LIMPETS OF THE FAMILY LEPETIDAE (MOLLUSCA: PATELLOGASTROPODA) OF THE SHELF OF THE FAR EASTERN SEAS OF RUSSIA AND ADJACENT WATERS

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ABSTRACT

The family Lepetidae Gray, 1850 is a small group of the sea limpets widely distributed from tidal zone to abyssal. The monophyly of the family is supported by both morphological traits and molecular- phylogenetic analyses, but phylogenetic relations within family remain unsolved. The current phylogenetic analyses based on four gene markers (COI, 16S, 12S and H3) revealed a high supported clade including the Far Eastern lepetids. The ***Sagamilepeta sagamiensis*** is a sister species to ***Lepeta caeca*** and should be transferred into the genus ***Lepeta*** on the basis of morphological traits and genetic distances. ***Cryptobranchia*** is a separate genus which differs from ***Lepeta*** in both radular morphology and phylogenetic position. In addition, the analyses revealed second undescribed species of the genus ***Limalepeta*** which has amphiboreal distribution.

Fig. 1. Localities of speciments used for the current research

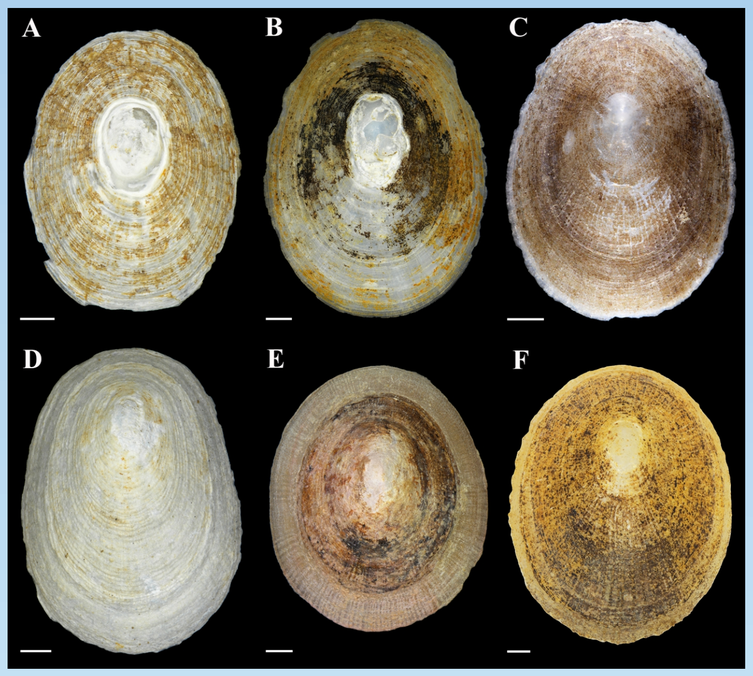

Fig. 2. Shells of the lepetids species used in the analysis. (A) *Lepeta caeca caeca* (White Sea); (B) *Lepeta caeca pacifica*, (Sea of Japan, Russia); (C) *Sagamilepeta sagamiensis* (Sagami Bay); (D) *Cryptobranchia concentrica* (Sea of Okhotsk, Magadan); (E) *Cryptobranchia kuragiensis* (Sea of Japan, Russia); (F) *Limalepeta lima* (Sea of Japan, Russia). Scale bar 1 mm.

Table 1. Primers and their annealing temperatures

Gene (Marker)	Primers	Sequences (5`–3`)	Annealing temperature
COI	dgLCO-1490 / dgHCO-2198	GGTCAACAAATCATAAAGAYATYGG / TAAACTTCAGGGTGACCAAARAAYCA	52 °C
12S	12S97L (F) / 12Smb	AACYCAAAGRACTTGGCGGT / CAGAGAGTGACGGGCGATTTGT	55 °C
16S	16LRN13398 / 16SRHTB	CGCCTGTTTAACAAAAACAT / ACGCCGGTTTGAACTCAGATC	55 °C
Histone H3	H3af / H3ar	ATGGCTCGTACCAAGCAGACVGC / ATATCCTTRGGCATRATRGTGAC	60 °C

INTRODUCTION

To the current data, the family **Lepetidae** includes 7 genera with 21 recent species, distributed mostly in polar, boreal and notal areas of the World Ocean. Their unique trait is the fusion of four inner lateral teeth of radula into the “central tooth”. According to Moskalev (1977), morphology of radula can be used as the main characteristics in lepetid taxonomy. Such approach allowed to divide North Pacific lepetids into four genera: ***Cryptobranchia*** Middendorff, 1851, ***Limalepeta*** Moskalev, 1977, ***Lepeta*** Gray, 1842 and ***Propilidium*** Forbes & Hanley, 1849 (Fig. 2). Nowadays ***Cryptobranchia*** is considered as a junior synonym to the genus ***Lepeta***, whereas ***Limalepeta*** remains a separate one. ***Sagamilepeta*** Okutani, 1987 is also regarded as a separate genus, but its differences from ***Lepeta*** seem to be quite unclear.

MATERIAL AND METHODS

Samples were collected in different localities in the Sea of Okhotsk, the Sea of Japan, the Bering sea, and the White sea (Fig. 1). The shell and radula were studied and photographed with usage of light microscopes Axio Lab.A1, Nikon SMZ25, and scanning electron microscope (SEM) Zeiss Sigma 300VP. Total genomic DNA was extracted from a piece of mantle or foot muscle tissue with a chelating resin Chelex 100. Primers and their annealing temperatures are listed in Table 1.

RESULTS

MORPHOLOGY

SHELL

The shell appearance of lepetids has few differences between species because it lacks the color pattern typical for most of sea limpets (Fig. 2). However, the shell sculpture is well distinguishable and somedifferences among examined species.For ***Lepeta caeca*** (O.F. Müller, 1776), the sculpture is formed by radial ridges consisting of rounded tubercles (Fig. 3A, B).

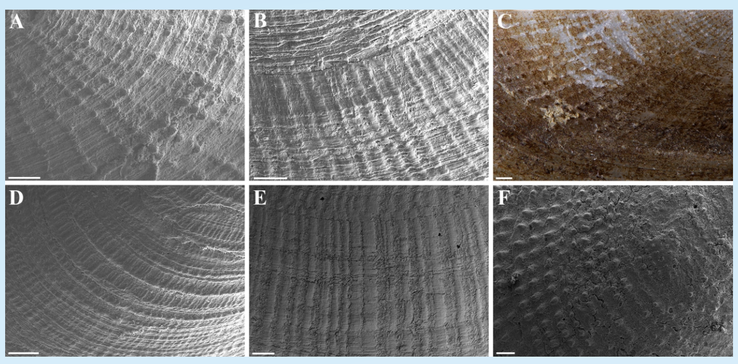

Fig. 3. Shell sculpture of the lepetids species used in the analysis (A, B, D–F – SEM, C – light microscope). (A) *Lepeta caeca caeca*; (B) *Lepeta caeca pacifica*; (C) *Sagamilepeta sagamiensis*; (D) *Cryptobranchia concentrica*; (E) *Cryptobranchia kuragiensis*; (F) *Limalepeta lima*. Scale bar: A, C–F, 200 μm; B, 100 μm.

The tubercles are more strongly marked on smaller shells than on large ones. The similar sculpture can be observed in ***Sagamilepeta sagamiensis*** (Kuroda & Habe, 1971) (Fig. 3C) and ***Limalepeta lima*** (Dall, 1918) (Fig. 3F). Radial ridges of ***Cryptobranchia kuragiensis*** (Yokoyama, 1920) are flattened and do not have tubercles (Fig. 3E). ***Cryptobranchia concentrica*** (Middendorff, 1848) has strongly developed concentric ridges which break the radial ones (Fig. 3D).

RADULA

The “central tooth” of all examined species is formed of two pairs of merged lateral teeth. This tooth has one large medial and two small lateral cusps. Within ***L. caeca*** and ***S. sagamiensis***, all three cusps are triangle (Fig. 4A, D), except for worn out teeth. As for ***C. concentrica***, ***C. kuragiensis***, and ***L. lima***, all cusps are rectangular. The medial cusp of ***C. concentrica*** and ***C. kuragiensis*** is significantly larger than the lateral ones (Fig. 4B, E), while cusps of ***L. lima*** are all quadrangular and equal in size (Fig. 4C, D). Both pairs of maginal teeth of ***L. caeca***, ***L. lima***, and ***S. sagamiensis*** have smooth edges (Fig. 4A, C, D, F), while edges of ***C. concentrica*** and ***C. kuragiensis*** have pectinate denticles (Fig. 4B, E).

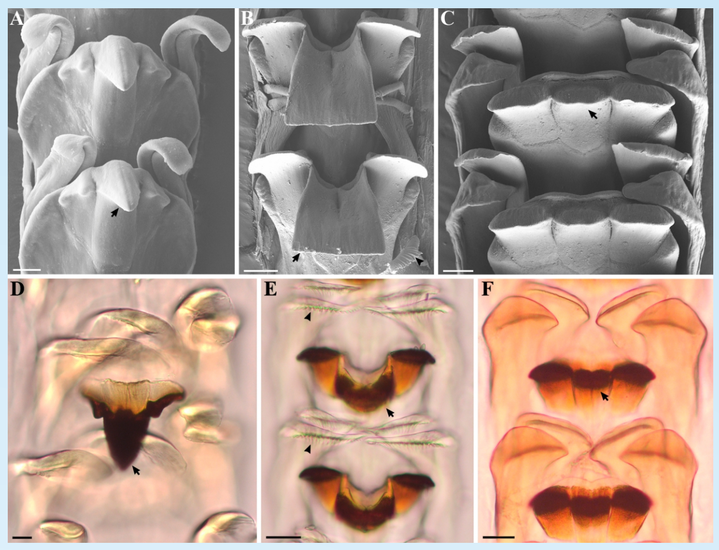

Fig. 4. Radula of the lepetids species used in the analysis (A–C – SEM, D–F – light microscope). (A, D) *Lepeta caeca*; (B, E) *Cryptobranchia kuragiensis*; (C, F) *Limalepeta lima*. Arrows show median cusp of the "central tooth", arrowheads show denticles on edge of the marginal teeth. Scale bar 20 μm.

MOLECULAR PHYLOGENETIC ANALYSES

The trees based on COI gene fragments, reconstructed with the Bayesian Inference (BI) and Maximum Likelihood (ML) methods, have similar topologies, resolutions, and nodal supports (Fig. 5). The clade Lepetidae is low supported by BI method (posterior probability values (pp) = 0.91), but highly supported by ML method (bootstrap values (BS) = 93%). The position of the genera ***Bathylepeta*** and ***Iothia*** in the trees and their relations with other genera of Lepetidae remains unsolved. However, the Far Eastern lepetids form a highly supported clade (pp = 1; BS = 87%) which includes two sister clades: (1) genera ***Sagamilepeta*** + ***Lepeta*** (pp = 0.96; BS = 89%) and (2) species of genera ***Limalepeta*** + ***Cryptobranchia*** (pp = 0.99; BS = 96%).The clade ***Sagamilepeta*** is low supported (pp = 0.72; BS = 76%), while clade ***Lepeta*** is highly supported (pp = 0.99; BS = 90%). ***Lepeta*** also forms two separated clades: highly supported ***Lepeta caeca pacifica*** Moskalev, 1977 (pp = 0.99; BS = 90%) and low supported ***Lepeta caeca caeca*** (pp = 0.81; BS = 71%). The ***Limalepeta*** clade (pp = 0.99; BS = 92%) includes two sister clades: (1) ***Limalepeta*** **sp**. (pp = 0.99; BS = 92%) and (2) ***Limalepeta lima*** (pp = 1; BS = 100%) The ***Cryptobranchia*** (pp = 0.99; BS = 96%) also forms two sister clades: (1) ***Cryptobranchia concentrica*** (pp = 1; BS = 100%) and (2) ***Cryptobranchia kuragiensis*** (pp = 1; BS = 98%).

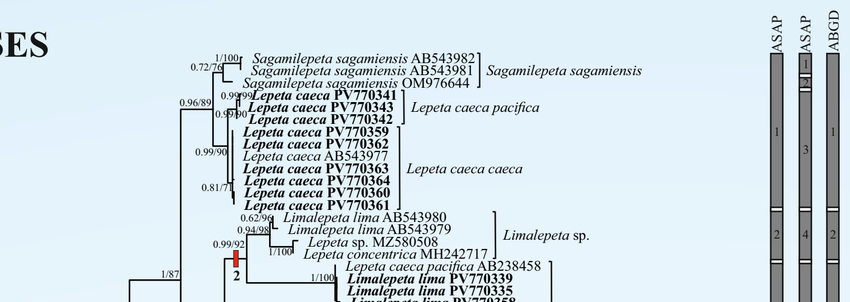

Fig. 5. Bayesian inference tree based on the Cytochrome Oxidase I sequence dataset. Numerals at nodes indicate posterior probabilities from Bayesian inference and bootstrap supports from Maximum likelihood analysis, respectively. Specimens studied in this work are highlighted in bold. Blocks on the right indicate species delimitation analyses results, numbers refer to respective operational taxonomical units. Synapomorphies (red rectangulars): 1, rectangular median cusp of radula; 2, quadrate and non-protruding median cusp of radula.

The COI-based haplotype networks corroborated the results of phylogenetic analyses. Within genus ***Limalepeta***, the samples form two heterogeneous species: ***Limalepeta lima*** and ***Limalepeta*** **sp.**, differed in 45–65 substitutions (Fig. 6). Genera ***Lepeta*** and ***Sagamilepeta*** have differences in 24–50 substitutions (Fig. 7). The ***Lepeta caeca*** specimens also form two heterogeneous groups (***L. caeca caeca***, ***L. caeca pacifica***) with differences of 9–17 substitutions. (Fig. 7).

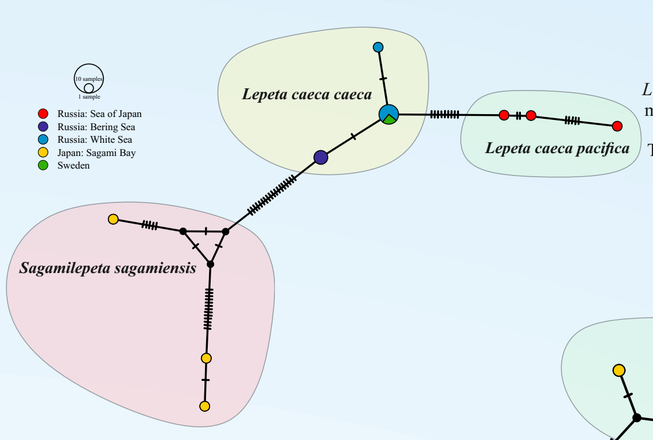

Fig. 6. COI-based haplotype network of *Lepeta* species produced with median-joining method in PopART. Colors of circles refer to the geographic origin of each haplotype. The relative size of circles is proportional to the number of sequences of the particular haplotype.

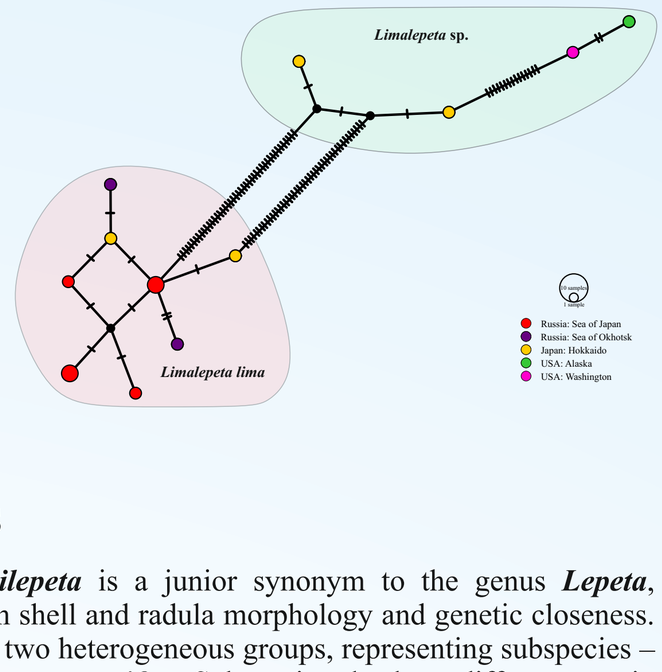

Fig. 7. COI-based haplotype network of *Limalepeta* species produced with median-joining method in PopART. Colors of circles refer to the geographic origin of each haplotype. The relative size of circles is proportional to the number of sequences of the particular haplotype.

The BI- and ML-trees inferred from the combined sequences of three mitochondrial markers (COI, 16S rRNA and 12S rRNA) as well as trees based on combined COI and H3 markers display similar topologies for Far Eastern lepetids and other clades.

CONCLUSIONS

1. The genus ***Sagamilepeta*** is a junior synonym to the genus ***Lepeta***, on the basis of uniform shell and radula morphology and genetic closeness.
2. ***Lepeta caeca*** forms two heterogeneous groups, representing subspecies – ***L. caeca caeca*** and ***L. caeca pacifica***. Subspecies also have diffenerences in shell sculpute, their geographic boundaries require clarification.
3. ***Cryptobranchia*** is a valid genus, separated from ***Lepeta*** genetically, morphologically (differences in shape of the “central tooth”, morphology of the edges of marginal teeth and shell sculpture), and even ecologically (***Cryptobranchia*** species inhabit depths of 0–80 m, ***L. caeca*** – 70–820 m).
4. The analyses revealed the second undescribed species of ***Limalepeta*** that includes two genetically different, and probably isolated groups of samples from Japan and Alaska. There are no morphological data about this species, but it greatly expands the geographical ranges of the genus ***Limalepeta***.