



Research

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Phylogeny

Conquest of the deep, old and cold: an exceptional limpet radiation in Lake Baikal

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Lake Baikal is the deepest, oldest and most speciose ancient lake in the world. The lake is characterized by high levels of molluscan species richness and endemism, including the limpet family Acroloxidae with 25 endemic species. Members of this group generally inhabit the littoral zone, but have been recently found in the abyssal zone at hydrothermal vents and oil-seeps. Here, we use mitochondrial and nuclear data to provide a first molecular phylogeny of the Lake Baikal limpet radiation, and to date the beginning of intra-lacustrine diversification. Divergence time estimates suggest a considerably younger age for the species flock compared with lake age estimates, and the beginning of extensive diversification is possibly related to rapid deepening and cooling during rifting. Phylogenetic relationships and divergence time estimates do not clearly indicate when exactly the abyssal was colonized but suggest a timeframe coincident with the formation of the abyssal in the northern basin (Middle to Late Pleistocene).

1. Introduction

Ancient lakes are key sources of biodiversity and have taken an important role in improving our understanding of speciation and adaptive radiation (e.g. [1]). Lake Baikal is one of the most famous ancient lakes, because it is the world's deepest (max. depth: 1642 m [2]) and oldest (*ca* 30 Myr [3,4]) freshwater lake and home to 2595 (1455 endemic) different animal taxa known to science [5]. The remarkably old age of Lake Baikal led to the assumption that some of the endemic species flocks might be as old as the lake itself. However, first molecular studies instead suggested considerably younger ages for the majority of taxa (see review by Sherbakov [3]). This, in turn, raised the question whether the onset of radiation may be related to post-Pliocene speciation events when the lake attained its present tectonic and climatic setting after a series of alternating cool and warm periods (e.g. [4,6]). Perhaps the most important environmental change has been the complete oxygenation of Lake Baikal, potentially allowing both the colonization of the abyssal and diversification in deeper water of several taxa including oligochaetes [7], amphipods [8] and cottoid fishes [9].

The lake is inhabited by 148 gastropod species, of which 78% are endemic [10]. The majority of these occur in the photic zone (0–100 m), while only nine species are found in deeper water (aphotic zone) between 100 and 1380 m [11]. Limpets of the family Acroloxidae are found in the Holarctic, with only one representative in North America (*Acroloxus coloradensis*) and a

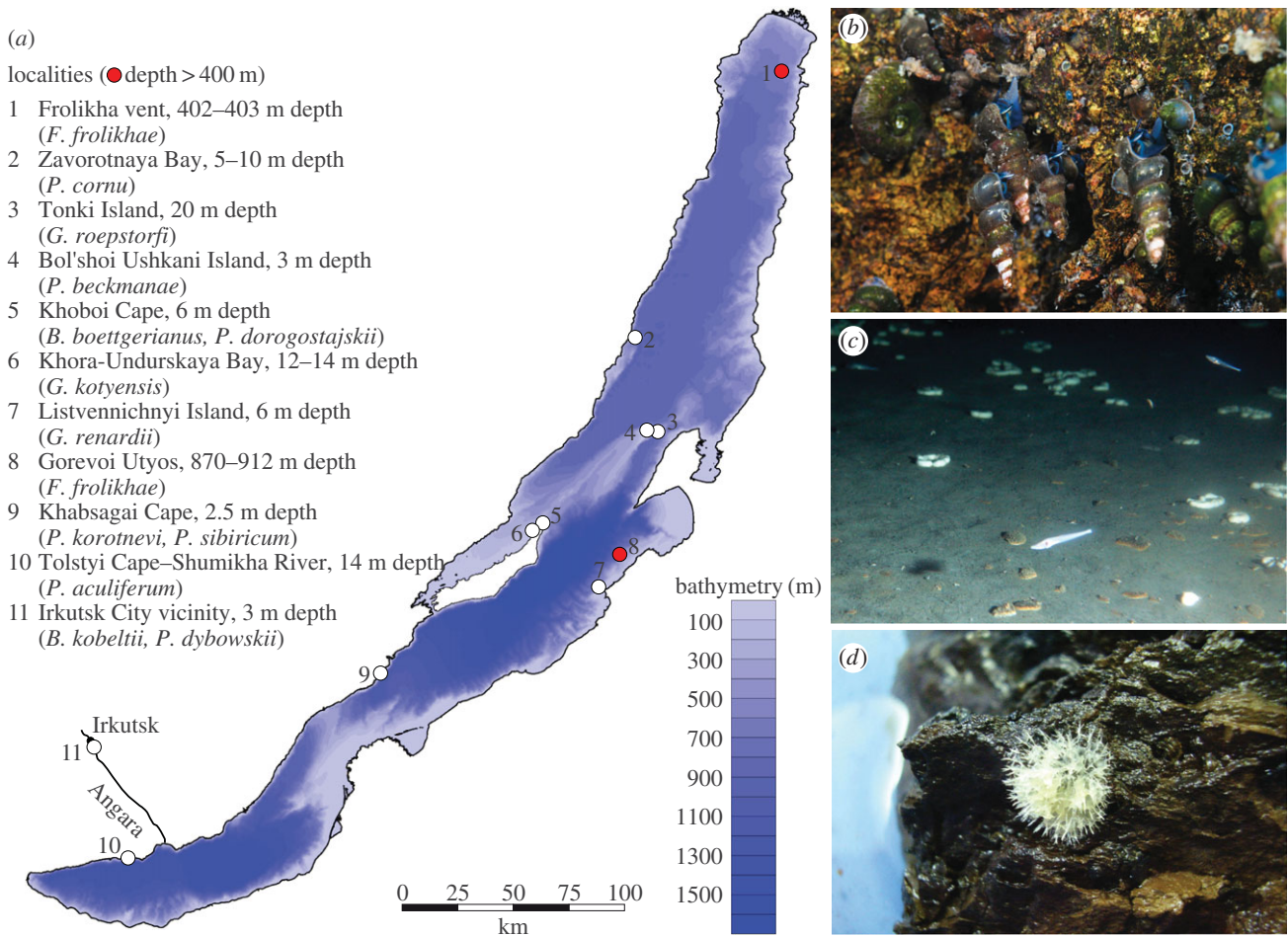


Figure 1. (a) Bathymetric map of Lake Baikal (<http://users.ugent.be/~mdbatist/intas/intas.htm>) including sample sites of Lake Baikal species. For full species names, see figure 2. Habitat pictures: (b) littoral, southern Baikal, 17 m depth, mollusc community on rocks, (c) abyssal, Frolikha Bay, northern Baikal, 407 m depth, silty bottom with bacterial and sponge mats and (d) abyssal, Gorevoi Utyos, central Baikal, 912 m depth, sponge on bitumen. (Online version in colour.)

few widespread species occurring across Europe (*A. lacustris*, *A. oblongus* and *A. shadini*). Further species are described from the Adriatic region, Turkey and Far East Russia, but the highest biodiversity is found in two ancient lakes, Lake Ohrid (three to four species [12]) and Lake Baikal (25 endemic species originally described in three endemic genera [13]; see the electronic supplementary material for taxonomic remarks). Furthermore, in these two lakes, limpets and other pulmonate snails have colonized the sublittoral and abyssal [10,14,15]. Such colonizations of deep water, particularly into the abyssal zones, represent a remarkable exception for pulmonate snails, as it generally requires the adaptation to both higher pressure and lower oxygen levels. However, since Lake Baikal is oxygen-saturated throughout the water column, limpets not only inhabit the shallow photic zone (the majority of species occur in depths of 1–40 m), but also have been recently recorded from hydrothermal vents and oil-seeps in the lake's northern and central basin at 340–430 and 912 m depth [11,16].

Here, we provide the first molecular phylogeny of the Lake Baikal acroloxid species to test whether the deep-water species falls within an endemic Lake Baikal limpet clade, and investigate whether these Baikal species form a species flock. Specifically, these analyses could identify if the flock has diversified in the littoral and started to colonize the abyssal. Moreover, we use fossil-calibrated molecular-clock analyses to estimate ages for the Lake Baikal clade and related taxa across the Holarctic.

2. Material and methods

(a) Taxon sampling, DNA extraction, amplification and sequencing

DNA of 30 specimens representing 19 species was isolated using a standard protocol for molluscs [17]. We included 13 species from Lake Baikal (figure 1) plus additional species from nearby localities outside the lake, the Amur region and specimens of *A. coloradensis*; *A. lacustris* from Germany and Albania was used as outgroup (see the electronic supplementary material, table S1 for specimen information and GenBank accession numbers). Two mitochondrial (COI, 16S rRNA) and two nuclear loci (28S rRNA, H3) were amplified (see the electronic supplementary material, table S2) and visualized on an ABI 3730 XL sequencer (Life Technologies) using a BigDye Terminator Kit (Life Technologies).

(b) Phylogenetic and molecular-clock analyses

16S rRNA sequences were aligned using MAFFT [18] and concatenated with the 28S rRNA, COI and H3 sequences, resulting in a final alignment of 2181 bp. Phylogenetic analyses were conducted using RAxML BlackBox [19] using GTR + Γ for each of the four partitions (see the electronic supplementary material, figure S1). Estimation of divergence times was performed in BEAST v. 1.8.0 [20] using a single fossil calibration point, a fossil of *A. coloradensis* (ca 2.2–3.0 Myr), which provides the lower constraint on the divergence of *A. coloradensis* and its sister group (including *A. arachleicus*, *A. likharevi*, *A. victori* and *A. baicalensis*). A lognormal

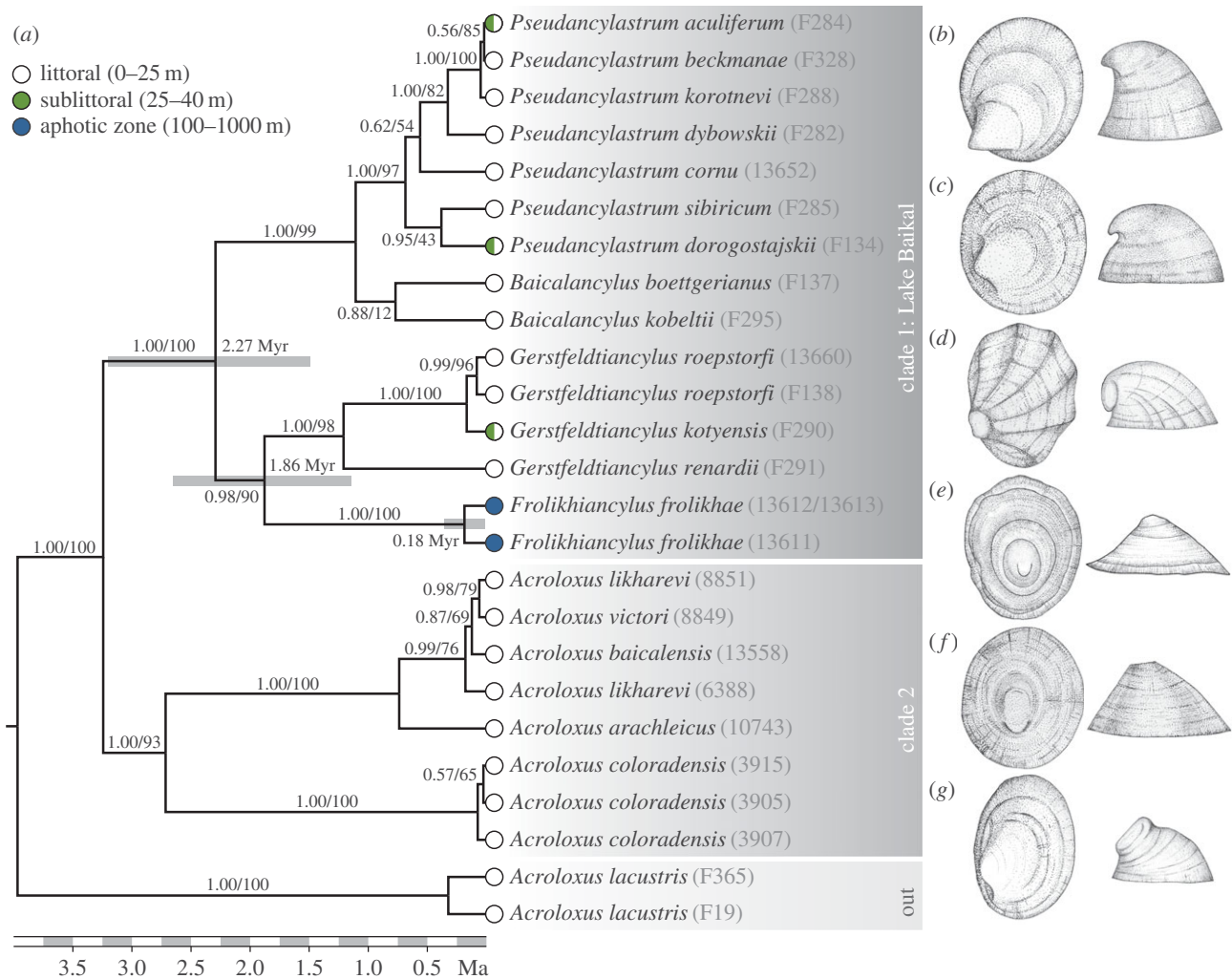


Figure 2. (a) BEAST MCC tree showing acroloid relationships. Numbers at nodes represent estimated divergence times for selected nodes (including the 95% credibility intervals); numbers on branches denote RAxML bootstrap values and BEAST posterior probabilities. Circles at tips represent vertical distribution documented for each species. Habitus pictures of selected Baikalian species (from [22]; not to scale): (b) *P. beckmanae*, (c) *P. dorogostajskii*, (d) *B. boettgerianus*, (e) *G. roepstorfi*, (f) *G. renardii* and (g) *F. frolikhae*. (Online version in colour.)

distribution was used with a mean of 0.0, a s.d. of 0.6 and an offset of 2.0 (see the electronic supplementary material for details, table S3 for substitution models applied and figure S2 for unconstrained BEAST MCC trees).

3. Results and discussion

The topology indicates that clade 1 (Lake Baikal species) may represent a species flock, as the group is endemic, monophyletic and species-rich (e.g. [21]). Intra-generic relationships are not well resolved in several cases; however, each of the genera examined represents a reciprocally monophyletic group, with *Baicalancylus* + *Pseudancylastrum* and *Gerstfeldtancylus* + *Frolikhiancylus* as potential sister groups (figure 2 and see the electronic supplementary material for some taxonomic remarks). Furthermore, the phylogeny shows that Lake Baikal's sister group (clade 2) includes *A. coloradensis* from North America plus a monophyletic clade comprising *A. arachleicus* (Lake Arakhlei), *A. likharevi* and *A. victori* (Far East Russia), and *A. baicalensis* (Siberian-Amur species). This Holarctic distribution of closely related species is suggestive of dispersal across a Bering land bridge. However, testing such biogeographic hypotheses requires a denser dataset including the remaining Palaearctic species and consideration of dispersal means, and is thus not within the scope of this study.

The fossil-calibrated BEAST analysis suggests that clade 1 started to diversify in the post-Pliocene *ca* 2.27 Myr (95% HPD, highest posterior density: 1.49, 3.20 Myr) and thus much later than the lake's origin. This process was potentially triggered by rapid deepening and cooling, associated with rifting (e.g. [6]) and is congruent with patterns observed in other invertebrate taxa [3,23,24]. The colonization of the hydrothermal vents and oil-seeps by *Frolikhiancylus* may have occurred in the Pleistocene, given divergence time estimates for both the split of the two populations (*ca* 0.18 Myr) and the time of the split to the sister group (*ca* 1.86 Myr; figure 2). Interestingly, this timeframe can be related to the formation of the abyssal in the northern basin *ca* 0.4–1.6 Ma [25]. Information on vertical distribution documented for each species (figure 2), unfortunately, does not allow a clear colonization scenario to be drawn. However, the majority of species examined inhabit the photic zone, and thus it is possible that the colonization of the deeper waters has been a single event. The two localities examined along Lake Baikal's east shore are about 260 km apart (linear distance) and *Frolikhiancylus* has recently only been found in two out of nine deep-water habitats sampled [11]. Furthermore, major lake-level fluctuations are not recorded for Lake Baikal which could potentially have driven geographical isolation of ancestral populations. We thus conclude that the abyssal was colonized from the littoral along with the

adaptation to the specific deep-water habitats (hydrothermal vents and oil-seeps).

Ethics. Collection of specimens was conducted in accordance with national and provincial guidelines and permits.

Data accessibility. Detailed information and data used for all analyses are available as electronic supplementary material. Supplementary files (RAXML tree file, BEAST xml and MCC tree files) are available at the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.p0dn0>.

Authors' contributions. C.A. conceived and designed the study. A.A.S., T.Y.S. and L.A.P. collected specimens, identified species and contributed habitat and habitus images. B.S., A.A.S. and C.C. performed laboratory work, analysed sequences and performed phylogenetic analyses. C.C. prepared the figures. B.S. and C.A. wrote the article and revised the first draft with input from A.A.S., C.C., T.Y.S. and

L.A.P.; all authors discussed the results and gave final approval for publication.

Competing interests. We declare we have no competing interests.

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