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Rapid evolution of sessility in an endemic species flock of the freshwater bivalve *Corbicula* from ancient lakes on Sulawesi, Indonesia

Thomas von Rintelen* **and Matthias Glaubrecht**

Museum of Natural History, Humboldt University Berlin, Invalidenstrasse 43 10115 Berlin, Germany
**Author for correspondence (thomas.rintelen@rz.hu-berlin.de).*

The fauna of ancient lakes frequently contains taxa with highly derived morphologies that resulted from *in situ* radiation of lacustrine lineages with high antiquity. We employed a molecular mtDNA phylogeny to investigate this claim for corbiculid freshwater bivalves in two ancient lake systems on the Indonesian island Sulawesi. Among the otherwise mobile corbiculid species flock, only one taxon, *Pososstrea anomioides*, in the ancient Lake Poso exhibits a unique habit, i.e. cementing one valve to the substrate. Our data show that *Corbicula* on Sulawesi is polyphyletic, with the endemic riverine taxa in terminal position, and the lacustrine species flock being paraphyletic. Surprisingly, *Pososstrea* is not confirmed as a genus distinct from *Corbicula* and genetic distances suggest a rather recent origin from the only other corbiculid species endemic to Lake Poso, the non-cementing *Corbicula possoensis*. While the cementing *anomioides*, despite its unique behavioural and morphological characteristics, clusters together with non-sessile *Corbicula* species, the latter exhibit strong genetic distances in the absence of morphological disparity and fall into several genetically rather distinct clades. These findings suggest that developmental plasticity of animals in ancient lakes rather than the antiquity of lineages might account for the unique morphology of some species.

Keywords: ancient lakes; molecular phylogeny; rapid evolution; bivalves; Sulawesi

1. INTRODUCTION

Ancient lakes are hotspots of aquatic biodiversity and provide fascinating insights into evolutionary processes (Fryer 1996; Martens 1997; Rossiter & Kawanabe 2000), as exemplified by recent hypotheses on the origin of cichlid and snail species flocks (Streelman & Danley 2003; Rintelen *et al.* 2004; Wilson *et al.* 2004). A long-standing assumption has been the antiquity of ancient lake organisms and, closely correlated, a usually highly derived morphology (Brooks 1950; Boss 1978; Nishida 1991). While current research has indicated the potential for rather rapid speciation and diversification in organisms prone to sexual selection (e.g. the cichlids; Danley & Kocher 2001; Kocher 2004), new work on a classical invertebrate species flock, the Lake Tanganyika paludomid gastropods, has confirmed an ancient origin of disparity and diversity even predating lake formation (Wilson *et al.* 2004).

On the Indonesian island Sulawesi (the former Celebes) (figure 1*a*), two ancient lake systems of tectonic origin and an age of 1–2 myr (Rintelen *et al.* 2004), *viz*. the five lakes of the Malili system and Lake Poso (figure 1*b*,*c*), host a range of fishes and invertebrate radiations. The mollusc assemblages of these lakes were discovered more than a century ago, and immediately proposed to represent an ancient fauna with, for example, a peculiar array of endemic gastropods with three different highly derived groups in Lake Poso (Sarasin & Sarasin 1898). While recent studies on the endemic gastropod species flocks in the lakes have revealed several adaptive radiations within the pachychilid *Tylomelania* (Rintelen *et al.* 2004), the bivalves have received scant attention so far.

One family only, the Corbiculidae (Mollusca: Bivalvia: Veneroidea), is represented in the Sulawesi lakes with seven described species in two genera. All but one species belong to *Corbicula* Mühlfeld, 1811, a brooding and notoriously invasive taxon with internal fertilization. While most *Corbicula* species are simultaneous hermaphrodites, the Sulawesi taxa are gonochoric (Glaubrecht *et al.* 2003). In addition to this mobile species assemblage, Bogan & Bouchet (1998) only recently described a second monotypic and conchologically highly distinct corbiculid genus *Pososstrea*. Endemic to Lake Poso, *Pososstrea anomioides* Bogan & Bouchet 1998 represents the only sessile, i.e. cementing member of the family. Moreover, *P. anomioides* is the only sessile freshwater bivalve species besides the phylogenetically distant unionidoidean family Etheriidae in Africa, South America and India. Adding to the mobile *Corbicula possoensis* Sarasin & Sarasin, 1898 endemic to Lake Poso, this unique shell morphology and mode of living have been suggestive of a long independent evolution of *Pososstrea* (see Bogan & Bouchet 1998).

A recent morphological revision (Glaubrecht *et al.* 2003), while finding support for the existence of two riverine species on the island, recognized only three of the six lacustrine species described from the Malili lakes. However, morphological species delimitation in *Corbicula* is generally difficult, a statement perhaps best illustrated by the wide range of species diversity estimates in these freshwater bivalves: around 70 species are described and currently used in the literature (e.g. Glaubrecht *et al.* 2003; Korniushin 2004; Lee *et al.* 2005), while Morton (1986) recognized only two variable and widespread species, of which but one inhabits freshwater.

In this paper, we use a molecular phylogeny to test the assumptions of the expected antiquity of *Pososstrea* and its phylogenetic, i.e. taxonomic distinction from the non-cementing *Corbicula* in Lake Poso. Additionally, we aim to test the molecular support for morphology-based species descriptions in corbiculids

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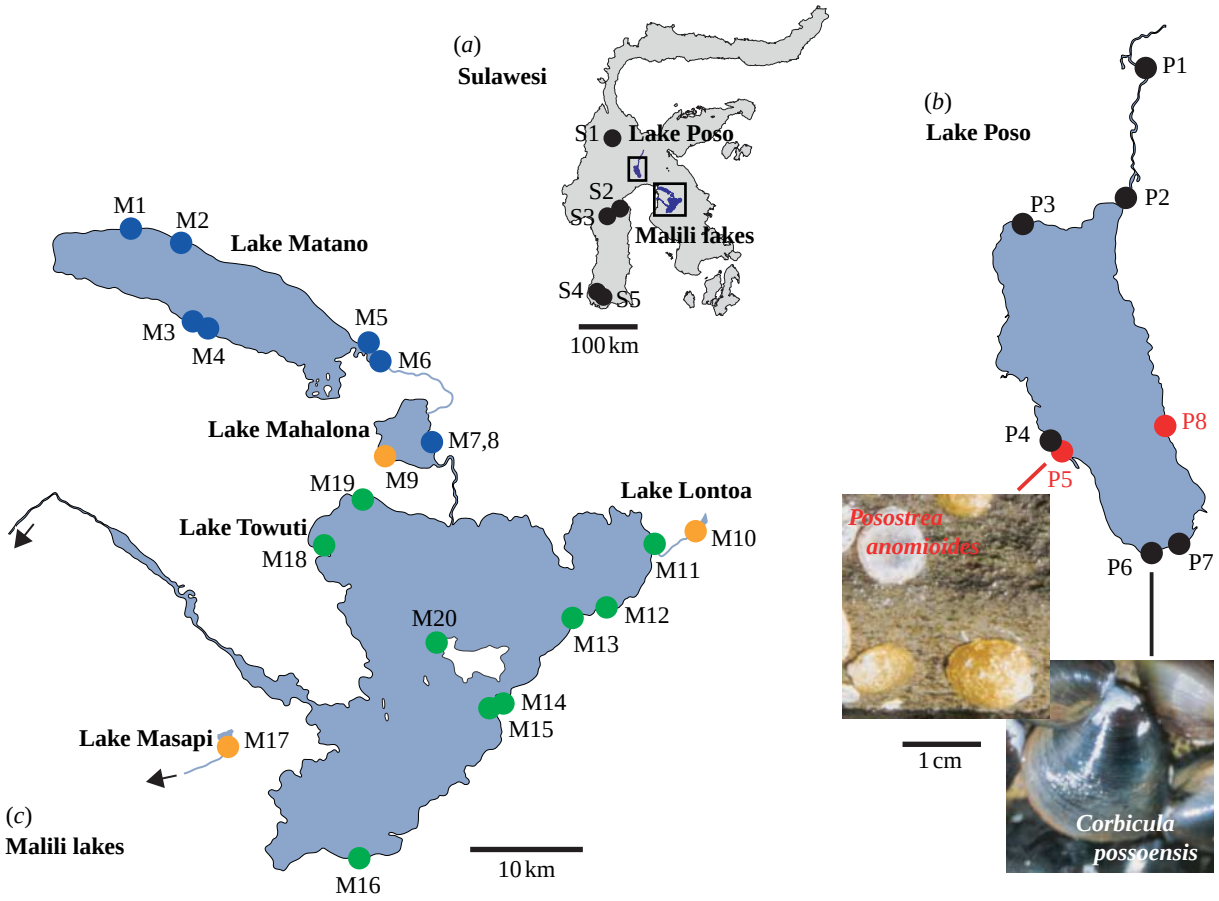

Figure 1. Sulawesi, the ancient lakes and sampling stations. (*a*) Sulawesi. (*b*) Lake Poso. (*c*) Malili lake system.

on Sulawesi in general, and particularly in the other ancient lake system, the Malili lakes.

2. MATERIAL AND METHODS

The material used for this study was collected by the first author in the Malili lakes in September–December 2002, September 2003 and January 2005, and the Lake Poso samples were taken by both authors in March and August 2004. All specimens are preserved in 70–96% ethanol. They were assigned to described species based on shell morphology following the taxonomic revision of the endemic Sulawesi corbiculids by Glaubrecht *et al.* (2003). Voucher specimens are deposited in the Museum of Natural History, Berlin (ZMB); see electronic supplementary material for accession numbers.

DNA was extracted exclusively from foot tissue. For the molecular phylogeny a *ca* 710 bp gene fragment of the mitochondrial cytochrome oxidase subunit I gene was amplified and sequenced on an ABI 377 or ABI 3130 DNA sequencer using universal primers (LCO1490 and HCO2198; Folmer *et al.* 1994) as outlined in Glaubrecht *et al.* (2003). In addition, some published sequences (Siripattrawan *et al.* 2000; Glaubrecht *et al.* 2003) were taken from GenBank, and all new sequences have been deposited there as well (see electronic supplementary material for accession numbers and sequence provenience). *C. madagascariensis* has been chosen as outgroup based on a more comprehensive phylogeny (Glaubrecht *et al.* 2003).

The sequence alignment of 614 bp was created by eye with BioEdit 5.09 (Hall 1999). The phylogeny has been estimated using three methods: maximum-parsimony (MP) with PAUP 4.0b010 (Swofford 2002), maximum-likelihood (ML) with Treefinder (Jobb 2005) and Bayesian inference (BI) with MrBayes 3.1 (Ronquist & Huelsenbeck 2003). The MP analysis was done using a full heuristic search with random addition (10 replicates) and tree bisection-reconnection (TBR), and the same settings were used in a MP bootstrap analysis (100 replicates). For the ML and BI analyses, an appropriate model of sequence evolution was selected using MrModeltest 2.2 (Nylander 2004), and consequently based on the Aikaike Information Criterion the HKY+I+Γ model was employed. The ML analysis was done with the Treefinder default settings, and 1000 RELL bootstrap replicates. For the BI analysis, posterior probabilities of phylogenetic trees were estimated by a 1 000 000 generation Metropolis-coupled Markov chain Monte Carlo algorithm (four chains, chain temperature = 0.2), with parameters estimated from the dataset. A 50% majority-rule consensus tree was constructed following a 25% generation burn-in to allow likelihood values to reach stationarity.

3. RESULTS

The molecular phylogeny (figure 2) reveals the Sulawesi corbiculids to be polyphyletic: the riverine and lacustrine species are neither sister groups nor even closely related but form distantly related clades. Each of these two clades comprises species endemic to Sulawesi and several other populations or species from (mostly mainland) Asia, i.e. neither the riverine nor the lacustrine species from Sulawesi form a monophylum.

The lacustrine species are a paraphyletic group, as the Lake Poso corbiculids comprise two distinct genetic lineages: in the gene tree, two populations of *C. possoensis* from the southern part of the lake (figure 1*b*, P6,7 and figure 2, Poso I) are not closely related to the other lake corbiculids (figure 2, Poso II and Malili), but are sister to a group comprising all other Sulawesi corbiculids and several populations or species from mainland eastern Asia, Japan and Java.

Despite its unique morphological habit, the sessile *P. anomioides* in Lake Poso does not represent a distinct genetic lineage, but together with some populations of *C. possoensis* represents a second mtDNA clade (figure 2, Poso II). Within that clade, the genetic distance between *anomioides* and *possoensis*

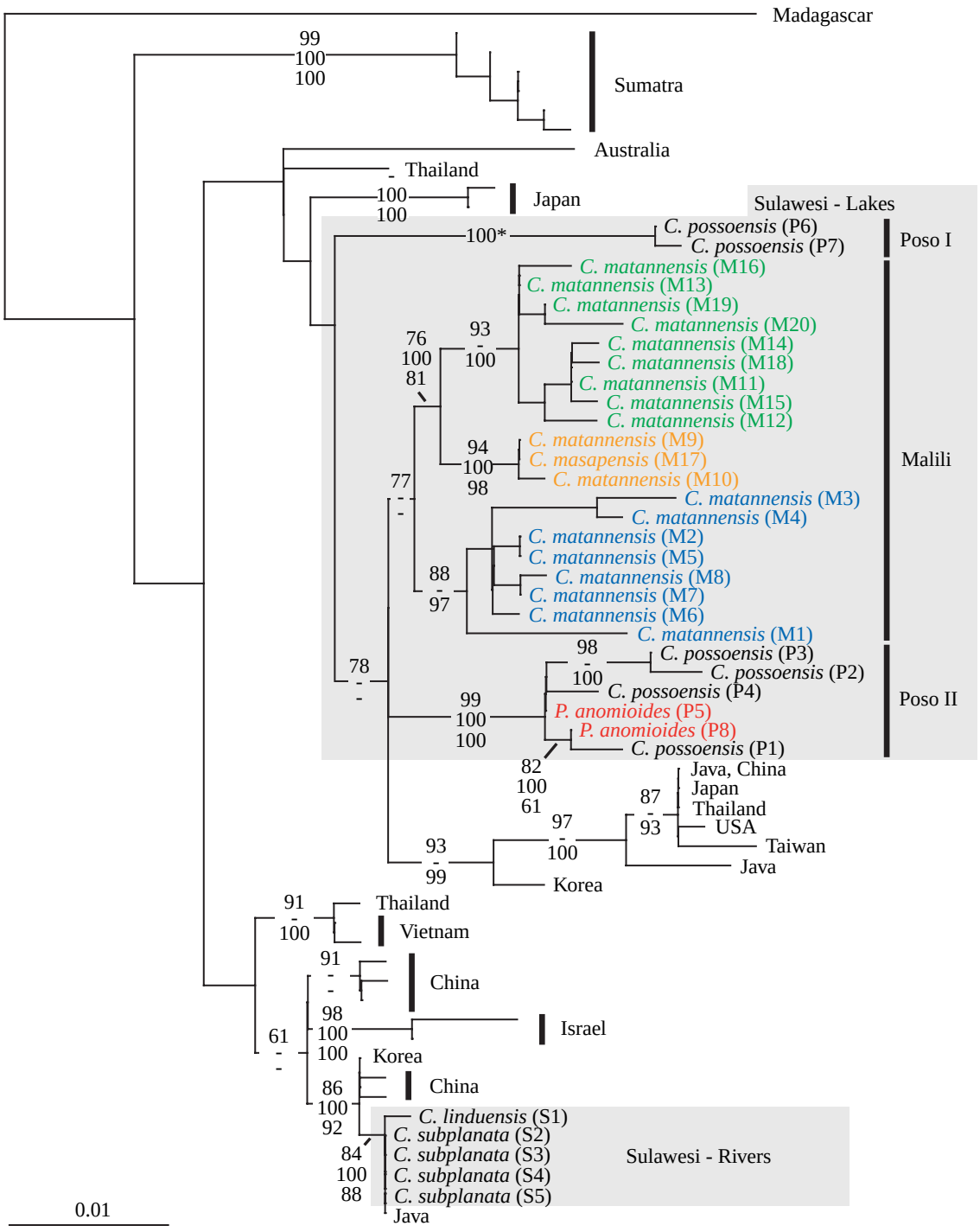

Figure 2. Phylogenetic relationships of Sulawesi corbiculids. Maximum-likelihood phylogram, the number groups on branches are, from top, maximum-likelihood (RELL)- and maximum-parsimony bootstrap values and Bayesian Inference posterior probabilities. The asterisk indicates that the given number is identical for all three support values. The colours and sampling site codes for the Sulawesi taxa correspond to those in figure 1 and the letter prefixes indicate the area (M, Malili lakes; P, Lake Poso; S, Sulawesi rivers). The scale bar indicates the number of substitutions per site.

is even less than among *possoensis* (0.33–0.97 versus 0.33–1.30, *p*-distance in %).

In contrast to the Lake Poso corbiculids, the Malili lake taxa form a monophylum (figure 2, Malili), albeit with low statistical support. Among populations attributed *prima facie* to the species *C. matannensis*, we found a strong geographical pattern with three largely allopatric mtDNA clades. In addition to a clade inhabiting Lake Matano and Lake Mahalona, a second clade comprises all studied specimens from Lake Towuti. The latter is sister to a clade comprising one *C. matannensis* population from Lake Mahalona and *C. loehensis* from two small satellite lakes adjacent to Lake Towuti.

Genetic distances among the populations of the two endemic riverine species, *C. subplanata* and *C. linduensis*, are much lower than those among the lacustrine species within a lake system (0.0 versus 1.4 (Malili) and 1.8 (Poso) within the lake clades, mean *p*-distances in %), even though the sampling points of the sequenced riverine specimens are widely spread across the island (figures 1*a* and 2, Sulawesi Rivers).

Indeed, in *C. subplanata* only one haplotype is present, which is also shared by a population on Java.

4. DISCUSSION

Surprisingly, our results do not support the hypotheses we tested. On a larger scale, the riverine corbiculid species on Sulawesi apparently represent an independent colonization of the island without any closer relation to the lacustrine radiations. We even anticipate that the Sulawesi riverine species may not be endemic at all, because genetically the widespread *C. subplanata* is indistinguishable from one *C. javanica* population from Java. This supports earlier assumptions by Glaubrecht *et al.* (2003) that *C. javanica* and *C. subplanata* may be conspecific, possibly also with the widespread *C. fluminea*. However, we consider it difficult to place taxonomic decisions purely on mtDNA data because of the peculiarities of mitochondrial inheritance found in corbiculids and bivalves in general (see e.g. Pfenninger *et al.* 2002; Lee *et al.* 2005). Nevertheless, a general pattern found in our and previous studies (Park & Kim 2003) is the occurrence of identical or closely related haplotypes over large geographic distances, indicating both excellent dispersal abilities in concert with the highly invasive nature of *Corbicula*.

Given this, it is rather surprising on a finer spatial scale to find well-defined genetic clades among the lacustrine species of Sulawesi. For example, the sequence divergence within the geographically restricted lineages in the Malili lakes (in particular the Towuti and Matano/Mahalona subclade) actually largely exceeds that found within and among the more widespread riverine taxa not only on Sulawesi, but even among taxa distributed from Thailand to Japan. This remarkable genetic pattern found here for the Malili populations further underscores the need to reconsider current morphological species assignments in these lakes.

In contrast, the situation in Lake Poso presents a more challenging pattern. First, the morphologically rather uniform *C. possoensis* is at best paraphyletic, as its populations fall into two very distinct genetic clades. Second, despite its highly derived morphology and substrate preference, *P. anomioides* is not genetically distinct from *C. possoensis*. Our data hint at a mtDNA gene tree–species tree conflict, a phenomenon possibly caused either by hybridization or incomplete lineage sorting (Nichols 2001). Irrespectively, we conclude from the molecular evidence that *P. anomioides* does not represent an independent lineage, but instead a terminal taxon of species status within one of the *Corbicula* subclades. Consequently, the nomenclatorial distinction of a separate corbiculid genus *Pososstrea* is futile.

The case provides just another example of the perils of equating a unique and possibly derived morphological condition with distinct taxonomic status and phylogenetic implications. Incidentally, the same misapprehension has also led to confusion in the pachychilid gastropods from Sulawesi. In addition to the pantropically widespread so-called 'melaniid' gastropods, Sarasin & Sarasin (1897) erected a separate genus *Tylomelania* for three presumably derived and distinct gastropod species from Lake Poso and Poso River only. (To the extent that this case is comparable to the corbiculids here, it should be noted that the concept of *Tylomelania* has been revised to now comprise all pachychilid taxa endemic to Sulawesi; see Rintelen & Glaubrecht (2005)). Given a century of research between the descriptions of *Tylomelania* and *Pososstrea*, and in light of the conviction that taxonomic status significantly influences evolutionary interpretations and thinking, we suggest that it is about time that we stop perpetuating disadvantageous remnants of typological thinking, with its systematic consequences.

With respect to the unusual sessile habit of *Corbicula anomioides*, we anticipate that it evolved rapidly from the mobile corbiculids in Lake Poso. We expect this assumption to hold true, even if introgressive hybridization could be shown to have caused the genetic pattern described above, as the likelihood of introgression depends on the geographic proximity and taxonomic age of the involved taxa. We might also speculate upon the possibility of an involvement of sympatric speciation in the recent origin of *C. anomioides* in Lake Poso. However, it would prove extremely difficult to rule out allopatric speciation here, given the size of the lake and the lack of data on its palaeohydrology. Irrespectively, despite the long existence of this lake, sessility among these freshwater bivalves apparently is a recently derived feature, suggesting that developmental plasticity of animals in ancient lakes rather than the antiquity of lineages might account for their unique morphology.

Similar phenomena on an albeit less spectacular level among the endemic radiations of the viviparous gastropod *Tylomelania* in the ancient lakes of Sulawesi (Rintelen *et al.* 2004) indicate that morphological evolution and speciation can occur very rapidly in ancient lake invertebrates, even though sexual selection plays no part. More studies on these often neglected species flocks overshadowed by their fish counterparts should consequently help to increase our understanding of radiation processes.

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