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Freshwater shrimp–sponge association from an ancient lake

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Shrimp–sponge associations occur frequently in marine ecosystems, serving as model systems for the evolution of eusociality. Here, we describe the first known instance of such association in freshwater from an ancient lake in Indonesia. The shrimp *Caridina spongicola* forms an exclusive and probably commensal association with a yet undescribed spongillinid sponge. Phylogenetic and ecological data suggest a comparatively recent origin of both taxa. Climatic fluctuations may have facilitated speciation and occasional hybridization of the shrimp species, which is derived from a rock-dwelling ancestor. Their extremely localized occurrence in an increasingly disturbed area makes both taxa a conservation priority.

Keywords: shrimp–sponge association; ancient lake; recent speciation; ecology; Sulawesi

1. INTRODUCTION

Shrimps inhabiting sponges are well known in marine ecosystems, and members of different families (e.g. Alpheidae, Palaeomonidae) form specific, obligate associations with various sponge species (Arndt 1933). Sponge-dwelling snapping shrimps (*Synalpheus*) represent one of the most speciose crustacean genera, and have independently evolved eusociality in some species (Macdonald *et al.* 2006). In contrast to this well-established marine diversity, no sponge-inhabiting shrimp had so far been found in freshwater. Here, we describe the first instance of a shrimp–sponge association in an ancient lake in Indonesia.

Ancient lake faunas are outstanding among all other freshwater biota owing to their often peculiar species, which are usually distinct from that of surrounding freshwater habitats. The Indonesian island Sulawesi has two ancient lake systems (figure 1*a*), Lake Poso and the Malili lake system, with typical *lacustrine* species assemblages and a fair share of endemic and unique forms. Among these are a cementing bivalve (Rintelen & Glaubrecht 2006) and limpet-like pulmonates that live epibiontic on other snails (Albrecht & Glaubrecht 2006). Our discovery of an exceptional shrimp–sponge association in one of the lakes as the only such record in freshwater further confirms the peculiar nature of the lakes' fauna.

Electronic supplementary material is available at http://dx.doi.org/10.1098/rsbl.2006.0613 or via http://www.journals.royalsoc.ac.uk.

2. MATERIAL AND METHODS

The shrimp material and the Sulawesi sponges used for this study were collected in the Malili lakes in September 2003 (by K.v.R. & T.v.R., shrimps and sponges), March 2004 (by T.v.R. & M.G., sponges only), August 2004 (by K.v.R. & T.v.R., shrimps and sponges) and January 2005 (by K.v.R., T.v.R & Y.C., shrimps only). Prior to the preservation in 95% ethanol, the shrimp specimens were separated based on substrate and colour pattern, which has been photographically documented in the field. Voucher specimens of shrimps and sponges are deposited in the Museum of Natural History, Berlin (ZMB), see electronic supplementary material for accession numbers.

DNA was extracted exclusively from abdominal tissues (shrimps) and excised interior tissues (sponges), respectively. For the molecular phylogeny of *Caridina*, two mitochondrial gene fragments, 861 bp of the cytochrome oxidase subunit I (COI) and approximately 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 (5′-GCTGCTAATTTTAT ATCTACAG-3′) and COI-R-Car (5′-TGTGTAGGCATC TGGGTAATC-3′), and atyid-specific primers 16S-F-Car (5′-TG CCTGTTTATCAAAAACATGTC-3′), 16S-R-Car (5′-AGATAG AAACCAACCT-GGCTC-3′) and 16S-R-Car1 (5′-GAAAGATAG AAACTAACCTGGCT-3′). For the sponges, the entire nuclear 18S rRNA gene was amplified using primers 18a_mdl (5′-AACC TGGTTGATCCTGCCAGT-3′) and 18b_mdl (5′-TGATCCTT CTGCAGGTTCACC-TAC-3′; Medlin *et al.* 1988).

The orthologous DNA sequences for each group were aligned using default settings, by Clustal W, v. 1.81 (Thompson *et al.* 1994), and optimized by eye. For *Caridina*, the aligned sequence sets of COI (781 bp) and 16S (546 bp) were combined into a single alignment after separate analyses yielded congruent topologies. *Caridina lanceolata* Woltereck, 1937 from the Malili lakes was used as an outgroup. The alignment of the sponge sequences has a length of 1702 bp and three marine taxa (*Spongia officinalis*, *Mycale fibrexilis* and *Halichondria melanodocia*) were chosen as the outgroup.

The phylogenies have been estimated by Bayesian inference with MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003). Appropriate models of sequence evolution were selected using MrModeltest v. 2.2 (Nylander 2004), and consequently the HKY $+\text{I}+\Gamma$ model (*Caridina*) and the GTR $+\text{I}+\Gamma$ (sponges) were employed. The posterior probabilities of phylogenetic trees were estimated by 1 000 000 (*Caridina*) and 2 000 000 (sponges) generation Metropolis-coupled Markov chain Monte-Carlo algorithms (four chains, chain temperature=0.2), with parameters estimated from the dataset. A 50% majority-rule consensus tree was constructed following a 50% burn-in (5000 and 10 000 trees, respectively). The likelihood value plots output by MrBayes were used to check for the stationarity of parameters.

All sequences have been deposited in GenBank; see electronic supplementary material for accession numbers.

3. RESULTS

The recently described decapod freshwater shrimp *Caridina spongicola* (Zitzler & Cai 2006) from one of the Malili lakes of Sulawesi (figures 1*b* and 2*a*) is an obligate sponge-associate. It dwells exclusively on and in a yet undescribed sponge of the suborder Spongillina (figure 2*c*). The sponge is soft tissued and can reach a diameter of up to approximately 0.2 m. *Caridina spongicola* can occur in high densities, the maximum number of specimens found in a single-dissected sponge was 137 (mean 28.73, $n=15$ sponges examined). Both shrimp and sponge show an extreme degree of endemism, being restricted to part of the outlet bay (figure 1*b*) of the largest and the southernmost Malili lake, Lake Towuti, where they occur in a depth of 3–5 (−10) m.

The shrimp clade based on data from an mtDNA phylogeny (figure 2*b*) comprises four morphologically distinguishable species (*C. spongicola*, *Caridina spinata*, *C.* sp. 1 and *C.* sp. 2), each with a highly specific body

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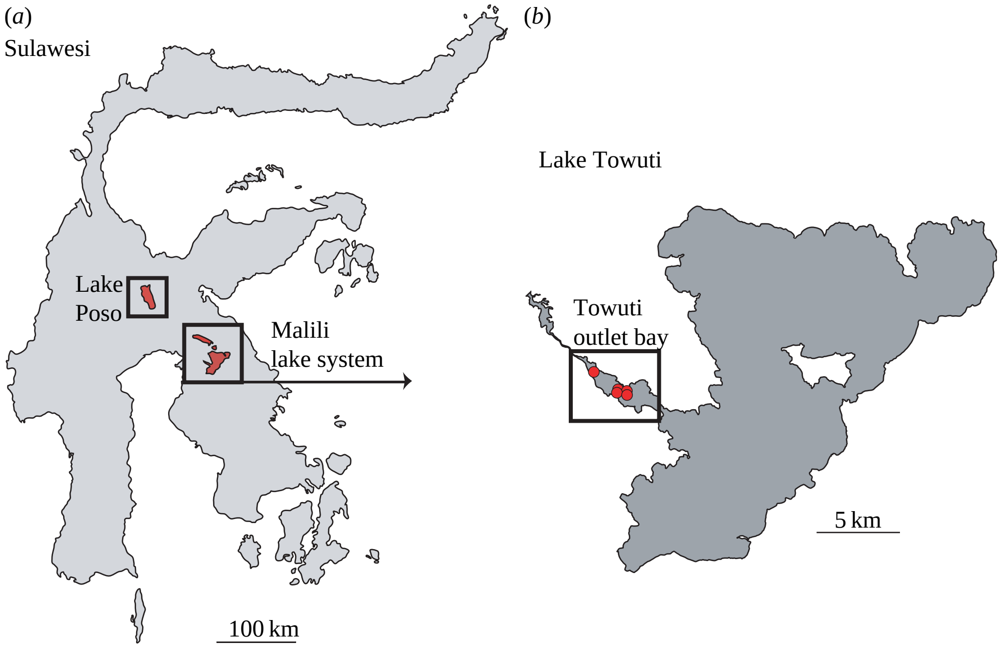

Figure 1. The Indonesian island of Sulawesi. (*a*) The two ancient lake systems of Sulawesi. (*b*) Occurrence of *Caridina spongicola* on its sponge host in the outlet bay of Lake Towuti, the largest and the southernmost of the Malili lakes.

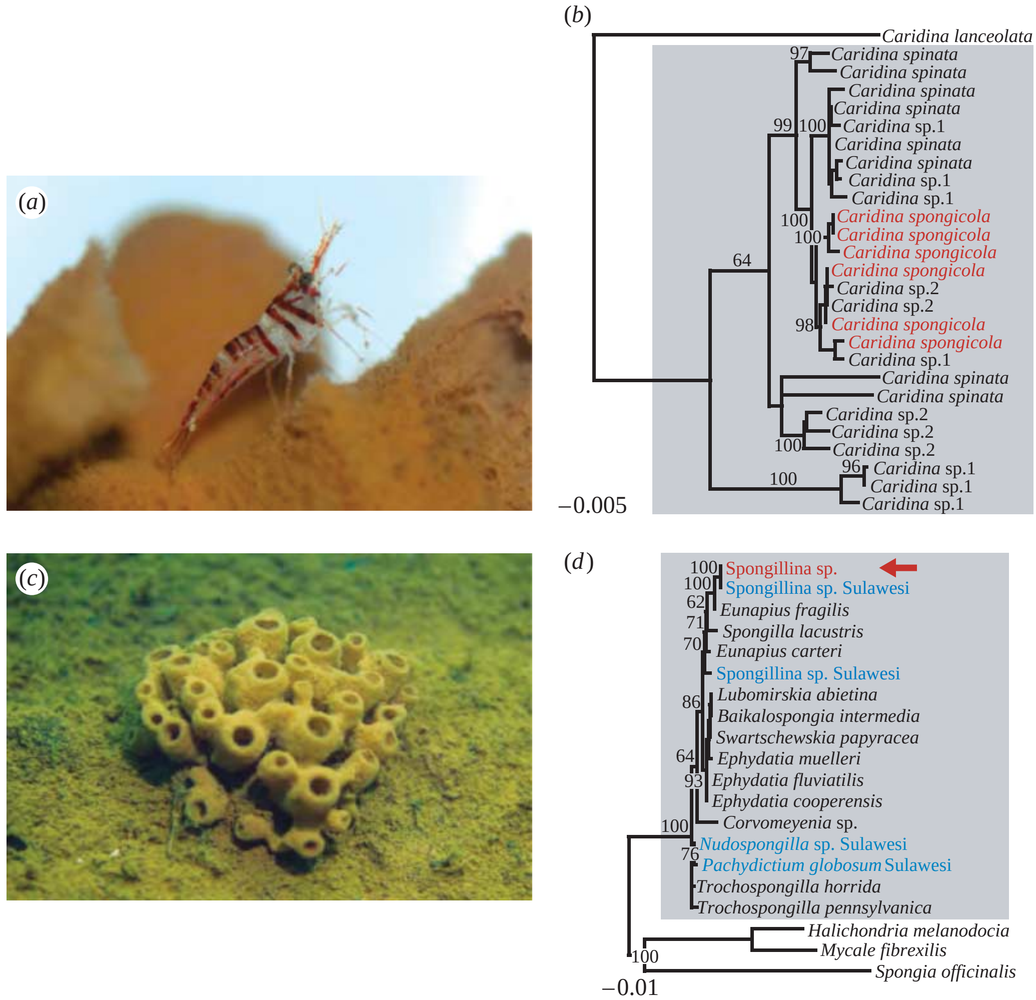

Figure 2. *Caridina spongicola* and its sponge associate. (*a*) *Caridina spongicola* in its natural habitat. Carapace length of adult specimen approximately 1.8–2.8 mm. (*b*) mtDNA phylogeny of Lake Towuti rock-dwellers (grey box) and sponge-dweller (red) based on 16S and COI (outgroup *C. lanceolata*). (*c*) Habitus of Spongillina sp. in the outlet bay of Lake Towuti. (*d*) Position of spongillinid species inhabited by shrimps (red arrow) and other Sulawesi sponges (blue) in a nuclear phylogeny of freshwater sponges (grey box) based on 18S. The sponges referred to as Spongillina sp. cannot yet be assigned to a genus within that group. Scale in (*b*,*d*) is substitutions per site.

coloration, but extensively shared haplotypes. All are endemic to Lake Towuti and dwell exclusively, except for *C. spongicola*, on hard substrate (rocks).

With its prominent oscula (figure 2*c*), the Towuti sponge resembles its marine cousins, but 18S rRNA data (figure 2*d*) clearly support a derived phylogenetic position within the freshwater taxon Spongillina, separated from the remaining Sulawesian freshwater sponges (Spongillina sp., *Nudospongilla* sp. and *Pachydictium globosum*).

4. DISCUSSION

The morphological peculiarity of the sponge obviously represents a case of parallel evolution between this freshwater lineage and marine taxa. The recent origin inferred for the sponge is concordant with data from an mtDNA phylogeny of the Malili lake shrimps indicating that *C. spongicola* has a comparatively recent origin as well. The topology either suggests an incomplete lineage sorting or introgression, both common phenomena among young species (Funk & Omland 2003).

The exact nature of the association between *C. spongicola* and the spongillinid remains to be clarified. The shrimps seem to use the sponge cavities as shelter and its inherent accumulation of diatoms as a food supply (Zitzler & Cai 2006), thus rather indicating commensalism. However, our observations so far do not allow us to place a definite decision as to commensalism versus alternatives, such as mutualism or parasitism.

Field observations suggest a possible scenario for the origin of the sponge-inhabiting specialist, as in the Towuti clade all species except this are rock-dwellers. While the water level of the lake is usually varying 1–2 m between seasons, exceptionally higher amplitudes occur as, for example, in January 2005, when a lower water level resulted in the exposure of shallow water rock areas. This caused significant changes in species assemblages. While *C. spongicola* was not directly affected due to the comparatively deep occurrence of the sponge, a rock-dwelling species was, uniquely, found on the sponges as well. We therefore anticipate that *C. spongicola* is derived from a rock-dwelling ancestor and climatic effects may have played a role in facilitating speciation and in addition provide an opportunity for occasional hybridization.

The shrimp–sponge association described here is unique in freshwater. On the shrimp side, this is the most extreme specialization found in the adaptive radiation of *Caridina* in the ancient lakes of Sulawesi. Their extremely localized occurrence, however, makes both shrimp and sponge rather vulnerable to extinction by human influences. Imminent threats are habitat destruction due to canalization work in the outlet area for the hydroelectric dams of a large nickel mine operating in the area, and possibly aquarium trade, where *Caridina* is a well sought-after pet due its partially flamboyantly coloured species. The Malili lakes have recently received much interest from this side (Chris Lukhaup 2006, personal communication). Protective measures should be taken to ensure not only the existence of the two beautiful species but also to enable further research on the evolution of specialization and interspecific association in this ancient lake model system that can continue to improve our understanding of the origin of these traits.

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