

Molecular phylogeny and diversification of freshwater shrimps (Decapoda, Atyidae, *Caridina*) from ancient Lake Poso (Sulawesi, Indonesia)—The importance of being colourful

Kristina von Rintelen^{*}, Thomas von Rintelen, Matthias Glaubrecht

Museum of Natural History, Humboldt University Berlin, Invalidenstrasse 43, 10115 Berlin, Germany

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Abstract

Ancient Lake Poso on the Indonesian island Sulawesi hosts a highly diverse endemic fauna, including a small species flock of atyid *Caridina* shrimps, which are characterized by conspicuous colour patterns. We used a mtDNA based molecular phylogeny to test the assumption of a monophyletic origin and intralacustrine radiation of the species flock and to assess the species specificity of some colour morphs. Our data reveal a rapid radiation of *Caridina* in the entire Poso drainage system, but provide no strong evidence for a monophyletic radiation of the lake species. Nevertheless each lacustrine species shows a varying degree of substrate or trophic specialization, usually considered a hallmark of adaptive radiation. Two distinct colour forms previously attributed to a single species, *C. ensifera*, lack distinguishing qualitative morphological characters, but are shown to be two different species. In contrast, morphologically rather distinct lake species lacking specific colour patterns may be hybridizing with riverine taxa. These results suggest that colour may play a similar role in species recognition and possibly speciation in ancient lake *Caridina* as hypothesized, e.g. for some African cichlids.

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1. Introduction

Ancient lakes, with their often highly diverse and speciose endemic fauna, are fertile grounds for the study of diversification processes, particularly speciation. Intralacustrine and adaptive radiation is generally regarded as the major mode of origin of ancient lake species flocks (e.g. Martens, 1997; Rossiter and Kawanabe, 2000; Fryer, 2006). The fauna of ancient Lake Poso in the central highlands of the Indonesian island Sulawesi (Fig. 1) comprises a fascinating and rich assemblage of endemic freshwater organisms with several species flocks, e.g. ricefishes (Parenti and Soeroto, 2004), hydrobioid gastropods (Haase and Bouchet, 2006) and pachychilid gastropods (Rintelen et al., 2004). The hypothesis of an adaptive radiation in

the lake has only been tested for two mollusc species flocks, and been supported for the pachychilids, but rejected for corbiculid bivalves (Rintelen and Glaubrecht, 2006).

The freshwater shrimp genus *Caridina* H. Milne Edwards, 1837 (Decapoda, Atyidae) is represented in Lake Poso and its catchment by currently three described species: *C. ensifera* Schenkel, 1902 and *C. sarasinorum* Schenkel, 1902 from the lake itself and *Caridina acutirostris* Schenkel, 1902 from surrounding rivers (Schenkel, 1902; Chace, 1997). All three are morphologically distinct, possessing the typical feeding appendages (chelipeds) associated with a characteristic feeding behaviour described for *Caridina* (Fryer, 1960), i.e. small food particles are swept from the substrate by the setae of the very mobile chelipeds and are passed with extreme rapidity to the mouthparts. Schenkel (1902) already mentioned differences in the cheliped morphology between *C. ensifera* and *C. sarasinorum*, e.g. stouter chelae of the first chelipeds in the latter.

^{*} Corresponding author. Fax: +49 30 20938565.

E-mail address: Kristina.Rintelen@museum.hu-berlin.de (K. von Rintelen).

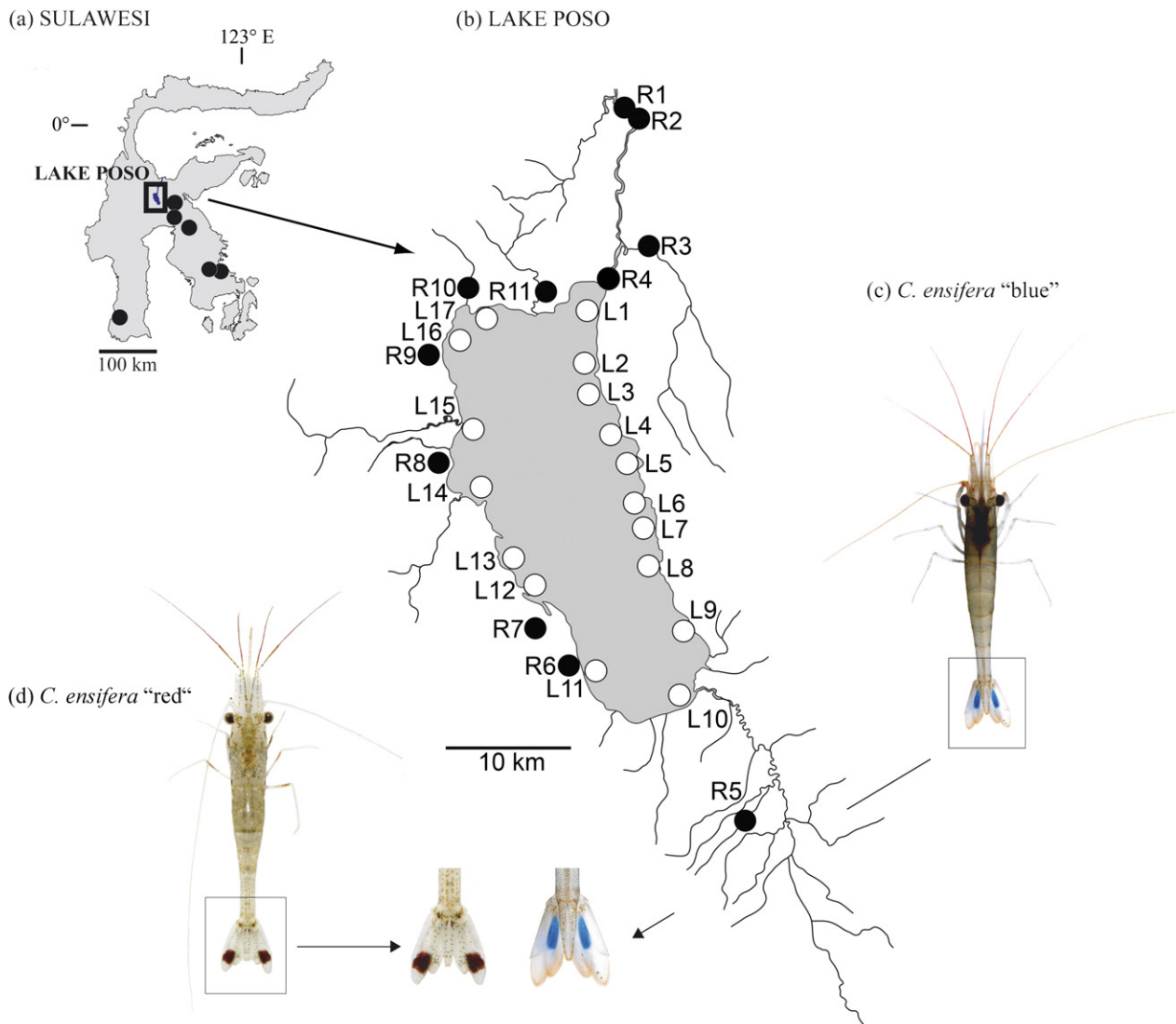


Fig. 1. Sulawesi and Lake Poso, sampling and colour morphs (see Table 1 for further details). (a) Sulawesi, sampling sites outside of Lake Poso. (b) Lake Poso and catchment, lacustrine (white dots; L1–17) and riverine sampling sites (black dots; R1–11). (c) and (d) *C. ensifera* colour morphs, enframed tailfans are shown in detail: “blue” with an elongated blue patch on the distal part of each endopod (upside down V-shape); “red” smaller red spot on the distal part of each exopod. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Besides the differences in trophic morphology, i.e. the chelipeds, the most characteristic feature of Lake Poso *Caridina* is the conspicuous body colouration recently discovered by field observation of living specimens (Rintelen and Rintelen, pers. observ.). One species in particular, *Caridina ensifera*, not only shows the most obvious colour pattern among all species in the lake, but is the only one with two easily distinguishable colour morphs (“red” and “blue”; Fig. 1c, d), which have not been reported before. The respective pattern is equally pronounced in both sexes and already distinctive in juveniles. This observation is *a priori* equally compatible with the assumption of intraspecific polymorphism or the existence of ‘cryptic’ species.

While *Caridina* is known to show species specific colour patterns in some taxa, for example *C. spongicola* from the Malili lakes (Zitzler and Cai, 2006), the second ancient lake

system of Sulawesi, or *C. trifasciata* Yam and Cai, 2003 from Hong Kong (Yam and Cai, 2003), species specificity has been doubted in other cases, such as for the striking colour forms of some widespread species used in aquarium trade (for example the *Caridina serrata* species group; Andreas Karge and Werner Klotz, pers. comm.). The lack of a well-established taxonomy in *Caridina* is a major obstacle in this respect, and species distinction is often difficult. Recent studies using molecular data indicate, however, that morphologically cryptic species among atyid shrimps may be common as for instance shown in Australian *Caridina* (Page et al., 2005).

Whereas the lack of data in *Caridina* prevents the study of the evolutionary role of body colouration, it is a well-documented feature in the lacustrine radiations of fishes, especially African cichlids, with regard to its potential

importance in speciation and radiation (e.g. Danley and Kocher, 2001; Seehausen and Schluter, 2004; Genner and Turner, 2005).

Here, we use a molecular phylogeny in combination with morphological and ecological data (i) to test the general assumption of an adaptive radiation for Lake Poso *Caridina*; (ii) to seek molecular support for morphology-based species descriptions and for yet undescribed forms found in new samples, in particular (iii) to investigate the significance of the two colour morphs of *C. ensifera*. In order to achieve the first aim, we use the definition of Schluter (2000) and apply the phylogenetic (common ancestry and rapid speciation) and ecological (phenotype-environment correlation) criteria he proposed to detect an adaptive radiation.

2. Materials and methods

Material from Lake Poso (17 localities) and its drainage (11 localities) was sampled by the authors in March and August 2004 and October 2005 (Fig. 1b and Table 1). Comparative samples were collected in other parts of Sulawesi (Fig. 1a and Table 1). Prior to preservation in 95% ethanol, lacustrine specimens were separated based on colour pattern, which has been photographically documented in the field. Voucher specimens are deposited in the Museum of Natural History, Berlin (ZMB); see Table 1 for accession numbers.

Fourteen morphometric measurements (compare Zitzler and Cai, 2006) were taken from $n = 40$ individuals each of both morphs of *C. ensifera* (see Supplementary Table 2 for details) using a stereo microscope with an ocular micrometer. Scanning electron microscopy was used to study the chelipeds from $n = 2$ –5 critical point dried specimens of each species.

DNA was extracted exclusively from abdominal tissue. For the molecular phylogeny two mitochondrial gene fragments, 861 bp of cytochrome oxidase subunit I (COI) and c. 560 bp of the large ribosomal subunit (16S) were amplified and sequenced on an ABI 3130 DNA sequencer using *Caridina*-specific primers COI-F-Car and COI-R-Car, and atyid-specific primers 16S-F-Car, 16S-R-Car and 16S-R-Car1 (Rintelen et al., 2007).

The orthologous DNA sequences obtained were aligned, using default settings, by CLUSTAL W, v. 1.81 (Thompson et al., 1994), and optimized by eye. The aligned sequence sets of COI (781 bp) and 16S (546 bp) were combined into a single concatenated alignment after this was not rejected ($P = 0.06$) by an incongruence length difference test (Farris et al., 1994) implemented in PAUP*4.0b010 as a partition-homogeneity test (Swofford, 2002).

Caridina typus H. Milne Edwards, 1837 from South and Southeast Sulawesi was used as outgroup.

Phylogenetic analyses were performed using Maximum Parsimony (MP) with PAUP*4.0b010, Maximum Likelihood (ML) with Treefinder (Jobb, 2005) and Bayesian

Inference (BI) with MrBayes 3.1 (Ronquist and Huelsenbeck, 2003). MP analyses were done with indels coded as fifth base and using a full heuristic search with random addition (100 repetitions) and tree bisection–reconnection, the same settings were used in a MP bootstrap analysis (1000 replicates). For the ML and BI analyses the appropriate models of sequence evolution (GTR + I + Γ for COI and the HKY + I + Γ for 16S) were selected using MrModeltest 2.2 (Nylander, 2004) both by Likelihood Ratio Tests and based on the Akaike Information Criterion. The two genes were set as partitions in the concatenated dataset and analyses run with the model specified for each partition separately. The ML analysis was done with Treefinder default settings, and 1000 bootstrap replicates. For the BI analysis posterior probabilities of phylogenetic trees were estimated by a 2,000,000 generation Metropolis-coupled Markov chain Monte Carlo algorithm (4 chains, chain temperature = 0.2), with parameters estimated from the dataset. A 50% majority-rule consensus tree was constructed following a 50% burn-in (10,000 trees) to allow likelihood values to reach stationarity, which was assessed using the value plots output by MrBayes.

All sequences have been deposited in EMBL Nucleotide Sequence Database (EMBL-Bank); see Table 1 for accession numbers.

3. Results

3.1. Species delineation in Lake Poso based on morphology

Based on the newly collected material we can distinguish five morphologically distinct species (Table 2): three from the lake itself, *C. ensifera*, *C. sarasinorum* and *C. spec. A*, a new lacustrine species (compare Fig. 1, L1–17), as well as two from the surrounding rivers, *C. acutirostris* and *C. spec. B*, a yet undescribed riverine species (Fig. 1, R1–11).

As mentioned in the introduction, *C. ensifera* comprises two colour forms, “blue” and “red” (Fig. 1c and d). In a discriminant analysis specimens are assigned to these morphs at 100% using 14 morphometric characters (see Supplementary Table 2), whereas no qualitative characters separate them when alcohol bleached material is studied. In this respect the morphometric data of *C. ensifera* “red” matches the original description of *C. ensifera* Schenkel, 1902.

All three lacustrine species differ strongly in their cheliped morphology (Fig. 2 and Supplementary Table 2). While *C. ensifera* has notably longer and more slender chelipeds than the stout feeding appendages of *C. sarasinorum*, they are uniformly slender in *C. spec. A* with unusually long brushes that make this species very different from other members of the genus *Caridina* from Sulawesi in general (Yixiong Cai, pers. comm.).

3.2. Ecological preferences of lacustrine species

The three lacustrine species not only differ in trophic morphology, but partly in their ecological preferences.

Table 1
Sample provenance, museum and EMBL-Bank accession numbers

Taxon	Locality data (compare Fig. 1)	Accession numbers		
		Museum	EMBL-Bank	
			COI	16S
<i>Caridina acutirostris</i> Schenkel, 1902	R9	ZMB 29439	AM747728	AM747637
			AM747729	AM747638
	R5	ZMB 29440	AM747730	AM747639
			AM747731	AM747640
<i>Caridina cf. acutirostris</i> Schenkel, 1902	Central Sulawesi, Ensa River	ZMB 29309	AM747726	AM747635
			AM747727	AM747636
<i>Caridina ensifera</i> Schenkel, 1902 “blue morph”	L8	ZMB 29207	AM747732	AM747650
	L10	ZMB 29251	AM747733	AM747713
	L12	ZMB 29260	AM747735	AM747651
	L10	ZMB 29290	AM747736	AM747641
	L7	ZMB 29292	AM747739	AM747649
	L3	ZMB 29306	AM747740	AM747652
	L16	ZMB 29325	AM747741	AM747653
	L17	ZMB 29382	AM747743	AM747642
	L11	ZMB 29385	AM747745	AM747643
	L1	ZMB 29393	AM747748	AM747644
	L13	ZMB 29394	AM747749	AM747645
	L4	ZMB 29395	AM747750	AM747646
	L5	ZMB 29400	AM747754	AM747647
	L14	ZMB 29405	AM747756	AM747648
<i>Caridina ensifera</i> Schenkel, 1902 “red morph”	L10	ZMB 29248	AM747737	AM747655
	L9	ZMB 29253	AM747734	AM747654
	L6	ZMB 29291	AM747738	AM747664
	L17	ZMB 29381	AM747742	AM747656
	L16	ZMB 29384	AM747744	AM747657
	L4	ZMB 29389	AM747746	AM747658
	L11	ZMB 29392	AM747747	AM747659
	L13	ZMB 29396	AM747751	AM747660
	L1	ZMB 29397	AM747752	AM747661
	L2	ZMB 29399	AM747753	AM747662
	L15	ZMB 29404	AM747755	AM747663
<i>Caridina lanceolata</i> Woltereck, 1937	South Sulawesi, Malili lake system	ZMB 29113	AM747757	AM747665
<i>Caridina opaensis</i> Roux, 1904	Southeast Sulawesi, Humbuti River	ZMB 29340	AM747758	AM747678
<i>Caridina sarasinorum</i> Schenkel, 1902	L10	ZMB 29068	AM747759	AM747687
	L8	ZMB 29137	AM747760	AM747690
	L7	ZMB 29201a	AM747761	AM747688
	L7	ZMB 29201b	AM747762	AM747689
	L9	ZMB 29261	AM747763	AM747679
	L9	ZMB 29288	AM747764	AM747680
	L16	ZMB 29383	AM747765	AM747681
	L11	ZMB 29386	AM747766	AM747682
	L1	ZMB 29388	AM747767	AM747683
	L15	ZMB 29402	AM747768	AM747684
	L2	ZMB 29403	AM747769	AM747685
	L14	ZMB 29406	AM747770	AM747686
	<i>Caridina spec. A</i>	L7	ZMB 29060a	AM747771
L7		ZMB 29060b	AM747772	AM747675
L7		ZMB 29060c	AM747773	AM747667
L8		ZMB 29252	AM747777	AM747666
L12		ZMB 29258	AM747779	AM747677
L9		ZMB 29289	AM747780	AM747673
L11		ZMB 29387	AM747784	AM747668
L4		ZMB 29390	AM747785	AM747669
L1		ZMB 29391	AM747786	AM747670
L5		ZMB 29398	AM747787	AM747671
L14		ZMB 29401	AM747788	AM747672
L13		ZMB 29456	AM747803	AM747676

Table 1 (continued)

Taxon	Locality data (compare Fig. 1)	Accession numbers		
		Museum	EMBL-Bank	
			COI	16S
<i>Caridina spec. B</i>	R11	ZMB 29159	AM747774	AM747699
			AM747775	AM747700
	R2	ZMB 29254	AM747778	AM747710
	R3	ZMB 29407	AM747789	AM747701
	R8	ZMB 29441	AM747792	AM747705
			AM747793	AM747706
	R6	ZMB 29442	AM747794	AM747702
			AM747795	AM747703
	R7	ZMB 29443	AM747796	AM747704
	R10	ZMB 29444	AM747797	AM747697
			AM747798	AM747698
	R2	ZMB 29445	AM747799	AM747711
			AM747800	AM747712
<i>Caridina spec. C</i>	South Sulawesi, Kawata River	ZMB 29234	AM747776	AM747691
	South Sulawesi, Cerekang catchment	ZMB 29297	AM747781	AM747692
<i>Caridina spec. D</i>	Central Sulawesi, Puawu River	ZMB 29438	AM747790	AM747695
			AM747791	AM747696
	Central Sulawesi, Stream at road Tomata-Beteleme	ZMB 29307	AM747782	AM747693
			AM747783	AM747694
<i>Caridina typus</i> H. Milne Edwards, 1837	South Sulawesi, Bantimurung waterfall	ZMB 29092	AM747724	AM747633
	Southeast Sulawesi, River at Tinobu	ZMB 29011	AM747725	AM747634

Table 2
Species from Lake Poso and catchment, based on morphology

Taxon	Occurrence	Remarks
<i>Caridina acutirostris</i> Schenkel, 1902	Riverine	
<i>Caridina ensifera</i> Schenkel, 1902	Lacustrine	Contains two colour forms: “blue” (Fig. 1c) “red” (Fig. 1d)
<i>Caridina sarasinorum</i> Schenkel, 1902	Lacustrine	
<i>Caridina spec. A</i>	Lacustrine	
<i>Caridina spec. B</i>	Riverine	

C. sarasinorum and *C. spec. A* occur in smaller numbers on various kinds of substrate (i.e. wood, rocks, leaf litter, macrophytes, but never pelagic) and both are lacking species specific colour patterns; depending on the substrate their colouration varies slightly. *C. ensifera* is abundant in the lake with both colour forms, which frequently occur in sympatry (see Supplementary Table 1). Both also show obvious differences in their choice of substrate and behaviour: *C. ensifera* “blue” is rather stationary and mainly collected from hard substrate (wood, rocks), whereas *C. ensifera* “red” is often found in pelagic swarms or sporadic on various kinds of substrates (hard and soft, for example sand or macrophytes). It also generally has the highest density of all shrimps in the lake and is often caught by local fishermen (Rintelen and Rintelen, pers. observ.).

3.3. Molecular phylogeny of Lake Poso shrimps

The topologies obtained by all three methods of phylogenetic analysis are largely identical (Fig. 2; Supplementary Figs. 1 and 2). The molecular phylogeny (Fig. 2) reveals a well-supported monophyletic group comprising all five species from the lake and its catchment. In a terminal position this clade also includes two haplotypes of *C. cf. acutirostris* from another catchment area (Tomori) as sister group to *C. acutirostris* from the Lake Poso catchment, though.

The three lacustrine species do not form one monophyletic group, but constitute three separate clades (Fig. 2). On the species level, the majority of sequenced specimens of the lacustrine species *C. sarasinorum* and *C. spec. A* each form a monophyletic group, while two haplotypes of each species cluster with sequences of the riverine species *C. spec. B*. The two colour forms of *C. ensifera* are both monophyletic and appear in two highly supported clades that are not sister groups. This correlates with their morphological and ecological differences (see above). *C. ensifera* “red” is rather sister group to *C. sarasinorum*.

Of the two riverine species occurring in the Lake Poso catchment area only the haplotypes of *C. acutirostris* form a monophyletic group. In contrast, *C. spec. B* appears polyphyletic in the tree, even though the sequenced specimens and populations, respectively, are morphologically largely indistinguishable.

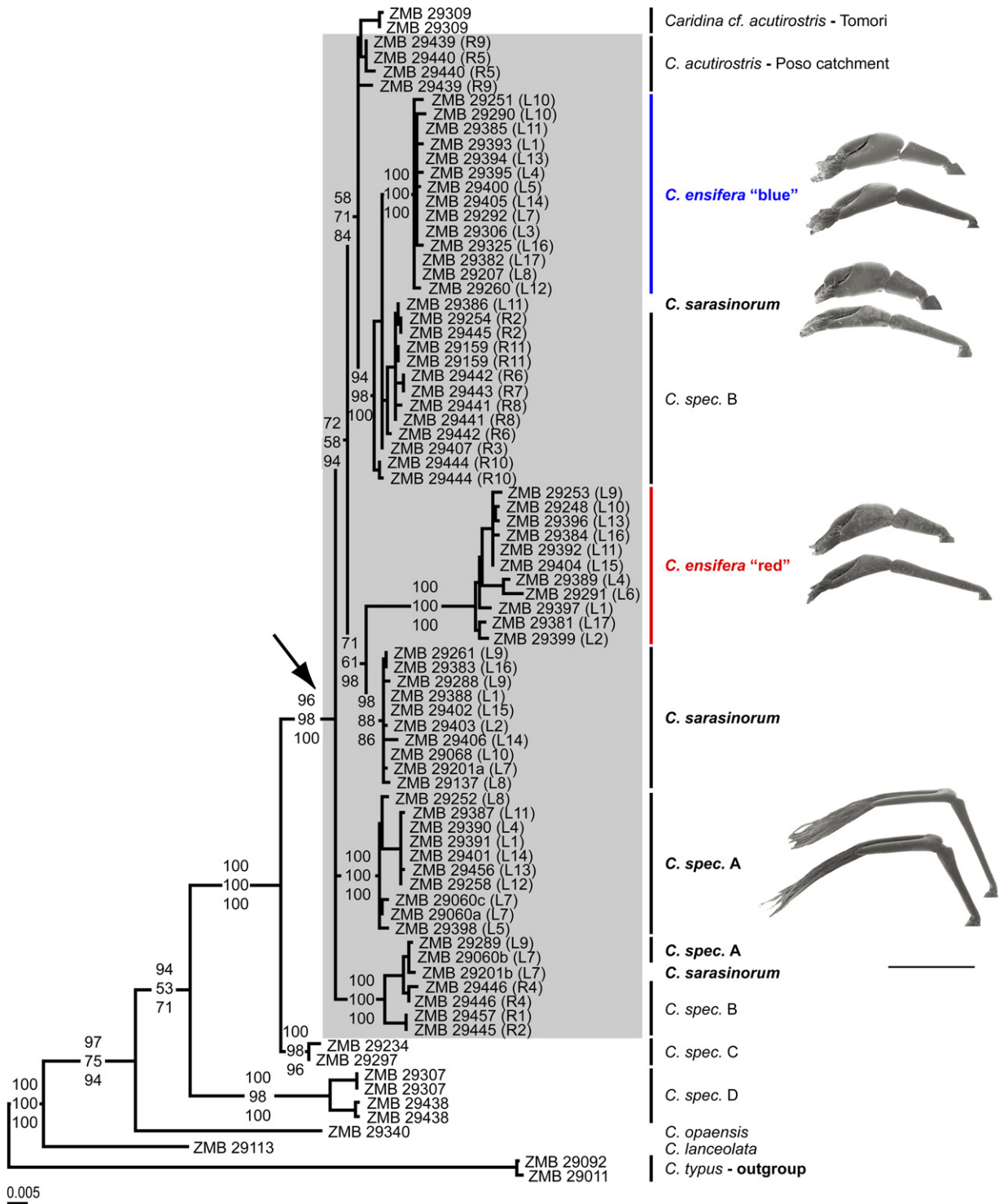


Fig. 2. Bayesian Inference phylogram (mtDNA, 16S and COI) of Lake Poso *Caridina*. Numbers on branches are, from top, MP and ML bootstrap values, and Bayesian posterior probabilities. The scale bar indicates the number of substitutions per site. The arrow indicates the origin of Poso species flock which is highlighted in dark. Lacustrine species are set in bold type and their respective trophic morphology is shown (SEM picture of both chelipeds; scale = 1 mm). For each sequenced specimen museum accession and sampling station numbers (in brackets) corresponding to the map in Fig. 1 are provided.

Overall sequence divergence (*p*-distance) within the entire Lake Poso and catchment clade does not exceed 3.7% (16S) and 7.7 (COI)%, respectively (Table 3), while within the clades formed by lacustrine species including

the two *C. ensifera* colour forms, maximum sequence divergence does not exceed 0.9% (16S, *C. ensifera* "red"). Sequence divergence is highest within the polyphyletic riverine species *C. spec. B* with 2.1% (16S) and 4.9 (COI)%.

Table 3
Maximum genetic divergences (*p*-distance, %) within the Poso clade

Taxon	COI	16S
Entire clade	7.7	3.7
<i>C. acutirostris</i>	1.2	0.7
<i>C. ensifera</i> “blue”	0.8	0.6
<i>C. ensifera</i> “red”	2.3	0.9
<i>C. sarasinorum</i> ^a	0.9	0.2
<i>C. spec.</i> A ^a	1.3	0.4
<i>C. spec.</i> B ^b	4.9	2.1

^a Distances have been calculated without the haplotypes placed with *C. spec.* B.

^b The distances for *C. spec.* B include the lacustrine haplotypes placed with this species.

4. Discussion

The molecular phylogeny reveals a rapid radiation of *Caridina* in Lake Poso and its catchment, but the data fail to provide conclusive evidence for a separate radiation within the lake. The three lake species do not form a monophylum, and the position of one lake species in a terminal position within a riverine clade (Fig. 2, *C. ensifera* “blue”) might indicate an instance of secondary lake colonization. However, the almost complete lack of support for all basal nodes in the whole Poso clade does not allow to make a strong statement about the number of lake colonizations. The grouping of some haplotypes of highly distinct lacustrine species with those of the riverine *C. spec.* B suggests the occurrence of introgressive hybridization or, though less likely, incomplete lineage sorting, which further complicates conclusions about the origin of lake species from riverine ones or *vice versa*. The short and almost completely unsupported basal branches strongly point to rapid cladogenesis in the whole Poso clade, though. In contrast to the almost simultaneous appearance of several highly distinct and well supported lineages within the catchment area, no clear genetic distinction between a lacustrine and riverine environment can be made. Such partial intermixture of lacustrine and riverine haplotypes has also been observed in the species flocks of the pachychilid gastropod *Tylomelania* in Lake Poso and Sulawesi’s Malili lake system (Rintelen et al., 2004).

The presence of Poso clade haplotypes outside of the lake system in the case of *C. cf. acutirostris* indicates that the species has secondarily dispersed beyond the area of the Poso radiation. Similar cases are known from other species flocks such as, e.g. the derivation of the Cocos finch from within the Galapagos finches (Sato et al., 1999).

On the species level, the match of morphologically delineated species to genetic clades is rather heterogeneous. While the majority of sequenced specimens of the lacustrine *C. sarasinorum* and *C. spec.* A. fall into monophyletic clades, some haplotypes group with those of riverine *C. spec.* B. The latter is polyphyletic itself, suggesting a need to reconsider its taxonomy.

Most strikingly, the two colour forms of *C. ensifera*, “blue” and “red” are not only found in well defined clades,

but are also not sister to each other. This provides strong evidence that these colour forms are indeed distinct species, particularly given the ecological and, albeit slight, morphological differences between the two morphs. Consequently, as the “red” form is *C. ensifera* as described by Schenkel (see results), the “blue” form awaits formal description as a new species and is further on referred to as such in the text.

The lack of misplaced haplotypes in the two colourful species formerly subsumed as *C. ensifera* suggests that colour or colour patterns play an important role in species recognition here and may also have been involved in speciation. Lake Poso provides a favourable setting for colour perception, being oligotrophic with clear water and a (Secchi disc) visibility of 9.3 m (Giesen, 1994). A role for sex-independent and colour-based assortative mating in speciation has been recently described for fishes in Lake Tanganyika cichlids (Salzburger et al., 2006) or Caribbean coral reef hamlets (Puebla et al., 2007). While colour vision in decapods is still poorly studied and the data for the Poso shrimp species are not sufficient to draw further reaching conclusions in that direction, this case at least indicates that similar mechanisms might be found in aquatic invertebrates.

In contrast, the non-monophyly of the lacustrine *C. sarasinorum* and *C. spec.* A and the riverine *C. spec.* B might indicate introgressive hybridization among these species, as the lack of distinctive colour patterns might facilitate ‘mating errors’ in these taxa. This hypothesis needs testing, however, e.g. by the study of potential contact areas between the species, such as the lake’s outlet, and the application of nuclear markers.

Irrespective of a potential involvement of colour in species diversification and despite the uncertainty concerning in the number of colonization events in Lake Poso, the occurrence of at least one speciation event within Lake Poso proper is highly likely. The likelihood of allopatric versus sympatric diversification is difficult to assess, though. In the Malili lake system, clear genetic differentiation has been found between allopatric populations of an endemic lacustrine species (*Caridina lanceolata* Woltereck, 1937) in two lakes (Roy et al., 2006), suggesting that speciation is at least partly driven by geographic separation. In contrast, our data show no significant genetic structuring within any Lake Poso *Caridina* species (assuming that the misplaced haplotypes owe their existence to introgression), which is perhaps not surprising given the lack of geographic subdivisions in essentially trough-like Lake Poso. However, given the size and depth of the lake (323 km², max. depth 450 m; Giesen, 1994) and the fact that the palaeohydrology of the lake is not known at all, the possibility of past allopatric settings cannot be ruled out at present.

While it remains uncertain if Lake Poso *Caridina* are an example of an *in situ* radiation, the four lacustrine species *C. ensifera* (the former “red” morph), *C. spec.* (the former “blue” morph of *C. ensifera*), *C. sarasinorum* and *C. spec.* A nevertheless show some hallmarks of adaptive radiation

sensu Schluter (2000): (i) diversification into conspicuously different phenotypes as compared to the riverine taxa *C. acutirostris* and *C. spec. B*, combined with (ii) different substrate preferences (i.e. phenotype-environment correlation), albeit weak, and, linked to the first two, (iii) coexistence of species in sympatry in the lake, whereas only one species is found in each river.

Especially the differences in cheliped morphology are probably pivotal in enabling species to coexist on different substrates in the lake. Ecotypic differentiation has, e.g. been suggested for *Caridina nilotica* Roux, 1833 from Lake Victoria (Hart et al., 2003) and adaptive chelipeds related to different ways of feeding for other atyid shrimps from Lake Tanganyika (Fryer, 2006). On Sulawesi, habitat specialization and an adapted trophic morphology have been described for *C. spongicola* Zitzler and Cai, 2006 from the Malili lakes, which is exclusively found on a freshwater sponge (Rintelen et al., 2007; Zitzler and Cai, 2006). While similar differences in the chelipeds morphology (Fig. 2) are found in Lake Poso species, there is no strong correlation between morphology and substrate. However, the unusual chelae of *C. spec. A* at least suggest trophic specialization in Lake Poso species as well.

In conclusion, our data support the notion of a rapid radiation of *Caridina* in the Poso system. The species of the lake proper, however, may not represent an *in situ* radiation, despite the fact that the lacustrine species are set apart by their body colouration and some degree of trophic specialization from the riverine species in the system. Colour seems to play an important role in species recognition and may prevent hybridization, which is likely in species with less distinct colour patterns. Comparative studies on the other species-flocks in the lake should enable comparisons among organisms with different intrinsic properties and contribute to our knowledge of diversification and adaptation processes in ancient lakes in general.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2007.07.002.

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