

Short communication

Molecular evidence for recent divergence of Lake Tanganyika endemic crabs (Decapoda: Platythelphusidae)

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1. Introduction

The East African Rift lakes are renowned as hotspots of endemism and as exemplary settings for studies on processes that generate biodiversity (Lowe-McConnell, 2003 and references therein). The cichlids in the East African lakes have received considerable scientific attention, leading to an increase of our understanding of the phylogenetic relationships of these fish, as well as of the mechanisms underlying the rapid radiations of their species flocks (Kocher, 2004; Kornfield and Smith, 2000). Species relationships and evolutionary processes in other endemic species-rich groups in Africa's Rift lakes, however, are less well studied and remain poorly understood (but see Martens, 1994; Michel, 2000; West and Michel, 2000; Wilson et al., 2004).

Lake Tanganyika is the only lake in the world that harbors a decapod crustacean radiation (Martens and Schön, 1999). The lake and its catchment are a hotspot of freshwater crab biodiversity, representing 44% of the species known from East Africa (N. Cumberlidge, personal communication). Of the 10 species of freshwater crabs endemic to Lake Tanganyika, nine are in the family Platythelphusidae (Cumberlidge et al., 1999; Marijnissen et al., 2004) and one, *Potamonautes platynotus* (Cunnington, 1907), is a member of the widespread African family Potamonautidae. The genus *Platythelphusa* shows extensive morphological disparity, which is especially striking compared to other African crab genera (*Potamonautes*, *Sudanonautes*) that

occupy much broader geographical ranges but show limited morphological differentiation (e.g., Bott, 1955; Cumberlidge, 1999). Several species of *Platythelphusa* exhibit morphological characters that are considered atypical for freshwater crabs, but are instead reminiscent of marine ancestry (von Sternberg and Cumberlidge, 1999). These unusual morphological features prompted the suggestion that platyhelphusids are among the most primitive of the extant species of African freshwater crabs. It was posited that they were able to retain a suite of plesiomorphic characters due to favorable marine-like environmental conditions in Lake Tanganyika (Bott, 1955; Cunnington, 1899; Moore, 1903). Alternatively, it was suggested that convergent evolution caused the unusual appearance of platyhelphusids (Cunnington, 1920; von Sternberg and Cumberlidge, 1999). These two hypotheses lead to fundamentally different predictions regarding (a) the phylogenetic placement of platyhelphusids with respect to other freshwater crabs and (b) the position of the most derived platyhelphusid within its own clade.

So far, only morphological characters have been used to infer evolutionary relationships of the platyhelphusid crabs (von Sternberg and Cumberlidge, 1999). Recent genetic analyses, however, indicate that the external morphological characters previously used in studies of African freshwater crabs are of limited power in resolving phylogenetic relationships (Daniels et al., 2002, unpublished data). Here, we use two mitochondrial markers (12S rRNA and 16S rRNA) to establish a genetic framework to test hypotheses on the origin of the morphological disparity and species diversity of Lake Tanganyika's endemic crabs.

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2. Materials and methods

Platythelphusid crabs were collected from 11 localities along the Tanzanian and Zambian shoreline of Lake Tanganyika between 2001 and 2003 (Table 1). We included representatives for eight of the nine species of the endemic Tanganyikan genus *Platythelphusa*. For seven of these we included multiple representatives, however a single individual of *Platythelphusa praelongata* was available (see Marijnissen et al., 2004). Extensive efforts to collect *Platythelphusa polita* were unsuccessful at the sites surveyed. Crabs were collected either by hand through SCUBA diving, or obtained from local fishermen (*Platythelphusa tuberculata*, *P. praelongata*, and outgroup taxa), and preserved in 95% ethanol. Vouchers were deposited at the Zoological Museum Amsterdam (ZMA De. 204594–96, De. 204686–95). Sequences from six mitochondrial and nuclear genes indicated that *Platythelphusa* nests robustly within East African potamonautid crabs (Daniels et al., unpublished data), so we included a range of potamonautids for outgroup sampling: the Tanganyikan endemic *P. platynotus*, the East African representatives *P. emini*, *P. lirrangensis*,

and *P. niloticus* and three species from southern Africa, *P. brincki*, *P. clarus*, and *P. depressus* (Table 1).

Total genomic DNA was extracted from muscle tissue of each crab, following standard procedures outlined by Daniels et al. (2002). Two primer sets, 12S rRNA (Kocher et al., 1989) and 16S rRNA (Palumbi et al., 1991), were used to amplify each of the two mitochondrial gene regions using standard polymerase chain reaction (PCR) methods. Individual 25 µl PCR contained 14.9 µl millipore water, 3 µl of 25 mM MgCl₂, 2.5 µl of 10× Mg²⁺ free buffer, 0.5 µl of a 10 mM dNTP solution, and 0.5 µl of each primer set (at 10 µM each), 0.1 µl of Taq polymerase, and 1 µl template DNA. PCR conditions were as follows: 3 min at 95 °C, then 32 cycles of 95 °C for 35 s, 48 °C for 45 s, 72 °C for 40 s, followed by 48 °C for 5 min, 72 °C for 10 min, and 20 °C for 10 min. PCR products were purified using a QIAquick PCR purification kit (Qiagen), then cycle sequenced following standard protocols (3 µl purified PCR product, 4 µl ABI PRISM fluorescent dye terminators, 3 µl of a 1 µM of primer solution), followed by analysis on an ABI 3100 automated DNA sequencer.

Table 1

Specimens of *Platythelphusa* and *Potamonautes* included in the phylogenetic analyses, localities where crabs were collected, and GenBank Accession numbers

Species	Code	Locality	Latitude, longitude	12S	16S	
<i>Platythelphusa armata</i> (A. Milne-Edwards, 1887)	JKB	Jakobsen	LT, TZ	4°54.87'S, 29°35.85'E	DQ203187	DQ203213
<i>Platythelphusa armata</i>	MBT	Mbita	LT, ZM	8°45.23'S, 31°05.14'E	DQ203188	DQ203214
<i>Platythelphusa armata</i>	UJJ	Ujiji	LT, TZ	4°58.00'S, 29°41.82'E	DQ203189	DQ203215
<i>Platythelphusa conculcata</i> (Cunnington, 1907)	HTP	Hilltop	LT, TZ	4°53.20'S, 29°36.90'E	DQ203190	DQ203216
<i>Platythelphusa conculcata</i>	JKB	Jakobsen	LT, TZ	4°54.73'S, 29°35.90'E	DQ203191	DQ203217
<i>Platythelphusa conculcata</i>	KIG	Kigoma	LT, TZ	4°53'21'S, 29°37.21'E	DQ203192	DQ203218
<i>Platythelphusa denticulata</i> (Capart, 1952)	KAB	Kabwe	LT, TZ	7°01.60'S, 30°33.00'E ^a	DQ203194	DQ203220
<i>Platythelphusa denticulata</i>	MZG	Mzungu	LT, TZ	4°55.05'S, 29°35.73'E	DQ203193	DQ203219
<i>Platythelphusa echinata</i> (Capart, 1952)	HTP	Hilltop	LT, TZ	4°53.45'S, 29°35.80'E	DQ203196	DQ203222
<i>Platythelphusa echinata</i>	MPL	Mpulungu	LT, ZM	N.A.	DQ203197	DQ203223
<i>Platythelphusa echinata</i>	UJJ	Ujiji	LT, TZ	4°58.75'S, 29°43.27'E	DQ203195	DQ203221
<i>Platythelphusa immaculata</i> (Marijnissen et al., 2004)	JKB	Jakobsen	LT, TZ	4°54.73'S, 29°35.90'E	DQ203199	DQ203225
<i>Platythelphusa immaculata</i>	KTB	Katabe	LT, ZM	4°54.21'S, 29°35.67'E	DQ203200	DQ203226
<i>Platythelphusa immaculata</i>	MBT	Mbita	LT, TZ	8°45.23'S, 31°05.14'E	DQ203198	DQ203224
<i>Platythelphusa maculata</i> (Cunnington, 1899)	KAS	Kasanga	LT, TZ	8°28.00'S, 31°08.60'E ^a	DQ203201	DQ203227
<i>Platythelphusa maculata</i>	KMJ	Kangamoja	LT, TZ	4°57.92'S, 29°41.20'E	DQ203202	DQ203228
<i>Platythelphusa maculata</i>	MPL	Mpulungu	LT, ZM	8°45.99'S, 31°06.40'E	DQ203203	DQ203229
<i>Platythelphusa praelongata</i> (Marijnissen et al., 2004)	MPL	Mpulungu	LT, ZM	8°45.22'S, 31°05.14'E	DQ203204	DQ203230
<i>Platythelphusa tuberculata</i> (Capart, 1952)	UJJ	Ujiji	LT, TZ	4°54.20'S, 29°30.00'E ^a	DQ203206	DQ203232
<i>Platythelphusa tuberculata</i>	MBT	Mbita	LT, ZM	8°44.91'S, 31°05.34'E	DQ203205	DQ203231
<i>Potamonautes emini</i> (Hilgendorf, 1892)	GMB	Gombe	LT, TZ	4°38.15'S, 29°37.81'E	DQ203207	DQ203233
<i>Potamonautes emini</i>	KIV	Ruzizi	LK, DC	N.A.	DQ203208	DQ203234
<i>Potamonautes niloticus</i> (H. Milne-Edwards, 1837)	SRD	N.A.	N.A.	N.A.	AY803496	AY803536
<i>Potamonautes lirrangensis</i> (Rathbun, 1904)	KIV	Ruzizi	LK, DC	N.A.	DQ203210	DQ203236
<i>Potamonautes lirrangensis</i>	MAL	Thumbi West	LM, MW	N.A.	DQ203209	DQ203235
<i>Potamonautes lirrangensis</i>	ZAM	Uazua	LT, ZM	N.A.	DQ203211	DQ203237
<i>Potamonautes platynotus</i> (Cunnington, 1907)	KAL	Kalemie	LT, DC	5°55.60'S, 29°11.60'E ^a	DQ203212	DQ203238
<i>Potamonautes clarus</i> (Gouws et al., 2000)	OLI	Oliviershoekpas	KZ, SA	N.A. ^b	AY042320	AY042241
<i>Potamonautes brincki</i> (Bott, 1960)	FER	Fernkloof	WC, SA	N.A. ^b	AY042322	AY042244
<i>Potamonautes depressus</i> (Krauss, 1843)	COL	Coleford	KZ, SA	N.A. ^b	AY042325	AY042247

DC, Democratic Republic Congo; KZ, Kwa Zulu Natal; LK, Lake Kivu; LM, Lake Malawi; LT, Lake Tanganyika; MW, Malawi; SA, South Africa; TZ, Tanzania; WC, Western Cape; ZM, Zambia; SRD, sequences provided by Savel Daniels; N.A., not available.

^a Indicates location not verified with GPS.

^b Samples from Daniels et al. (2002).

Sequences were aligned in CLUSTALX 1.81 (Thompson et al., 1997) under default settings and optimized manually (Page and Holmes, 1998). Although this methodology is widely used, it should be noted that it has received criticism due to its sensitivity to arbitrary selection of alignment parameters (e.g., Giribet, 2003, and references therein). Regions that could not be unambiguously aligned were identified, and the effect of omitting those from the analyses was tested. A partition homogeneity test was carried out in PAUP* 4b10 (Swofford, 2002) to test for congruence among the genes (Farris et al., 1994). MODELTEST 3.06 (Possada and Crandall, 1998) was used to determine the best-fit model of sequence evolution under the Akaike information criterion (AIC). Phylogenetic analyses were reconstructed using maximum likelihood and parsimony procedures in PAUP* 4b10 under default settings. Maximum likelihood and parsimony analyses were performed using heuristic searches and TBR branch swapping with 10 random additions. Bootstrap support was calculated over 100 and 1000 permutations for maximum likelihood and parsimony, respectively. Bayesian trees were inferred using MrBayes 3.0b4 (Ronquist and Huelsenbeck, 2003). Bayesian inference of phylogeny (BI) was performed using eight Markov chains, 10 million generations with a burn-in of 10%. The posterior distributions were approximated three times under the Bayesian approach, to determine successful convergence of the Markov chains. All the analyses above were performed on the separate data sets of 12S and 16S rRNA, as well as on the combined data set. To obtain an approximation of the relative timeframe of platythelphusid diversification a molecular clock was applied to the 16S rRNA sequence data set. A likelihood ratio test was carried out to the 16S rRNA data in PAUP* 4b10 prior to performing molecular clock inferences. Divergence time estimates were obtained by applying a rate of 0.0032–0.0045 substitutions per site per lineage per million years to corrected divergence values. This rate corresponds to estimates obtained for taxa in the marine crab genera *Sesarma* and *Uca*, which range from $\approx 0.65\%$ to $\approx 0.9\%$ pairwise sequence divergence per million years (Ma) for 16S rRNA (Schubart et al., 1998a,b; Sturmbauer et al., 1996).

3. Results

Both 12S rRNA and 16S rRNA exhibited heterogeneity in sequence variation. Exclusion of regions of ambiguous alignments, which were primarily between ingroup and outgroup taxa, resulted in no loss of ingroup phylogenetic signal. The combined 12S and 16S rRNA sequence data included 754 base pairs, with 127 variables and 66 parsimony informative sites. The results from the partition-homogeneity test were not significant, and thus the 12S and 16S rRNA genes were combined in the analyses. The best-fit maximum likelihood model was chosen using the AIC. The TVM+I maximum likelihood model was selected for 12S rRNA, and GTR+I for 16S rRNA, whereas the GTR+I+G model was selected for the combined data set.

Similarity of log likelihood values after burn-in, indicated that the Markov chains successfully reached convergence during all three Bayesian runs.

The platythelphusids from Lake Tanganyika form a well supported clade in all analyses (Fig. 1). *Potamonautes emini* and a clade consisting of *P. niloticus*, the Tanganyikan endemic *P. platynotus* and *P. lirrangensis* were placed sister to the Platythelphusidae. Average 12S rRNA and 16S rRNA sequence divergence (uncorrected *p*-distances) between *Platythelphusa* individuals was 1.21% (range 0.4–2.7%), 8.94% (range 0.1–11.5%) within the potamonautid outgroup, and the minimum divergence between the ingroup and outgroup was 7.56%. The hypothesis of a molecular clock was not rejected ($\chi^2 = 33.7$ *df* = 29 *P* > 0.05). Applying a molecular clock of 0.65–0.9% sequence divergence per Myr to the 16S rRNA sequence data suggests that the platythelphusid lineage separated from Potamonautidae approximately 9.0–6.5 Myr ago, while divergence of the platythelphusid clade is estimated to have taken place approximately 3.3–2.5 Myr ago (node C1 and C2, respectively in Fig. 1).

The combined 12S and 16S rRNA data set resolved some, but not all species relationships within Platythelphusidae (Fig. 1). The different phylogenetic reconstruction methods resulted in congruent topologies for the combined data set, however, separate analysis of the 12S and 16S rRNA sequences differ in the position of several individuals: (i) *P. armata* JKB (respectively unresolved, and sister to *P. armata* UJJ), (ii) *P. denticulata* MZG (in a clade with *P. maculata* KAB and *P. maculata* MPL, and sister to *P. maculata* KMJ), (iii) *P. maculata* KAS (in an unresolved clade with *P. armata* MBT, *P. armata* UJJ and *P. maculata* KMJ, and sister to *denticulata* KAB, and (iv) *P. immaculata* KTB (sister to *P. echinata* UJJ, and in a polytomy with *P. immaculata* and *P. conculcata*) (data not shown).

4. Discussion

The platythelphusids from Lake Tanganyika are the first example of a recent diversification among African freshwater crabs. The phylogenetic patterns recovered by our analyses of 12S and 16S rRNA mtDNA sequences reveal that Platythelphusidae is an unequivocal genetic clade, with surprisingly short internal branches. The endemic Tanganyikan potamonautid *P. platynotus* does not cluster with the platythelphusids, and instead it is sister to the widespread East African *P. lirrangensis*. The 12S and 16S rRNA mtDNA genes did not resolve phylogenetic patterns within Platythelphusidae well enough to confidently detect species level relationships. This was an unexpected result, because these mitochondrial markers have been successfully employed for resolving brachyuran crab species-level phylogenies (Bossuyt et al., 2004; Daniels et al., 2003; Schubart et al., 1998a). The limited sequence divergence (0.4–2.7%) between the platythelphusid taxa indicates that their lineages might have diverged too recently for the 12S rRNA and 16S rRNA

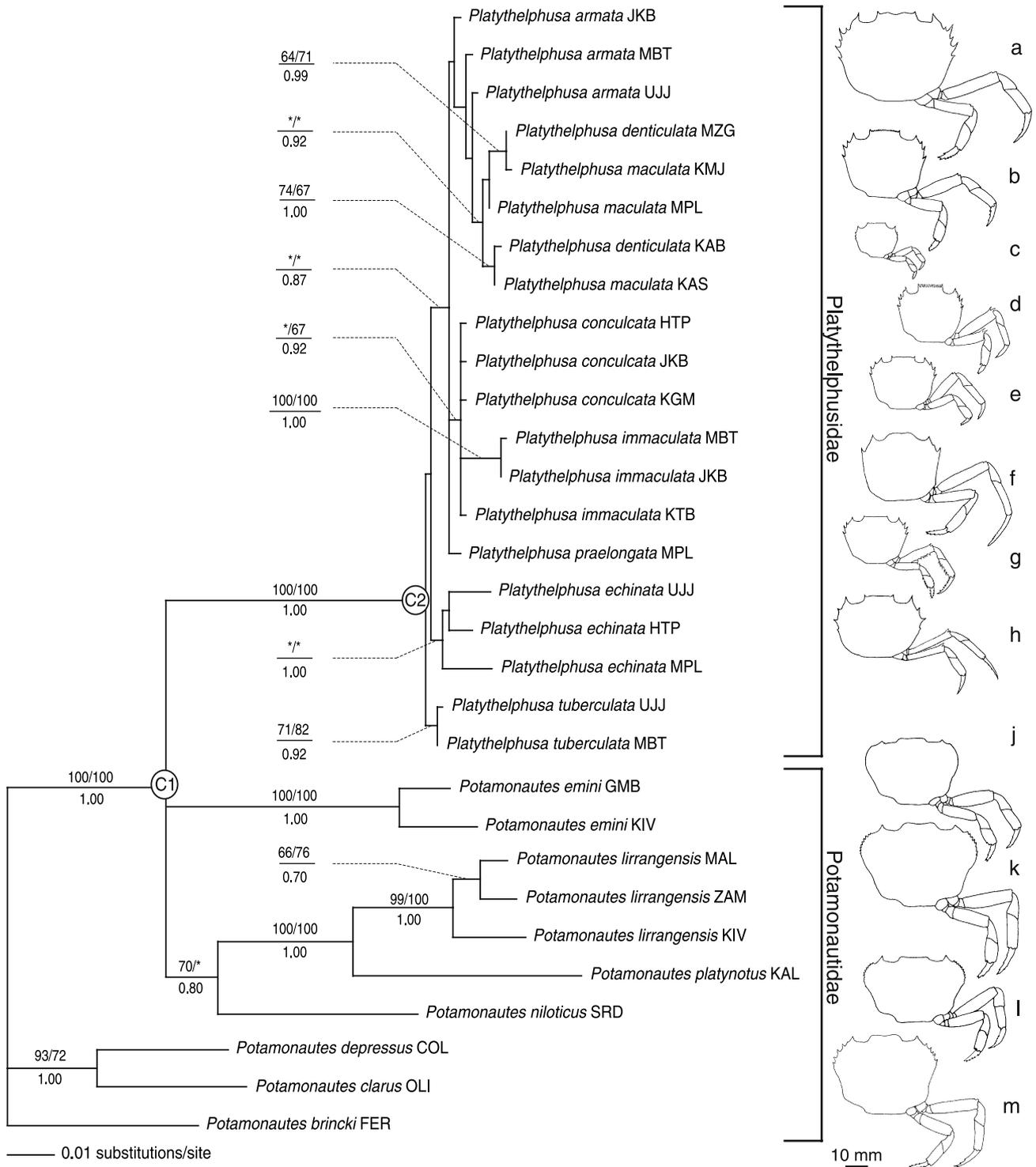


Fig. 1. Maximum likelihood tree of combined 12S and 16S rRNA mtDNA sequences, with branch lengths proportional to number of changes. Taxon labels indicate morphological species diagnosis and locality acronym (see Table 1). Numbers above nodes denote maximum likelihood and parsimony bootstrap support values, followed by Bayesian posterior probabilities. *Indicates support less than 60% in otherwise supported branches. Node C1 represents the origin of the platythelphusid lineage, estimated 9–6.5 Myr ago; C2 was estimated at 3.3–2.5 Myr (see Materials and methods). (a) *Platythelphusa armata*, (b) *P. denticulata*, (c) *P. maculata*, (d) *P. conculcata*, (e) *P. immaculata*, (f) *P. praelongata*, (g) *P. echinata*, (h) *P. tuberculata*, (j) *Potamonautes emini*, (k) *P. lirrangensis*, (l) *P. platynotus*, and (m) *P. niloticus*. a–h and l, endemic to Lake Tanganyika; j, k, and m, riverine species from eastern Africa.

to become fixed and provide sufficient phylogenetic signal, resulting in incongruence between the gene trees and the species tree (Neigel and Avise, 1986). Incomplete lineage sorting of ancestral polymorphisms is common in

newly evolved species, and has been reported recurrently in studies of the cichlid fish species flocks in the African rift lakes (Albertson et al., 1999; Moran and Kornfield, 1993; Takahashi et al., 2001).

A study of morphologically almost indistinguishable allopatric populations of *P. clarus* and *P. depressus* yielded several distinct clades, with 16S rRNA sequences divergences of 2.9–17.0% between lineages (Daniels et al., 2003). By applying a molecular clock of 0.65–0.9% pairwise divergence per Myr (Schubart et al., 1998b; Sturmbauer et al., 1996), the major cladogenetic events between *P. clarus* and *P. depressus* are estimated to have taken place between ≈ 8 –17 Myr ago, in the Miocene (recalculated from Daniels et al., 2003). Applying the same molecular clock to our data suggests that the divergence of the platythelphusid clade was initiated during the Pliocene. We present these dates for relative comparison only, and caution that substantial errors may be associated with vicariance dated molecular clocks. The molecular clocks that we used here were based on dating of the closure of the Panama land bridge of 3.1 Myr (Schubart et al., 1998b) and 3.0 Myr (Sturmbauer et al., 1996). Phylogenetic responses to closure of the Central American seaway have been shown to be unpredictable and often older than the commonly used 3–3.5 Myr geologic date for complete uplift of the Isthmus of Panama (e.g., Knowlton and Weight, 1998; Marko, 2002). Thus, the molecular clock for freshwater crabs is conservative, and other potential dates would provide an even younger time of divergence for the platythelphusid radiation. Evidently, the diversification of the endemic Tanganyikan clade occurred recently, and explanations for the morphological disparity of these crabs should be sought in situ.

The recent diversification (≈ 3.3 –2.5 Myr ago) of the platythelphusids underlines that the unusual morphologies of these crabs are not a reflection of retained plesiomorphies. This is corroborated by (a) cladistic analyses (von Sternberg and Cumberlidge, 1999), and recent analyses of six mitochondrial and nuclear genes (Daniels et al., unpublished), which place Platythelphusidae well within the African freshwater crabs, and (b) the placement of *Platythelphusa conculcata*, the species with the most ‘marine-like’ morphological characters (see von Sternberg and Cumberlidge, 1999), on short, internal branches instead of basal in the platythelphusid clade. There are several factors that can be causal to the morphological disparity of platythelphusids. Their diversification occurred in the context of the dynamic geological history of Lake Tanganyika (central basin initiation 9–12 Myr ago, three tectonic basins fused into single deep lake 5–6 Myr ago, followed by intermittent re-separation during episodes of dramatic water level fluctuations (Cohen et al., 1997; Scholz and Rosendahl, 1988; Tiercelin and Mondeguer, 1991)). This led to possibilities for speciation in allopatric populations on a range of geographic scales, ecological divergence in vacant niches and periods of potential secondary contact. The phylogenies of several cichlid lineages have been correlated with climatic events and fluctuations in lake level during the Pleistocene (Sturmbauer et al., 2001; Verheyen et al., 1996). Platythelphusids have markedly flattened carapaces, characteristic

for freshwater crab taxa that have adapted to a fully aquatic life (Cumberlidge, 1999, p. 279), and this could have resulted in limited dispersal between sub-basins during periods of lowest water levels. Future work with broader geographic sampling of crabs will allow testing the influence of physical barriers on Tanganyikan crab species boundaries.

Examples of ecological niche diversification among species of *Platythelphusa* provide clues to sympatric mechanisms that could be causal to their rapid radiation. For instance, several species exhibit clear habitat specificity. *P. maculata* has a small, rounded body and shows a preference for living in empty gastropod shells (Capart, 1952; Cunnington, 1899), whereas *P. tuberculata* shows affinity for deep lake floor habitats (Coulter, 1991; Marijnissen et al., 2004). *P. armata* is equipped with enlarged claws that were suggested to have coevolved with Lake Tanganyika’s heavily armoured gastropods (West et al., 1991). On the other hand, there is evidence of ecological niche overlap between morphologically distinctly different species (*P. conculcata*, *P. echinata*, and *P. immaculata*) that coexist in the same habitat (Marijnissen et al., unpublished data). Although the degree to which allopatric factors and sympatric speciation mechanisms have contributed to the diversification of platythelphusids remains speculative at present, these observations demonstrate that the Tanganyikan crabs show an unequivocal potential to serve as an invertebrate model system for studies of speciation in ancient lakes.

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