

Molecular diversity of freshwater crabs from Sulawesi and the sequential colonization of ancient lakes

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Abstract Ancient lakes represent one of the most stable freshwater environments on Earth, with a species richness clearly exceeding that of younger and more short-lived limnic habitats. In most cases, the biological colonization of old lake systems must have occurred via the surrounding rivers. Two ancient lake systems of Sulawesi (Malili lake system and Lake Poso) have been studied in terms of the taxonomy and phylogeny of freshwater crabs (Decapoda: Brachyura: Gecarcinucidae: Parathelphusinae). Both systems have been colonized twice independently, and in both systems we can find three trophic niches which are always occupied by different crab species: molluskivores, omnivores, and detritivores. In the present study, we reconstruct phylogenies of freshwater crabs from more than 20 river systems of Sulawesi. We thereby confirm two independent colonization events for both ancient lake systems, with subsequent radiations. The phylogenies imply that the lineages which

evolved into the molluskivore forms were the first ones to colonize, whereas omnivores and detritivores are derived from later colonization events and, based on their monophyletic relationship, resulted from minor lacustrine radiations. Most of the diversity of freshwater crabs from Sulawesi remains taxonomically undescribed. This study uncovers several undocumented phylogenetic units, with long independent evolutionary histories according to patristic distances.

Keywords Brachyura · Gecarcinucidae · *Parathelphusa* · Endemism · Sulawesi · River systems

Introduction

Southeast Asia is a well-known hotspot of biodiversity (Myers et al., 2000), with its insular part being subdivided into three regions: Sundaland, the Philippines, and Wallacea. Each of these regions shows a remarkably high degree of biodiversity (Sodhi et al., 2004). Within these areas, the freshwater systems have a high share of biodiversity and endemism (e.g., amphibians in Sodhi et al., 2004; Dudgeon et al., 2006). Therefore, animals that are restricted to freshwater habitats can be considered as good indicators for overall biodiversity and, conversely, we can assume to find high diversity of freshwater organisms within the hotspots of biodiversity in Southeast Asia. However, most of the studies on the biodiversity of Southeast

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Asia so far focused on terrestrial animals (Inger & Voris, 2001; Gorog et al., 2004; Steiper, 2006; Outlaw & Voelker, 2008; Meijaard, 2009; Blackburn et al., 2010; den Tex et al., 2010; Lim et al., 2010; Sheldon et al., 2010), and the aquatic diversity often still needs to be estimated and documented.

Freshwater crabs can be considered good zoogeographic indicators (Ng & Rodriguez, 1995). They are completely independent from the marine habitat in terms of their ontogeny (direct development), and they are probably restricted to their respective drainage system (e.g., Daniels et al., 2002b; Daniels, 2003), which leads to a high rate of endemism and, therefore, to high overall diversity. The freshwater crab fauna of Southeast Asia is of special interest, because of the insular habitats and past connections during lowered sea levels at the ice ages. Recently, Klaus et al. (2013) reconstructed the phylogenetic history and geographic origin of the crab genus *Parathelphusa* and related genera, postulating a center of origin on the island of Borneo and revealing high undocumented biodiversity. It also became evident that all representatives of Sulawesi are monophyletic, with inclusion of one ancestral lineage from southeast Borneo. But despite of these new insights and earlier studies by Schubart et al. (2008) and Schubart & Ng (2008), the phylogenetic history, phylogeographic structure, and genetic diversity of the freshwater crabs from Sulawesi remains mostly unknown.

The freshwater crabs of Sulawesi had evoked interest, due to the presence of different ecotypes and consequent sympatric diversity in the ancient lakes of the Central Plateau (Poso Lake and Malili lake system). These lake representatives belong to the genera *Parathelphusa*, *Nautilothelphusa*, *Migmathelphusa*, *Syntripsa*, and *Sundathelphusa* (see Chia & Ng, 2006), showing a high rate of lacustrine diversity within the relatively small area of these lake systems. This is paralleled by high diversity levels in freshwater shrimps (von Rintelen et al., 2010a, 2012), snails (von Rintelen et al., 2004, 2010b, 2012), and fishes (Herder et al., 2012a, b; von Rintelen et al., 2012), which have been shown to be the outcome of adaptive radiations. Previous studies on Asian freshwater crabs had shown that Sulawesian representatives of the genus *Sundathelphusa* belong to different phylogenetic lineages than the genus *Parathelphusa* (see Klaus et al., 2009, 2010), whereas *Nautilothelphusa*, *Migmathelphusa*, and *Syntripsa* are found to be phylogenetically

embedded within the genus *Parathelphusa* (see Schubart & Ng, 2008; Schubart et al., 2008). The latter authors reconstructed phylogenetic relationships of Sulawesi freshwater crabs, described a new species, and showed that colonization took place at least twice independently in both lake systems, with only minor subsequent radiations. However, only a small number of the overall freshwater drainage systems of Sulawesi and tributaries to the lakes were included in these studies. Thus, the real dimension of biodiversity that is to be expected within the freshwater crabs on Sulawesi and the potential colonization pathways must be considered in a larger framework. In a recent effort, we were able to obtain mtDNA sequences from freshwater crabs inhabiting more than 20 river systems of Sulawesi. This leads to a better insight into the real biodiversity of Sulawesi river systems. Although we are far from providing a complete picture of rivers from Sulawesi and small neighboring islands, due to the lack of available material, our aim is to show that the biodiversity in terms of evolutionary lineages is much higher than that currently recognized in the scientific literature (Bott, 1970). Currently, 13 species of *Parathelphusa* are considered valid for Sulawesi (Chia & Ng, 2006). Here, we examine and quantify the biodiversity in a number of freshwater systems on Sulawesi to clarify the evolutionary history and to estimate potential numbers of species of freshwater crabs on Sulawesi.

Materials and methods

Sampling and sequencing

Freshwater crabs were collected in Sulawesi during several expeditions between 2000 and 2008. They were preserved in ethanol and often walking legs were punctured with sterile needles for better alcohol penetration into the tissue. Many crabs were made available by Thomas and Kristina von Rintelen, Matthias Glaubrecht, and Fabian Herder. At the laboratories of the University of Regensburg, genomic DNA was isolated using a modified Puregene method (Gentra Systems, Minneapolis). In order to amplify the so-called “Folmer region” of the mitochondrial gene cytochrome oxidase subunit 1 (Cox1), polymerase chain reactions (PCR) were carried out with the primers LCO 1490 (GGT CAA CAA ATC ATA AGA TAT TGG) (Folmer et al., 1994), COL6b (ACA AAT

CAT AAA GAT ATY GG) and COH6 (TAD ACT TCD GGR TGD CCA AAR AAY CA) (Schubart & Huber, 2006; Schubart, 2009) and the following PCR profile: 40 cycles with 45 s 94°C, 1 min 48–50°C, and 1 min 72°C for denaturing, annealing, and extension, respectively. PCR products were purified with the Quick-Clean kit (Bioline) or Microcon filters (Millipore), cycle sequenced with the Big Dye Terminator v1.1 (AB Applied Biosystems) and analyzed with the automated capillary DNA sequencer ABI PRISM® 310 Genetic Analyzer (Applied Biosystems) (see also Schubart et al., 2008). In addition, 71 Cox1 sequences were obtained after sending tissue of samples to the Barcode of Life Database (BOLD) in Guelph (Canada) (see Ratnasingham & Hebert, 2007). All available Cox1 sequences of Sulawesi freshwater crabs originating from the previous studies on the relationship of the Gecarcinucidae or the colonization of the Sulawesi lakes were retrieved from GenBank (Schubart et al., 2008; Klaus et al., 2013: AB265250, AB601861, FM177599, FM177607–609, FM177611, FM177615–616, FM177618, FM177622, FM177625, FM177628–629, FM177635–643, GQ289661, GQ982588). Overall, this allowed aligning a total of 116 Cox1 sequences with a length of 658 base pairs using the software Bio-Edit (v.7.0.5.3) (Hall, 1999). Cox1 sequences from *Oziothelphusa hippocastanum* (GQ982588, Beenaerts

et al., 2010), *Sayamia cf. germaini* (AB265250, Shih et al., 2007), and *Siamthelphusa improvisa* (AB601861, Shih et al., 2011) were included as outgroups or reference for intergeneric genetic distances. The sampled geographic area of the ingroups covers more than 20 river systems from central and southern Sulawesi (Fig. 1). However, many river systems could not be included in this sampling and, therefore, the overall analysis remains incomplete.

Phylogenetic analyses

Two alignments, one for our complete set of sequences and one for all unique haplotypes from Sulawesi freshwater crabs of the genera *Parathelphusa*, *Nautilothelphusa*, *Migmatelphusa*, and *Syntripsa*, were converted with FaBox (Villesen, 2007) to Nexus files as input for Mr.Bayes (v. 3.2.1) (Huelsenbeck and Ronquist, 2001). The best evolutionary model was determined with MrModeltest (v.2.3) (Nylander, 2004) and MrMTgui (v.1.0) (Nuin, 2005) in PAUP (v.4.0b10) (Swofford, 2003). The evolutionary model that best described our data was selected with the Akaike information criterion (Posada & Buckley, 2004). For the Mr.Bayes run, we used 3 million generations with 4 chains (one heated) and a sample frequency of one tree per 1,000 generations.



Fig. 1 General map with sampling sites of the included specimens of freshwater crabs from Sulawesi

Patristic distances

Based on the smaller alignment (restricted to sequences of *Parathelphusa* and related genera from Sulawesi, i.e., *Parathelphusa* lineage), of 116 sequences, we used R (R Core Team, 2012) with the Ape package (v.3.0-6) (Paradis et al., 2004) to calculate patristic distances for each taxon pair along the final tree (Fig. 3). For determining an estimate of the number of potentially to be expected operational taxonomic units (OTUs), we initially applied a threshold of 0.16 as suggested in Lefébure et al. (2005).

Results

The alignment contained no indels or stop codons, making the inclusion of pseudogenes less likely. Analyzing the dataset with MrModeltest (Nylander, 2004) revealed GTR+G+I (Rodríguez et al., 1990) as the best fitting evolutionary model for the alignment containing only the *Parathelphusa* lineage and HKY+I+G (Hasegawa et al., 1985) as the best fitting evolutionary model for the alignment including *Sundathelphusa* and other outgroups. Therefore, these models were chosen for subsequent Bayesian inference analyses.

The resulting phylogenetic tree based on the larger alignment (Fig. 3) shows a deep early split between two clades, corresponding to *Sundathelphusa* on one hand, and *Parathelphusa* and allied genera on the other. As stated by Schubart & Ng (2008) and Klaus et al. (2013), the genera *Syntripsa*, *Migmathephusa*, and *Nautilothelphusa* are deeply nested within the genus *Parathelphusa*, which currently renders the genus paraphyletic (Figs. 3, 4). Hereafter, we will consider all these genera from Sulawesi as a monophyletic lineage and refer to it as the *Parathelphusa* lineage (see above).

The overall tree (Fig. 3) suggests an early colonization of Lake Poso by the genus *Sundathelphusa*, while the following colonization events of Lake Poso and the Malili lake system took place from within the *Parathelphusa* lineage more recently (Fig. 3, see also Schubart et al., 2008).

Even though there are only 14 valid species of *Parathelphusa* currently recognized for Sulawesi (see Chia & Ng, 2006), the clusters reflected in the topology of the *Parathelphusa* lineage phylogeny from Sulawesi (Fig. 4) and patristic distances along

this tree suggest a total potential number of up to 35 OTUs, if a threshold of 0.16 is applied as suggested by Lefébure et al. (2005).

The base of the tree for the *Parathelphusa* lineage (Fig. 4) is polytomous and formed by four big lineages. The first of these lineages consists of up to eight potential OTUs (Table 1, 1–6). Only two of them could be identified and connected to a valid species name, *Parathelphusa linduensis* Roux, 1904 (4b) and *P. lokaensis* De Man, 1892 (6). The second lineage contains three potential OTUs (Table 1, 7–9), and none of them could be assigned a valid species name.

The third lineage is the largest and can be subdivided into two clades and several groups. The first clade (10a–14) includes seven potential OTUs (Table 1, 10a–14). One of them could be identified as *Parathelphusa pareparensis* De Man, 1892 (13) whereas the others could not be assigned to one of the existing scientific names. The second clade within the third lineage contains three distinct groups. The first one (15a–24b) is formed by five OTUs (Table 1, 15a–24b) that are currently recognized as *Parathelphusa pallida* Schenkel, 1902 sensu lato (15a–17 and 23), a clade uniting *Parathelphusa ferruginea* Chia & Ng, 2006 and *Nautilothelphusa zimmeri* Balss, 1933 from lakes Towuti and Mahalona (19), a clade of *Nautilothelphusa zimmeri* from Lake Matano (20), an unnamed species of *Parathelphusa* from Lake Masapi (21), a clade of *Parathelphusa pantherina* Schenkel, 1902 (22), and two clades that could not be identified (24a and 24b) to species level. The second group consists of two clades (Table 1, 25–26) that could not be assigned to a scientific valid name (25 and 26). The third group is formed by four distinct clades (Table 1, 27–30), *Parathelphusa possoensis* Roux, 1904 (27), *Parathelphusa sarasinorum* Schenkel, 1902 (28), *Migmathephusa olivacea* Chia & Ng, 2006 (29), *Syntripsa matannensis* Chia & Ng, 2006, and *S. flavichela* Chia & Ng, 2006 (30). The fourth of the basal lineages of the *Parathelphusa* lineage on Sulawesi consists of three potential OTUs (Table 1, 31–33), of which only one could be linked to a species name, i.e., *Parathelphusa sorella* Chia & Ng, 2006 (33).

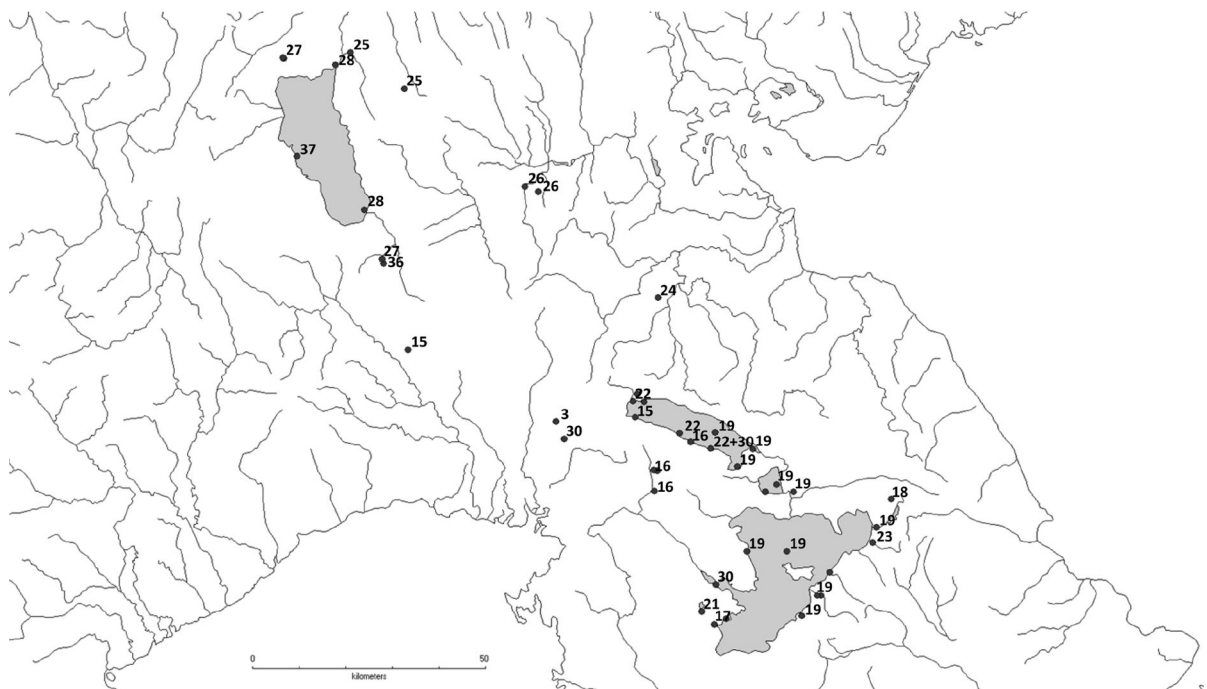
Unfortunately, for some samples (FM177635; R619-A06; R619-A07; R619-A10; R619-A11; R619-C01; R619-C02; R619-C03; R619-C04; R619-C07; R619-C08; R619-H10; R619-H11; Seb; Seb-1; Seb-3; ZRC2000.1681; ZRC2000.1684; ZRC2000.1695) we

Table 1 List of patristic distances between the closest relatives within the different clades according to the numbers in Figs. 3 and 4

1–6:												
	1	2	3a	3b	4a	4b	5	6				
1	0.0000	0.6471	0.8795	0.9323	1.0210	0.7792	1.2743	1.3384				
2	0.6471	0.0000	0.7537	0.8065	1.0348	0.7930	1.2881	1.3521				
3a	0.8795	0.7537	0.0000	0.2070	1.2671	1.0254	1.5205	1.5845				
3b	0.9323	0.8065	0.2070	0.0000	1.3199	1.0782	1.5733	1.6373				
4a	1.0210	1.0348	1.2671	1.3199	0.0000	0.6396	1.5387	1.6028				
4b	0.7792	0.7930	1.0254	1.0782	0.6396	0.0000	1.2970	1.3610				
5	1.2743	1.2881	1.5205	1.5733	1.5387	1.2970	0.0000	0.2989				
6	1.3384	1.3521	1.5845	1.6373	1.6028	1.3610	0.2989	0.0000				
7–9:												
			7			8			9			
7			0.0000			0.8761			0.9454			
8			0.8761			0.0000			1.0996			
9			0.9454			1.0996			0.0000			
10a–14:												
	10a	10b	11a	11b	12	13	14					
10a	0.0000	0.2347	0.2983	0.2433	0.3283	0.3041	0.3272					
10b	0.2347	0.0000	0.2914	0.2364	0.3214	0.2972	0.3203					
11a	0.2983	0.2914	0.0000	0.1299	0.2868	0.2626	0.2856					
11b	0.2433	0.2364	0.1299	0.0000	0.2317	0.2076	0.2306					
12	0.3283	0.3214	0.2868	0.2317	0.0000	0.2926	0.3156					
13	0.3041	0.2972	0.2626	0.2076	0.2926	0.0000	0.2915					
14	0.3272	0.3203	0.2856	0.2306	0.3156	0.2915	0.0000					
15a–24b:												
	15a	15b	16	17	18	19	20	21	22	23	24a	24b
15a	0.0000	0.2701	0.3588	0.4470	0.4825	0.5317	0.4869	0.4868	0.5158	0.3478	0.5999	0.5605
15b	0.2701	0.0000	0.2504	0.3386	0.3741	0.4233	0.3785	0.3784	0.4074	0.2394	0.4915	0.4521
16	0.3588	0.2504	0.0000	0.3710	0.4065	0.4557	0.4109	0.4108	0.4398	0.2718	0.5239	0.4845
17	0.4470	0.3386	0.3710	0.0000	0.4948	0.5440	0.4992	0.4990	0.5281	0.3601	0.6122	0.5728
18	0.4825	0.3741	0.4065	0.4948	0.0000	0.0659	0.2163	0.2161	0.3336	0.3291	0.5812	0.5418
19	0.5317	0.4233	0.4557	0.5440	0.0659	0.0000	0.2655	0.2654	0.3828	0.3783	0.6304	0.5910
20	0.4869	0.3785	0.4109	0.4992	0.2163	0.2655	0.0000	0.0782	0.3380	0.3335	0.5856	0.5462
21	0.4868	0.3784	0.4108	0.4990	0.2161	0.2654	0.0782	0.0000	0.3379	0.3334	0.5855	0.5461
22	0.5158	0.4074	0.4398	0.5281	0.3336	0.3828	0.3380	0.3379	0.0000	0.3624	0.6145	0.5751
23	0.3478	0.2394	0.2718	0.3601	0.3291	0.3783	0.3335	0.3334	0.3624	0.0000	0.4465	0.4071
24a	0.5999	0.4915	0.5239	0.6122	0.5812	0.6304	0.5856	0.5855	0.6145	0.4465	0.0000	0.1671
24b	0.5605	0.4521	0.4845	0.5728	0.5418	0.5910	0.5462	0.5461	0.5751	0.4071	0.1671	0.0000
25–26:												
					25							26
25					0.0000							0.1687
26					0.1687							0.0000

Table 1 continued

27–30:				
	27	28	29	30
27	0.0000	0.1998	0.2137	1.0220
28	0.1998	0.0000	0.1997	1.0080
29	0.2137	0.1997	0.0000	0.8983
30	1.0220	1.0080	0.8983	0.0000
31–33:				
	31	32	33	
31	0.0000	0.4709	1.1193	
32	0.4709	0.0000	1.1974	
33	1.1193	1.1974	0.0000	

**Fig. 2** Detailed map with sampling points around Lake Poso and the Malili lake system

were not able to achieve the exact coordinates and, therefore, they are not shown in Figs. 1 and 2.

Discussion

The overall phylogenetic tree (Fig. 3) shows that both the ancient lake systems (Malili lake system and Lake

Poso) have been colonized twice independently with subsequent radiations. A similar pattern of multiple independent colonizations and a subsequent radiation can also be found in other groups like gastropods (von Rintelen et al., 2004) and shrimps (von Rintelen et al., 2010a) from the ancient lakes in Sulawesi. In both systems, we can find three ecological niches which are always occupied by different crab species:

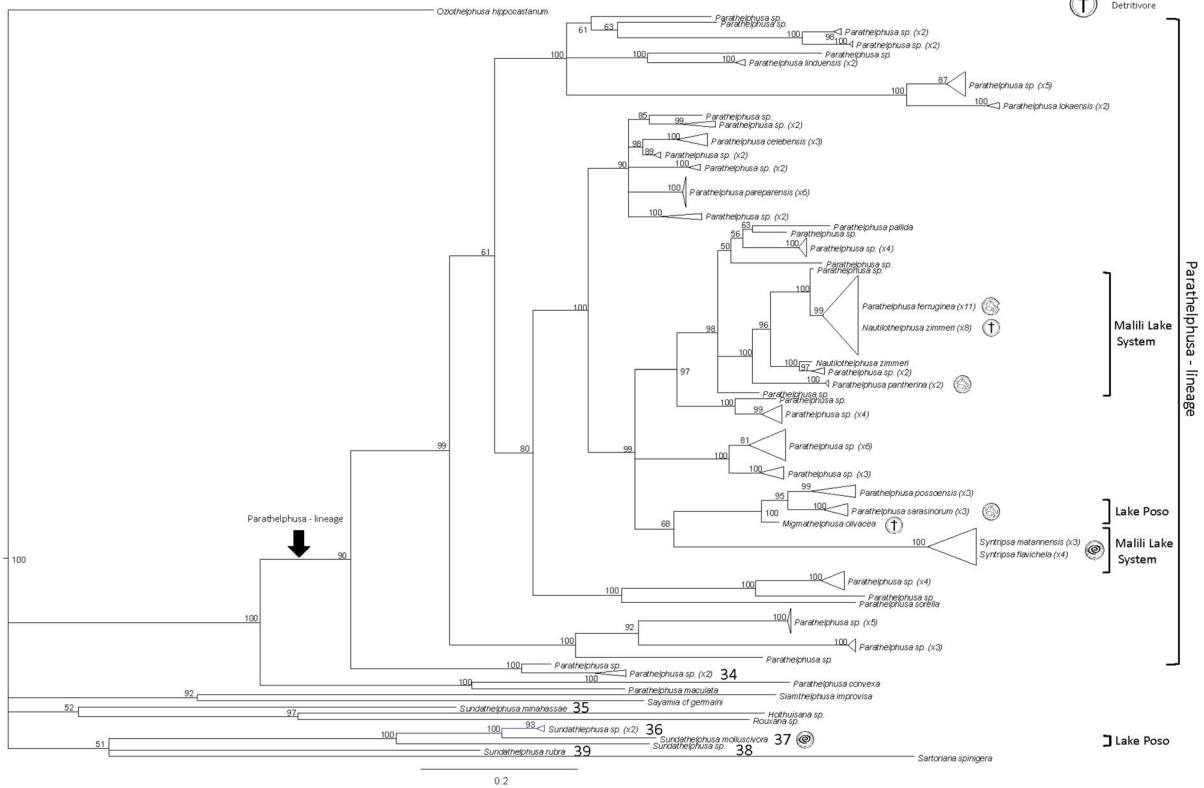


Fig. 3 Phylogenetic tree based on 658 base pairs from the mitochondrial Cox1 gene created with MrBayes (v.3.2.1) with 3 million generations, a sample frequency of 1,000, HKY+I+G as evolutionary model, and *Oziothelphusa hippocastanum* as

outgroup. The standard deviation of split frequencies by the end of the analysis was 0.006649. A more detailed version of the tree is available as electronic supplementary material

molluskivores, omnivores, and detritivores. This confirms the results based on 16S mtDNA and a much smaller dataset by Schubart & Ng (2008). Furthermore, the trees in Figs. 3 and 4 imply that the lineages which today are represented by molluskivore species were the first ones to colonize the lake, whereas omnivores and detritivores are derived from later colonization events and the outcome of local lacustrine radiations. This may be explained by the fact that mollusks represent the richest and most reliable source of proteins within the lake system and, therefore, the best available food source for the freshwater crabs. Von Rintelen et al. (2004) also described a long arms race between crabs and snails that may have driven diversification and adaptive radiation in the mollusks. The oldest lineage of the lake dwellers appears to be *Sundathelphusa molluscivora* Schubart & Ng, 2008,

which occupies the molluskivore niche in Lake Poso. The other niches in this lake are filled by species from the *Parathelphusa* lineage: *Parathelphusa sarasinorum* is the generalist in Lake Poso, but based on its chelar morphology also includes mollusks in its diet, whereas *Migmatelphusa olivacea* with its broadened last pair of legs occupies the niche of the soft sediment dwelling detritivore within this lake. In the Malili lake system, *Syntripsa flavichela* and *Syntripsa matannensis* are obviously the molluskivore forms, with notoriously large molariform armed chelae. *Parathelphusa ferruginea* and *Parathelphusa pantherina* appear to be the generalists, and *Nautilothelphusa zimmeri*, with the smallest forceps-shaped pincers, the detritivores.

The phylogenetic tree restricted to the *Parathelphusa* lineage as defined above (Fig. 4) reveals a huge proportion of cryptic diversity within the freshwater

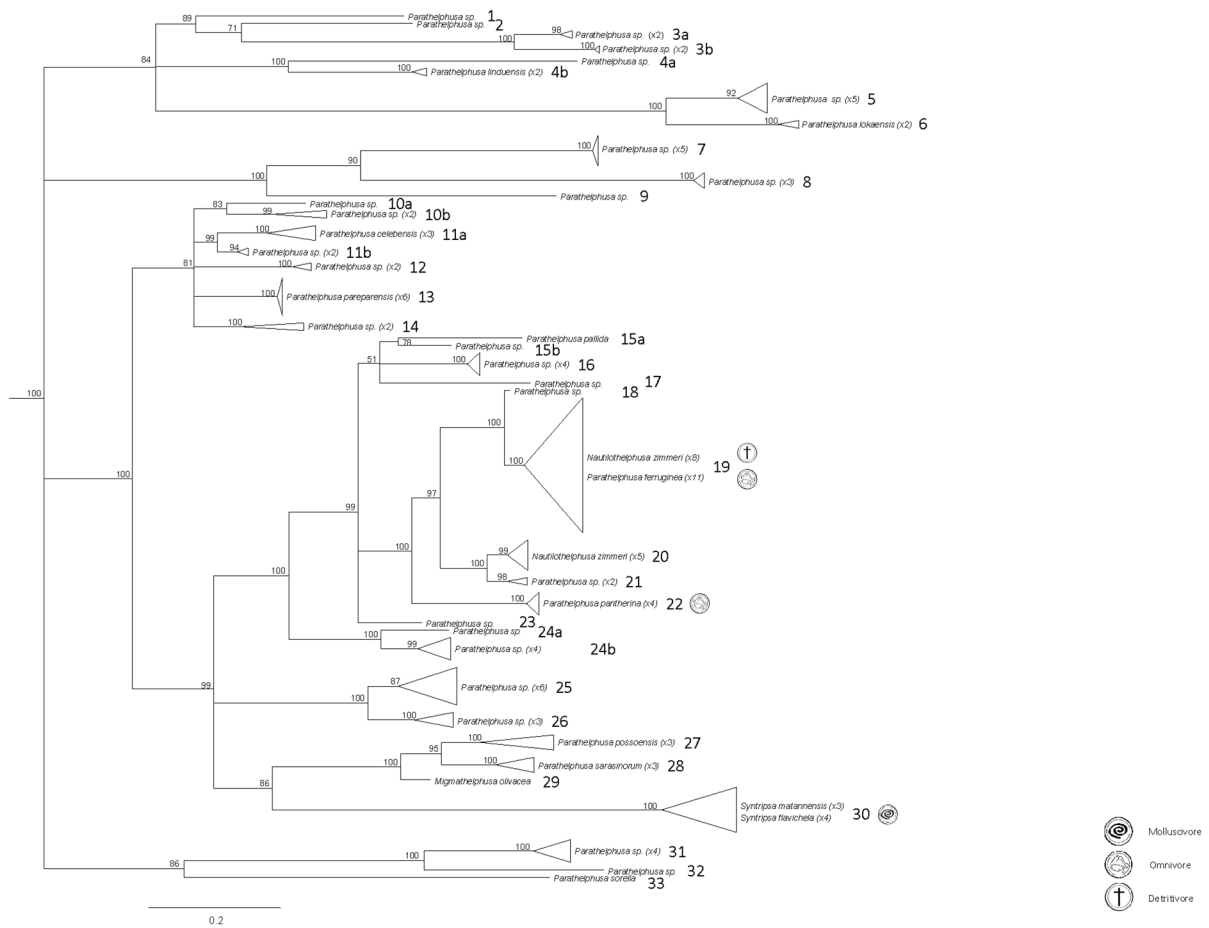


Fig. 4 Phylogenetic tree based on 658 base pairs from the mitochondrial Cox1 gene created with Mr.Bayes (v.3.2.1) with 3 million generations, a sample frequency of 1,000, GTR+G+I as evolutionary model, and a *Parathelphusa* sp. (34) from

Borneo as outgroup. The standard deviation of split frequencies by the end of the analysis was 0.006903. A more detailed version of the tree is available as electronic supplementary material

crabs of Sulawesi. The number of well-supported clades in the tree (Fig. 4) drastically exceeds the number of available scientific names for the genus *Parathelphusa*, which currently consists of 13 (see Chia & Ng, 2006). Even with our alignment lacking sequences from the type locality for *P. ceophallus* Ng, 1993; *P. tenuipes* Schenkel, 1902; and *P. crocea* Schenkel, 1902; the number of well-supported clades is clearly exceeding the number of available scientific names. The patristic distances, with the threshold of 0.16 (as applied by Lefébure et al., 2005) suggest up to 35 different potential OTUs within the sampled freshwater crabs of the *Parathelphusa* lineage from Sulawesi. Patristic estimates of species numbers are only rough estimates of taxonomic diversity and remain highly subjective and dependent on the threshold selected. Also relying on a

single mitochondrial marker like Cox1 can lead to a wrong estimation of biodiversity. Our clade 19 consists of *Parathelphusa ferruginea* and *Nautilothelphusa zimmeri* which cannot be separated from each other using Cox1 as mitochondrial marker due to possible mitochondrial introgression (see also Schubart et al., 2008). This could lead to an underestimation of biodiversity. Relying solely on Cox1 sequences, or other single-locus datasets, bears a risk of including pseudogenes or other paralogs, which would lead to an overestimation of biodiversity. We partly addressed this issue by controlling our sequence electropherograms for double peaks and testing for indels and stop codons to avoid inclusion of pseudogenes in the analysis. In any case, our aim was not to give exact species numbers as a measure for diversity within the *Parathelphusa* lineage.

Nevertheless, the bottomline message remains that the phylogeny reveals a huge potential for cryptic diversity within this group and on a long term will lead to a drastic increase of recognized species.

With a threshold for the patristic distance of 0.063, derived from our own data and corresponding to the distance between *Nautilothelphusa zimmeri* and a morphologically clearly distinct undescribed species of *Parathelphusa* from Lake Masapi (21), the number of potentially distinguishable OTUs rises to 43; Even with a more conservative estimate of threshold of 0.24 (subjective increase by 50% compared to the one used by Lefébure et al., 2005), still 23 OTUs could be postulated and many new species possibly described. Due to the fact that genetic distances must not be directly correlated to species boundaries and the use of only one single gene in the analysis, the patristic distances probably do not reflect the real number of valid species within our dataset, but they are a good indicator to show that there may be indeed a high number of unrecognized species hidden within the freshwater systems of Sulawesi. The real number of cryptic or unrecognized freshwater crab species in Sulawesi is probably even higher, because large parts of Sulawesi are still completely unsampled and leave a high potential for additional species being present in different drainage systems and on several neighboring islands. The Sulawesi *Parathelphusa* lineage has its next relatives on southeastern Borneo, as suggested by Klaus et al. (2013). Here we show that the monophyletic *Parathelphusa* lineage rapidly splits up into several distinct clusters. This indicates a very quick radiation into the different available niches after the arrival of their common ancestor from Borneo.

Also the biodiversity within the genus *Sundathelphusa* must be considered highly underrated until now, as crabs of this genus also show a high potential for endemism. Our study already reveals at least two undescribed species of *Sundathelphusa* (both here determined as “*Sundathelphusa* Mayoa,” see Fig. 3). To address this problem, further studies must be carried out to morphologically examine the different clades of freshwater crabs that are distinguished here.

A number of published studies already revealed a large proportion of cryptic diversity in freshwater crabs in South Africa (Daniels et al., 2001, 2002a, 2003, 2006; Daniels & Bayliss, 2012) belonging to the family Potamonautidae, in those along the Aegean region (Jesse et al., 2010, 2011), the Middle East (Keikhosravi

& Schubart, unpublished data) and East Asia (Shih et al., 2006; Shih & Ng, 2011) belonging to the family Potamidae, and in freshwater crabs from Borneo (Klaus et al., 2013) belonging to the Gecarcinucidae. In contrast, studies on neotropical freshwater crabs of the family Pseudothelphusidae showed a relatively low rate of endemism and diversity (Cook et al., 2008; Poettinger et al., 2011; Schubart et al., 2011; Rivera et al., unpublished data). This is probably due to the fact that the different families of freshwater crabs may not be monophyletic and colonized freshwater systems independently (see Tsang et al. unpublished data). Thus, they may differ in their ability to migrate over land. The neotropical Pseudothelphusidae seem to have a greater potential for dispersal over land and crossing hydrographic barriers. This may be due to adaptations of their branchial chamber to air breathing (see Díaz & Rodríguez, 1977). Therefore, they may be less geographically restricted and show less potential for endemism than Gecarcinucidae, Potamidae, and Potamonautidae, which constitute a monophyletic assemblage (Klaus et al., 2006, 2009, 2010; Marijnissen et al., 2006; Tsang et al., submitted). In contrast to the neotropical freshwater crabs, the *Parathelphusa* lineage from Sulawesi (this study) and representatives of *Parathelphusa* from Borneo (see Klaus et al., 2013) are genetically highly structured and have a strong potential for endemism.

Overall, it remains to be said that the species richness of Wallacea is remarkably high (Sodhi et al., 2004), but in the studied freshwater systems it is evidently far from being satisfactorily explored and documented. In order to obtain a complete picture of the real diversity of the freshwater crabs from Sulawesi and the colonization history of the island, far more comprehensive sampling and analyses must be carried out in the future.

Conclusion

Two large ancient lake systems on Sulawesi have been colonized twice independently by freshwater crabs. In both systems, the molluskivore niche was occupied by the lineages that colonized the corresponding lake system first and the following species evolved into detritivores and generalists within the lakes. In addition, our trees show that a huge part of the biodiversity within the freshwater crabs of Sulawesi remains undescribed. Even without sampling all of the

drainage systems and neighboring islands, the number of clades in the tree (Fig. 3) and potential species is clearly exceeding the number of available scientific names.

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