RESEARCH NOTE

Ecrobia grimmi in brackish Lake Sawa, Iraq: indirect evidence for long-distance dispersal of hydrobiid gastropods (Caenogastropoda: Rissooidea) by birds

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Mudsnail species of the genera *Hydrobia* and *Ecrobia* (junior synonym *Ventrosia*) belong to morphostatic or nonadaptive radiations. Although these genera are anatomically well defined, their species can only be confidently identified using molecular data. During the last decade, the status, distribution and evolutionary history of these coastal, brackish water taxa have been clarified in large part. In general, congeneric (sub)-species have nonoverlapping ranges replacing each other ecologically on large geographical scales (Wilke & Davis, 2000; Wilke, Rolán & Davis, 2000; Wilke & Pfenninger, 2002; Wilke, Pfenninger & Davis, 2002; Wilke, 2003; Kevrekidis, Wilke & Mogias, 2005). However, the discovery of isolated inland populations of these snails, such as an *Ecrobia* in Lake Sawa in Iraq, does not fit into this picture. Lake Sawa, which is located in the southwestern part of Iraq, is a mixomesohaline water body. It has a maximum depth of 5.5 m and no surficial influx or outflow. The lake is fed by groundwaters of the Euphrates and Dammam aquifers and its water chemistry, which is unique among Iraqi lakes, suggests that it is probably of relatively recent karstic origin and not a relic of a mid-Holocene marine incursion (Jamil, 1977; Naqash, Banat & Al-Shamee, 1977; Plaziat & Younis, 2005; Hassan, Al-Saadi & Alkam, 2006). The distances between Lake Sawa and the nearest populations of brackish-coastal hydrobiids are over 800 km to the Caspian Sea and over 900 km to the Mediterranean (Fig. 1).

The purpose of this note is to identify the hydrobiid snail that lives in Lake Sawa and to infer its origin based on molecular analyses. We also discuss, within this context, the importance of birds as vectors for the dispersal of coastal hydrobiid gastropods, which has been contested (e.g. Barnes, 1988).

Snails in Lake Sawa were collected by hand or washed from stones and fixed in *ca* 85% ethanol. Dissections indicated that they belonged to the genus *Ecrobia*. In order to identify the species we sequenced a 638-bp fragment of the cytochrome *c* oxidase subunit I gene (COI) of three specimens and compared it with that of congeners whose sequences were extracted from GenBank (for *E. ventrosa* we selected five individuals spread across its entire geographic range) as well as to unpublished sequences from our ongoing phylogenetic studies. *Hydrobia acuta acuta*, *Peringia ulvae* and *Salenthydrobia ferreri* served as outgroups (Table 1). DNA preparation and PCR followed standard procedures (Wilke *et al.*, 2006; Haase, 2008). For sequencing, PCR products were sent to AGOWA (www.agowa .de). Base composition was homogeneous, a prerequisite for model-based phylogenetic analyses. Phylogenetic analyses were conducted based on the optimality criteria maximum parsimony (MP) and maximum likelihood (ML) as well as by neighbour-joining (NJ) and Bayesian inference (BI). Optimal substitution models (HKY + Γ) were inferred using jModeltest

v. 0.1 (Posada, 2008) and MrModeltest v. 2.3 (Nylander, 2004), respectively. Trees were reconstructed in PAUP* v. 4b10 (Swofford, 2003) and MrBayes v. 3.1.2 (Ronquist & Huelsenbeck, 2003). For MP and ML, starting trees were obtained via stepwise addition. The addition sequence was random and random trees were used as starting points. Tree search went through 10,000 replicates, each limited to 10^8 rearrangements, which were done by TBR-branch swapping. Bootstrapping was performed with 10,000 (MP, NJ) and 1,000 (ML) replicates, respectively. For BI, four Metropolis-coupled Markov chains each searching the tree space in two parallel runs were sampled every 100th generation of a total of 2,000,000. The burn-in was conservatively set to 500,000 generations. At this point, the standard deviation of split frequencies had already dropped below 0.01, suggesting that both searches were already sampling around the same optimum. Convergence of parameters was monitored using Tracer v. 1.4 (A. Rambaut & A. Drummond, http://tree.bio .ed.ac.uk/software/tracer/) as well as the criteria implemented in MrBayes.

The three specimens from Lake Sawa had identical sequences. They differed from the Caspian sequence of *E. grimmi* in only three positions and therefore are most likely conspecific. However, assigning a specific name can only be done tentatively in this case owing to the large number of nominal hydrobiid taxa that were described from the Caspian and Aral Seas by Starobogatov and co-workers (see Filipov & Riedel, 2009, and references cited therein). Filipov & Riedel (2009) suggested that only a single species is involved, with the oldest available name for this snail being *Hydrobia grimmi* Clessin & Dybowski, 1888. Hence, we tentatively assign the hydrobiid snail that lives in the Caspian Sea and Lake Sawa to this species, pending detailed taxonomic study of the hydrobiid taxa of the Ponto-Caspian region.

The tree reconstructions are summarized in Figure 2 based on the ML topology, which had the highest resolution. The closest relative of *E. grimmi* was, as expected based on distributional evidence, *E. maritima*, which lives in the Black Sea and adjacent Mediterranean (Kevrekidis *et al.*, 2005). Surprisingly, the sister species of this clade was *E. truncata*, a Western Atlantic species. *Ecrobia ventrosa*, which is widely distributed in the Mediterranean and northeast Atlantic (Wilke & Davis, 2000), was sister to the above species. An unnamed species from the Tunisian island of Djerba branched off at the base of the tree; this will be dealt with in a comprehensive phylogeographic analysis of the entire genus that is in progress.

The support values of the four tree-reconstruction methods were very heterogeneous (Fig. 2). Posterior probabilities usually are expected to be higher than bootstrap values. However, this was only the case in two of the seven nodes supporting the species and their relationships. Two nodes were

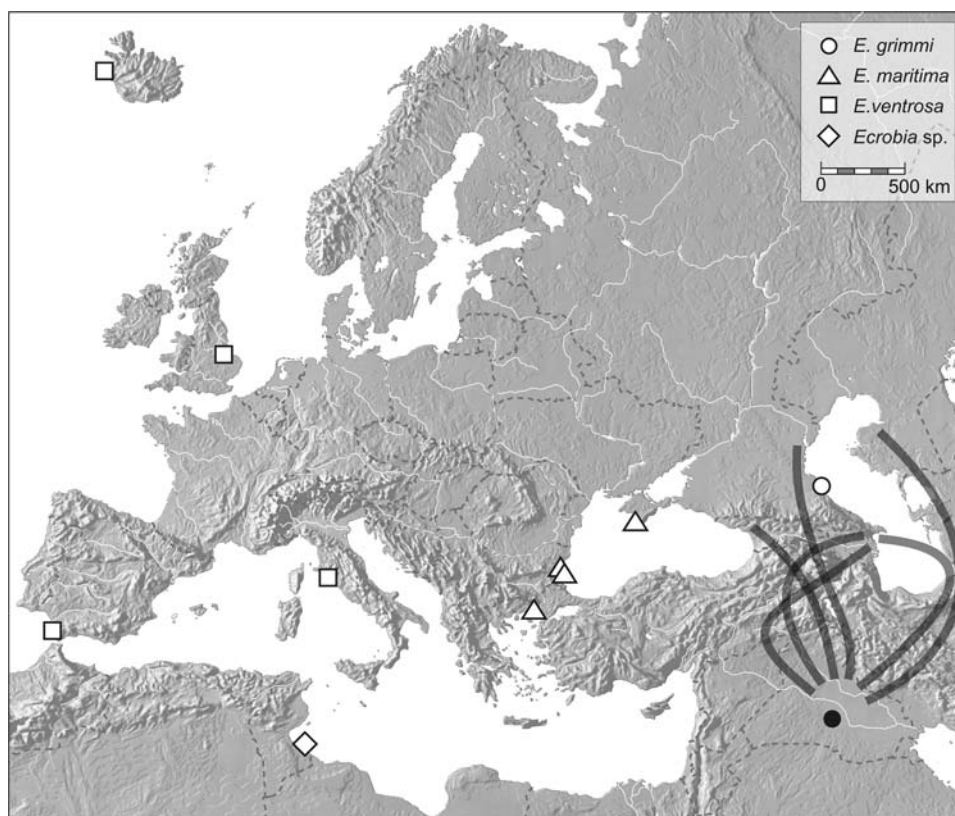Correspondence: M. Haase; e-mail: martin.haase@uni-greifswald.de

Figure 1. Map of Europe and adjacent regions showing *Ecrobia* sampling localities (excluding *E. truncata* from North America). The Lake Sawa population is shown as a black dot. The thickened lines show major bird migration routes from the Caspian and Black Sea to the greater Lake Sawa area (compiled from Combreau, Launay & Al Bowardi, 1999; SEEN, 2006; AEWA, 2008). The base map was generated using Mountain High Maps 2.5.

Table 1. Locality data (including latitude–longitude coordinates) and GenBank accession numbers for samples used in the phylogenetic analyses.

Species	Locality	Coordinates	Accession no.
Ecrobia grimmi	Iraq, Lake Sawa	31.302°N, 45.009°E	GQ505912
E. grimmi	Russia, Dagestan, Caspian Sea, Sulak Bay	43.301°N, 47.518°E	GQ505913
E. maritima	Bulgaria, bay between Strandscha and Kraimorie	42.430°N, 27.513°E	AF449216
E. maritima	Bulgaria, Nessebar	42.660°N, 27.720°E	AF253076
E. maritima	Bulgaria, Nessebar	42.660°N, 27.720°E	AF253078
E. maritima	Greece, Evros Delta	40.769°N, 26.054°E	AY616140
E. maritima	Ukraine, Sevastopol	44.610°N, 33.450°E	AY616139
E. sp.	Tunisia, Djerba	33.820°N, 11.070°E	AY616138
E. truncata	USA, NJ, Stone Harbour	39.500°N, 74.700°W	AF449217
E. ventrosa	Iceland, Galgahraun	64.467°N, 22.167°W	AF118341
E. ventrosa	Russia, Levin Navolok	66.317°N, 33.533°E	AF118346
E. ventrosa	Italy, Orbetello Lagoon	42.450°N, 11.217°E	AF118324
E. ventrosa	UK, Snettisham Lagoon	52.863°N, 0.460°E	AF118334
E. ventrosa	Spain, Bay of Cadiz	36.394°N, 6.138°W	AF118329
Hydrobia a. acuta	France, Étang du Prévost	43.513°N, 3.897°E	AF278809
Peringia ulvae	Germany, Altenbruch	53.850°N, 8.750°E	AF118293
Salenthydrobia ferreri	Italy, Porto Cesareo	40.283°N, 17.838°E	AF449201

recovered throughout the posterior distribution of tree samples of BI and in all MP bootstrap replicates; in one node, the MP bootstrap value was much higher than the respective posterior probability, and two nodes were not supported at all by BI. We assume that this was due to overcorrection of branch lengths, since the odd behaviour of posterior probabilities occurred on short branches. The same may hold for the bootstrap values resulting from ML. The on-average highest support came from MP with at least 90% in six of the seven deeper nodes considered. The sister relationship of *E. ventrosa* to *E. truncata*, *E. maritima* and *E. grimmi* was supported (but only weakly) by the distance analysis.
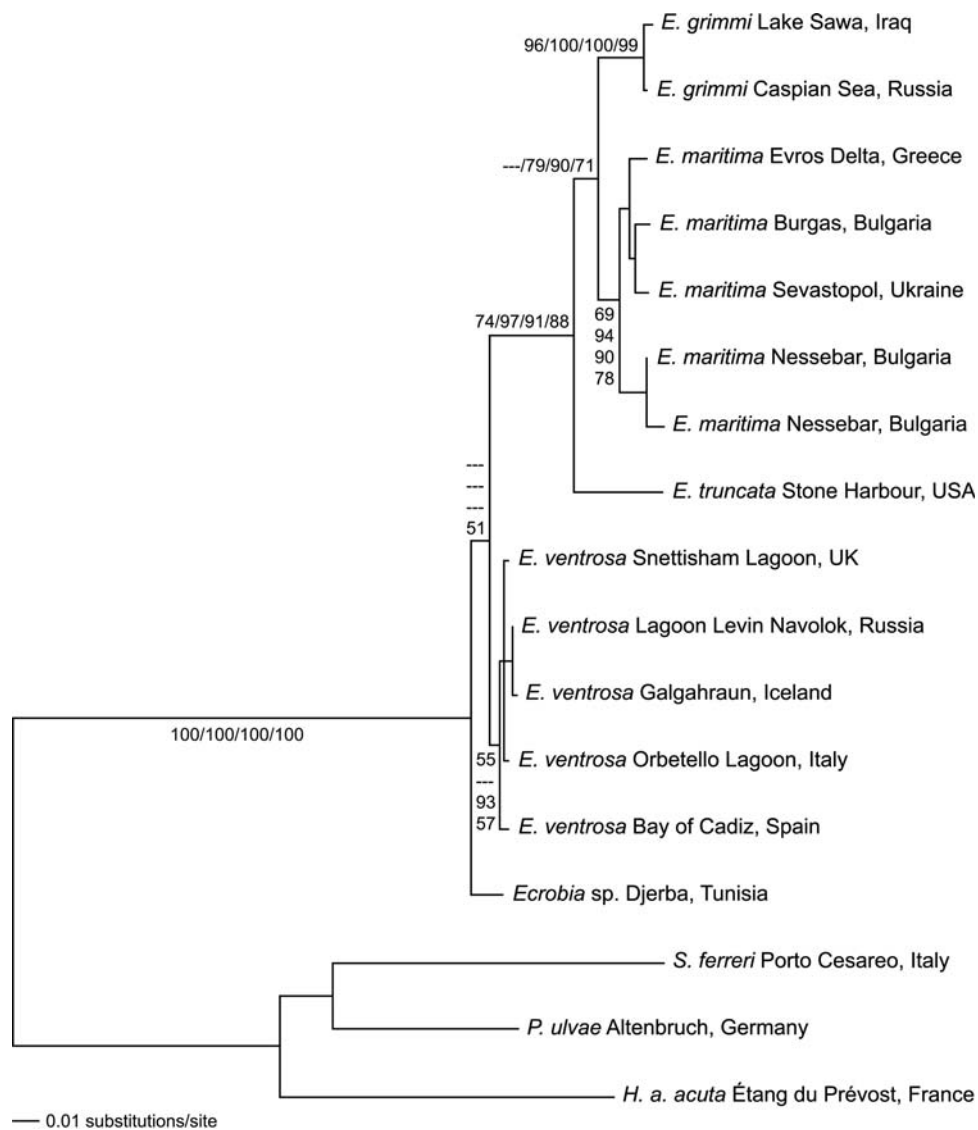
Figure 2. Maximum likelihood tree. Support values (bootstrap and posterior probabilities, respectively, as percentages) for species and deeper nodes: ML/BI/MP/NJ.

We are aware of a number of morphologically well-defined rissooidean congeners which are genetically cryptic (e.g. Haase, 2005; Haase, Wilke & Mildner, 2007). In the case of *Ecrobia* we are faced with the opposite situation: morphologically cryptic species that are genetically divergent. We employed three criteria in assigning species status within *Ecrobia*: species are monophyletic, separated by branches that are longer than those within species, and biogeographically discrete. Therefore, we did not distinguish the well-differentiated clades from the Black Sea and allocated both to *E. maritima*. The addition of more specimens and possibly also more loci might yield a clearer picture in this case. Based on our criteria it is clear, however, that within a morphologically cryptic radiation species that have recently evolved by peripatric or even sympatric speciation, which rendered their stem species paraphyletic, cannot be identified as above.

The biogeographical coherence of *E. grimmi* from the Caspian Sea and Lake Sawa in Iraq is not immediately obvious. Coastal hydrobiids are commonly found in brackish or marine land-locked lagoons close to the European and North-African coasts. Barnes (1988) suggested that East Anglian (UK) land-locked lagoonal populations were founded by rafting on algae or, in case of *P. ulvae*, by dispersal of planktonic larvae during occasional connections (by flooding) with the open sea, but he considered transport by water birds unlikely. However, coastal hydrobiids have been occasionally reported from brackish inland water bodies, e.g. North African oases and lakes surrounded by desert (Sattmann & Kinzelbach, 1988; Brown, 1994) or the Süße See in Central Germany (Wennrich, Meng & Schmiedl, 2007); these, like Lake Sawa, originated independently of and have never been connected with the sea. The ancestors of these populations presumably arrived by vectors connecting open coastal with inland waters, most likely water birds on their autumn migrations (e.g. Berthold, 2001; see also Fig. 1). Lake Sawa is frequented by migrating birds (M.D. Naser, personal observation), although there is no comprehensive account of its importance as a resting place for these animals. Wading birds such as the Caspian Plover *Charadrius asiaticus* (del Hoyo, Elliot & Sargatal, 1996) may have been responsible for the introduction of Caspian biota into Lake Sawa. In general, birds are known to be important vectors for long-distance transportation of aquatic organisms, including gastropods. Animals may get caught in the plumage, stick to the feet, or survive passage

through the gut of these birds (Rees, 1965; Charalambidou & Santamaria, 2002; Figuerola & Green, 2002; Green, Figuerola & Sanchez, 2002). Although there is no direct evidence for hydrobiids travelling with birds, this mechanism has been invoked to explain unusual distribution patterns of various rissooidean gastropods, e.g. the occurrence of tateids on several Pacific island groups (Haase, Gargominy & Fontaine, 2005; Haase, Ponder & Bouchet, 2006; Haase, Fontaine & Gargominy, in press; see Liu, Hershler & Clift, 2003 for a North American example). Hydrobiids can survive desiccation and anaerobic conditions for several days (Jensen, Latama & Mouritsen, 1996) and also tolerate passage through the gut of fishes (Aarnio & Bonsdorff, 1997). All circumstantial evidence therefore indicates that *E. grimmi* colonized Lake Sawa through transportation by water birds. It is possible, though, that the lake has not been colonized directly from the Caspian Sea. However, the only potential stepping stones would have been relictual water bodies of the mid-Holocene marine incursion (Plaziat & Younis, 2005), which still would imply bird-mediated dispersal over a similarly large distance.

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