameters. A 50% majority rule consensus tree was generated from the trees retained (after the burn-in trees were discarded), with posterior probabilities for each node estimated by the percentage of time the node was recovered. Convergence and mixing were monitored using the program Tracer v1.2 (Rambaut and Drummond, 2004). For the ML and Bayesian analyses, MODELTEST (Posada and Crandall, 1998) was used to find the appropriate substitution model of evolution for each gene and the combined data set. The best-fit maximum likelihood score was chosen using the Akaike Information Criterion (AIC) since this reduced the amount of unnecessary parameters that contribute little to describing the data by penalizing more complex models (Bernham and Anderson, 2002; Nylander et al., 2004). The Shimodaira and Hasegawa (1999) test and posterior probability as implemented in PAUP*4.0b10 (Swofford, 2002) was used to test the traditional taxonomic designations (comparing the unconstrained tree to the null hypothesis).

2.4. Divergence time estimation

Divergence times were estimated under the multi-locus Bayesian method of Thorne et al. (1998) and Thorne and Kishino (2002), as implemented in the multidivtime package (http://statgen.ncsu.edu/thorne/multidivtime.html), and the likelihood heuristic rate-smoothing algorithm of Yang (1997), as implemented in PAML3.14b3 (Yang, 1997; Yang and Yoder, 2003). The posterior distributions were approx-imated twice under the Bayesian approach and included a burn-in period of 5×10^5 steps, after which 10^6 samples were collected every 100 accepted states. The similarity of these two approximations indicated that the Markov chains successfully converged. Likelihood estimations were performed also twice using small values of 1e−6 and 0.5e−6 in the difference approximation of derivatives and similar results were also encountered. Three independent calibrations were used specifying upper and lower bounds for the Bayesian approach and fixed ages for the likelihood approach. A detailed discussion of the method is outlined in Yang and Yoder (2003) and used by Nagy et al. (2003), Porter et al. (2005), Vences et al. (2003), Yang (2004), and Yoder et al. (2003). Calibration point 1 (on Fig. 1) represents the maximum age estimation of the *Potamon* fossil from the upper Miocene (24 Mya), calibration point 2 (on Fig. 1) represents the geological age of the Seychelles Island at 70 Mya, while calibration point 3 (on Fig. 1) represents the *Potamonautes niloticus* fossil from six My old sediments from the lower Miocene of Kenya. The two fossil calibration points were set as the lower limit, while the geological age of the Seychelles was used as the upper limit in the divergence time estimations.

3. Results and discussion

Phylogenetic analysis performed on the mitochondrial and nuclear genes prior to combination into a single data matrix recovered highly congruent topologies (independent mt and nDNA tree topologies not shown). For MP, 956 parsimony informative characters were present that recovered a single tree with a tree length of 7346 steps, with a consistency index (CI) of 0.24 and a retention index (RI) of 0.47. The MP topology was identical to the ML and Bayesian topologies. For the combined data set the substitution model GTR (General Time-Reversible) + I (proportion of invariable sites = 0.4587) + Γ (gamma distribution shape parameter = 0.6915) had the following parameters, base frequencies A = 0.3378, C = 0.1402, G = 0.2017, and T = 0.3203, the rate matrix R (a) [A–C] = 0.4722, R (b) [A–G] = 6.2458, R (c) [A–T] = 1.5699, R (d) [C–G] = 1.5106, R (e) [C–T] = 2.5940, and R (f) [G–T] = 1.00.

Results derived from several phylogenetic methods (parsimony, maximum likelihood, and Bayesian inference) (Fig. 1) demonstrate that the Afrotropical freshwater crabs samples are monophyletic, based on our taxon sampling. However, none of the Afrotropical and Oriental families formed natural groups, suggesting the artificial nature of the current taxonomic designations. For example, the monophyletic Malagasy Potamonautidae formed a distinct clade from the African Potamonautidae while the East African Platythelphusidae was deeply nested within the African Potamonautidae. We did not recover a sister taxon relationship between the Seychellian and Indian freshwater crabs despite their present assignment to the same family (Gecarcinucidae); that is further incongruent with a Gondwanan fragmentation hypothesis. Instead, the Seychelles
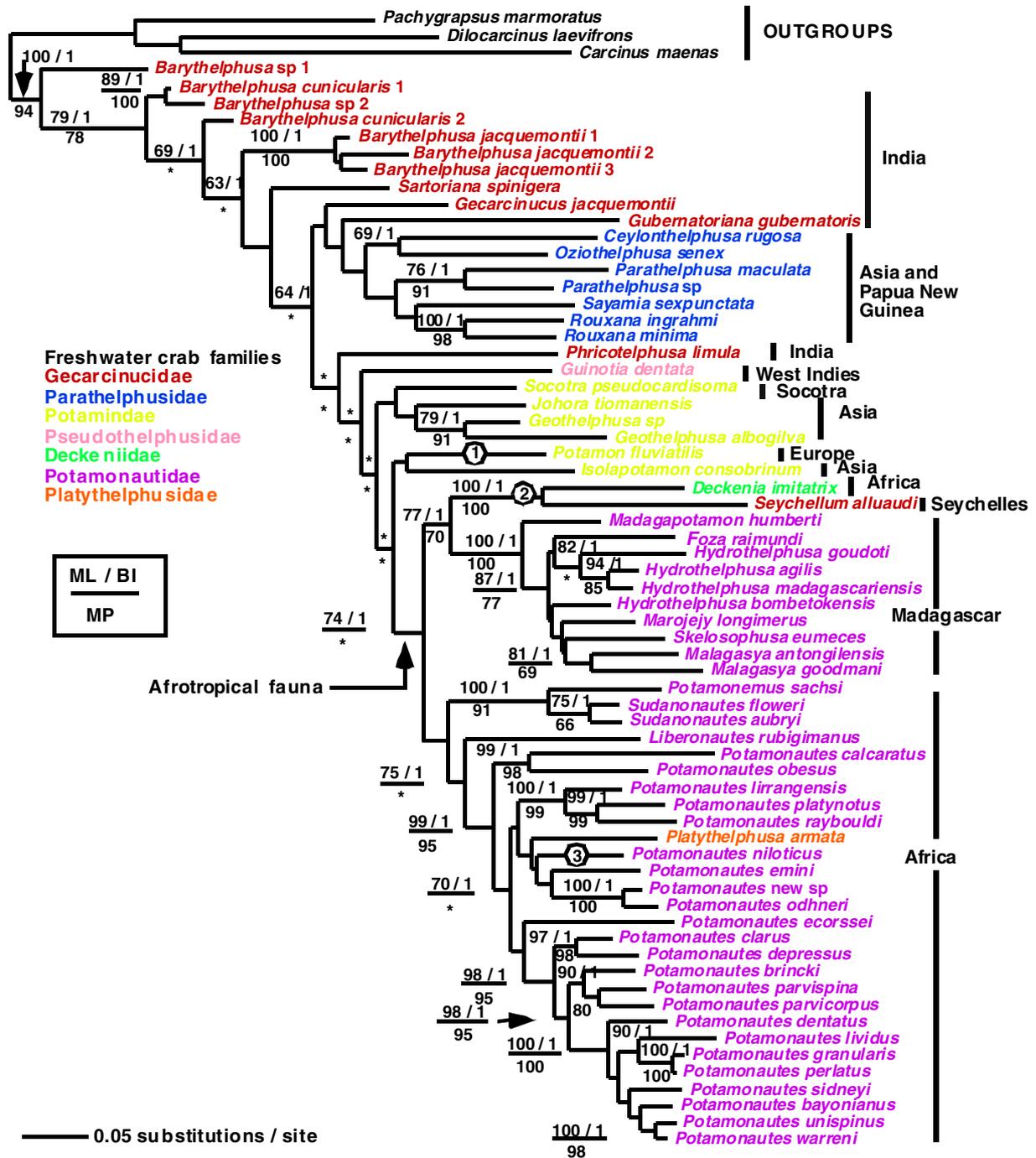
Fig. 1. A ML topology derived from the model GTR + I + Γ, with a likelihood score of 36685.95. The tree was rooted using three outgroups (*Dilocarcinus laevifrons*, *Pachygrapsus marmoratus*, and *Carcinus maenas*). The values above each node represent the bootstrap values for ML and posterior probability for Bayesian inferences respectively, while the values below each node represent the MP bootstrap values. Nodes marked with an asterisk had bootstrap values of <60% and posterior probabilities of <0.95.

taxon was closely related to an East African family (Deckiniidae), and formed a clade sister to the Malagasy freshwater crabs. Among the Indian and Asian Gecarcinucidae and Parathelphusidae we further found no evidence of distinct family groups. The estimated phylogram (Fig. 1) is completely irreconcilable with the expected area cladogram of continental fragmentation. Enforcing a strict Gondwanan fragmentation area cladogram consistently recovered a topology that was statistically significantly worse, and can

be rejected with confidence (Table 2) given these data. The evolutionary distinctiveness of the Afrotropical families from the Neotropical (South American and Meso-American) and the Oriental and Australasian families provides further evidence for a post-Gondwanan evolution of these freshwater crabs. A post-Gondwanan origin of Afrotropical freshwater crabs is further supported by the shallow corrected sequence divergence values between the African and Malagasy freshwater crab families. Divergence time

Table 2
Results from constraining phylogenetic relationships derived from the combined tree topology to test relationships between families and Gondwana distributions

Topological constraint	−1nL	Δ−1nL	P	pP
Unconstrained topology	35,892.17			
Monophyly of the African and Malagasy				
Potamonautidae	35,970	78.43	0.001	0.001
Monophyly of the Seychelles and Indian				
Gecarcinucidae	36,158.21	266.04	0.001	0.001

All values are significantly different from the unconstrained topology.

estimations, derived from the combined sequence data using a relaxed molecular clock and various biogeographic calibration points as well as fossil data and likelihood approaches provide further evidence for a recent, post Cretaceous evolution of freshwater crabs. Our divergence time estimations indicate that the radiation of the Afrotropical (African, Malagasy, and Seychelles fauna) freshwater crabs occurred ~78.6–75.03 Mya (Fig. 2). This geological period is characterized by the complete isolation of both Madagascar and the Seychelles from the African continent by substantial oceanic barriers that would

require transoceanic dispersal by freshwater crabs. Nevertheless, it is important to note that there are large standard deviations associated with these dates and many underlying assumptions (Graur and Martin, 2004). Moreover, the divergence dates are highly reliant on the calibration points included, and may therefore be markedly different when alternative reference points are used, however, a recent study by Porter et al. (2005) suggests that divergence time estimations using decapod fossils performed well with as few as one upper limit and a single deep limit calibration point.

The origin of the group must predate its earliest fossils and the present sparse fossil record for the freshwater crabs further complicates such estimates. Nevertheless, these results combined with the resulting phylogeny strongly contradict the long assumed ancient Gondwanan cladogenesis for freshwater crab families.

Contemporary freshwater crabs exhibit remarkable levels of drainage specificity (philopatry) however they are capable of limited long distance dispersal among aquatic terrestrial ecosystems (Daniels, 2003; Daniels et al., in press). This potentially renders freshwater crabs capable of rafting in hollow tree trunks under conditions of high humidity and rainfall. Transoceanic rafting, although
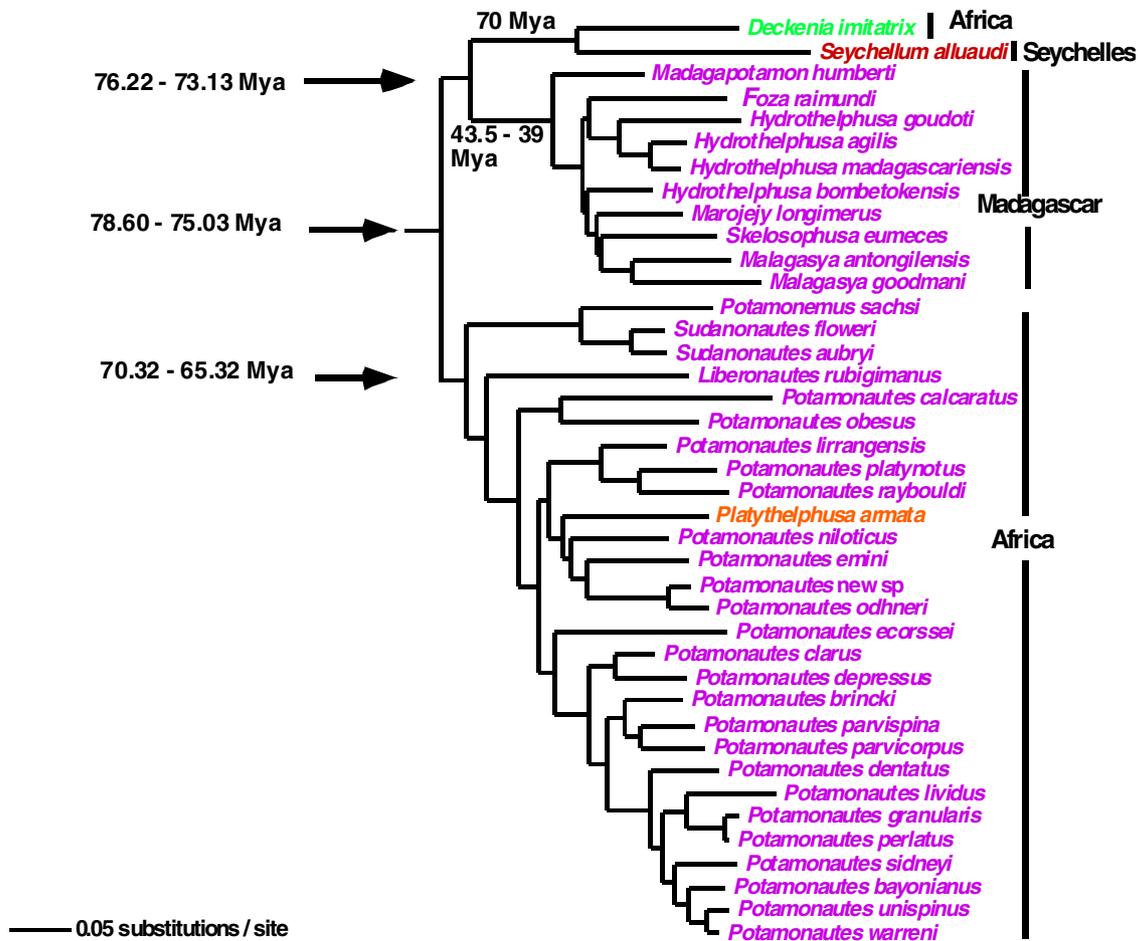

Fig. 2. The ML model based topology for the Afrotropical freshwater crab fauna with the divergence date estimations plotted onto each of the major nodes demonstrating when major cladogenetic events occurred.

apparently common among numerous vertebrates such as reptiles and mammal taxa between African and Madagascar (Raxworthy et al., 2002; Yoder et al., 2003) is limited, but nevertheless possible for freshwater invertebrates. For example, a recent study by Page et al. (2005) on the freshwater shrimp genus *Paratya*, suggests transoceanic dispersal to explain the biogeographic distribution of taxa. Noticeably, this genus is amphidromous and possesses planktonic larval phases that spend a certain amount of time in the marine environment. In addition certain adult *Paratya* species survive well in full strength seawater. Similarly, Murphy and Austin (2005) recently reported transoceanic dispersal for the freshwater prawn *Macrobrachium*. Physiologically, freshwater crabs such as *Potamonautes warreni* are known to withstand 14 parts per thousand seawater rendering them able to deal with limited exposure to saline environments (Morris and van Aard, 1998). It is likely that the ancestral freshwater crabs may have been able to withstand higher salinities compared with contemporary forms, since they were more closely related to marine taxa. The current distribution of freshwater crabs is more likely the result of the invasion of a marine ancestral group with a globally or circumtropical distribution that has since gone extinct, since these crabs cannot be allied to any living marine decapod families. It is widely believed that no marine crabs are closely allied to freshwater crabs and a comprehensive phylogeny for all brachyuran decapod crabs are lacking at this point in time. Alternatively, an older vicariance divergent event, prior to continental drift may explain the contemporary distribution of freshwater crabs. Our data cannot discern among any of these hypotheses.

The global absence of freshwater crabs from oceanic islands probably reflects the geologically recent age of these landmasses since the oldest volcanic island in the Afrotropical region adjacent to the African continent is less than 10 Myr old. For example, the islands in the Comores (Moheli and Grand Comoro, respectively) are between 0.13 and 5.4 Myr, while the estimated ages of the Mascarene Islands (Reunion, Mauritius, and Rodrigues) are 2.1, 8, and 10 million years for the three islands, respectively (McDougall and Chamalaun, 2001). Most of these oceanic or volcanic islands contain limited freshwater and have experienced several marine transgressions that would potentially have exterminated freshwater crab taxa. It is most probable that the ancestor of freshwater crabs became extinct prior to the development of these islands (because no extant marine crabs are thought closely related to freshwater crabs (Von Sternberg et al., 1999)), while true freshwater crabs have evolved recently and are capable of limited navigating of marine barriers, hence their absence from oceanic islands. Fossil evidence demonstrates the presence of the extant *Potamonautes niloticus* from the late Miocene, 6 Myr ago from Lake Albert in Uganda, while *Potamon* fossils from southern Europe, Africa and East Asia are known from the upper Miocene (Glaessner, 1969; Carriol and Secretan, 1992). The fossil record for the entire Neotropical region is younger than 4000 years (Rodriguez, 1986) providing

further corroborative evidence for the historically recent cladogenesis of the group.

Convergent morphological features, particularly those characters associated with the terminal segment of the mandibular palp generally used in freshwater crab classification at the family level is clearly an obstacle in its taxonomy that is likely to be mirrored in family level groupings in a number of brachyuran decapod crustaceans suggesting considerable taxonomic inaccuracies (Bossuyt et al., 2004; Martin and Davis, 2001). Novel morphological characters as well as a reinterpretation of existing taxonomic characters are required to accurately reflect family level relationships. A cautionary approach should be undertaken where biogeographic similarities derived from inaccurate taxonomy is inferred, underpinning the importance of coupling taxonomic practices with rigorous phylogenetic studies.

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