

# RHIZOCEPHALAN BARNACLES (CRUSTACEA: CIRRIPIEDIA) FROM PETER THE GREAT BAY OF THE SEA OF JAPAN

Darya D. Golubinskaya, Olga M. Korn

A.V. Zhirmunsky National Scientific Center of Marine Biology, Far Eastern Branch, Russian Academy of Sciences, Laboratory of Embryology, Russia, ddemchik@mail.ru

Rhizocephalan barnacles (Crustacea: Cirripedia: Rhizocephala) are extremely simplified parasites that infest mostly decapods and some other crustaceans. The structure of parasites is so reduced that only the morphology of the larvae reveals them as Crustacea. The rhizocephalan female consists of two functional parts: an external reproductive body (the externa) connected through a stalk to an internal system of trophic rootlets (the interna). Since the number of morphological characters of the externa is very limited, molecular analysis is required to correctly identify the rhizocephalan species. Larval morphology also provides the important information for taxonomy and phylogeny of this group. To study Rhizocephala, an integrative approach was used, including molecular and morphological methods that maximize the number of taxonomic characters. At present, at least ten rhizocephalan species belonging to four families occur in Russian waters of the Sea of Japan.

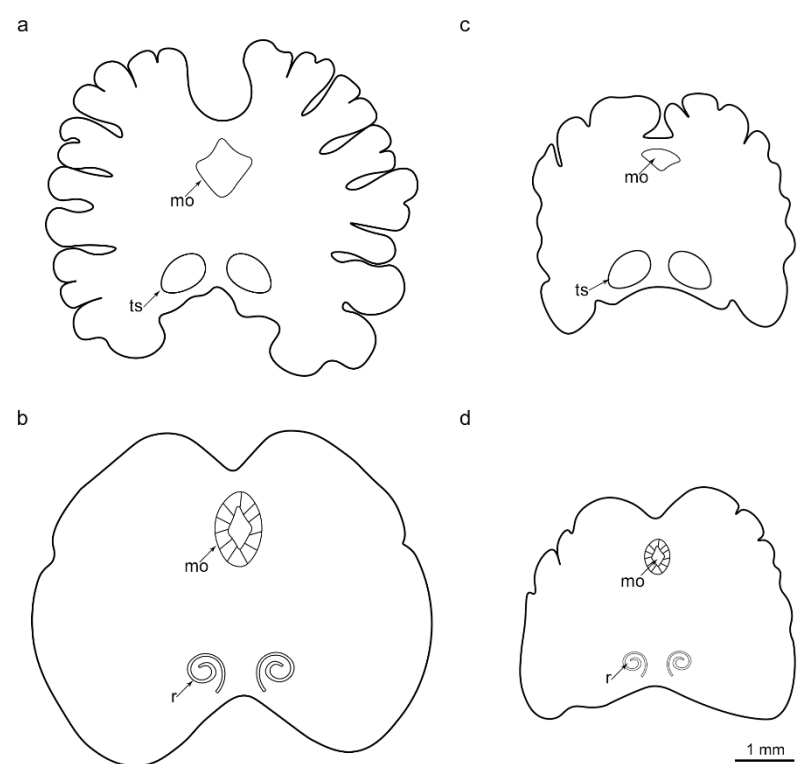


*Pachycheles stevensii* infested by *Lernaediscus rybakovi*



*Pachycheles stevensii* infested by *Lernaediscus kasyanovi*

Recently, two new species, *Lernaediscus rybakovi* and *L. kasyanovi*, parasitizing the porcellanid crab *Pachycheles stevensii* were found. Two *Lernaediscus* species differ well by molecular markers, by the size, color, the morphology of receptacles, by the retinacula type on the internal cuticle, and the presence of distinct marginal lobes in *L. kasyanovi*. *Lernaediscus kasyanovi* is rather rare compared to *L. rybakovi* (their prevalence on the crab *P. stevensii* is 2% vs 30%, respectively).

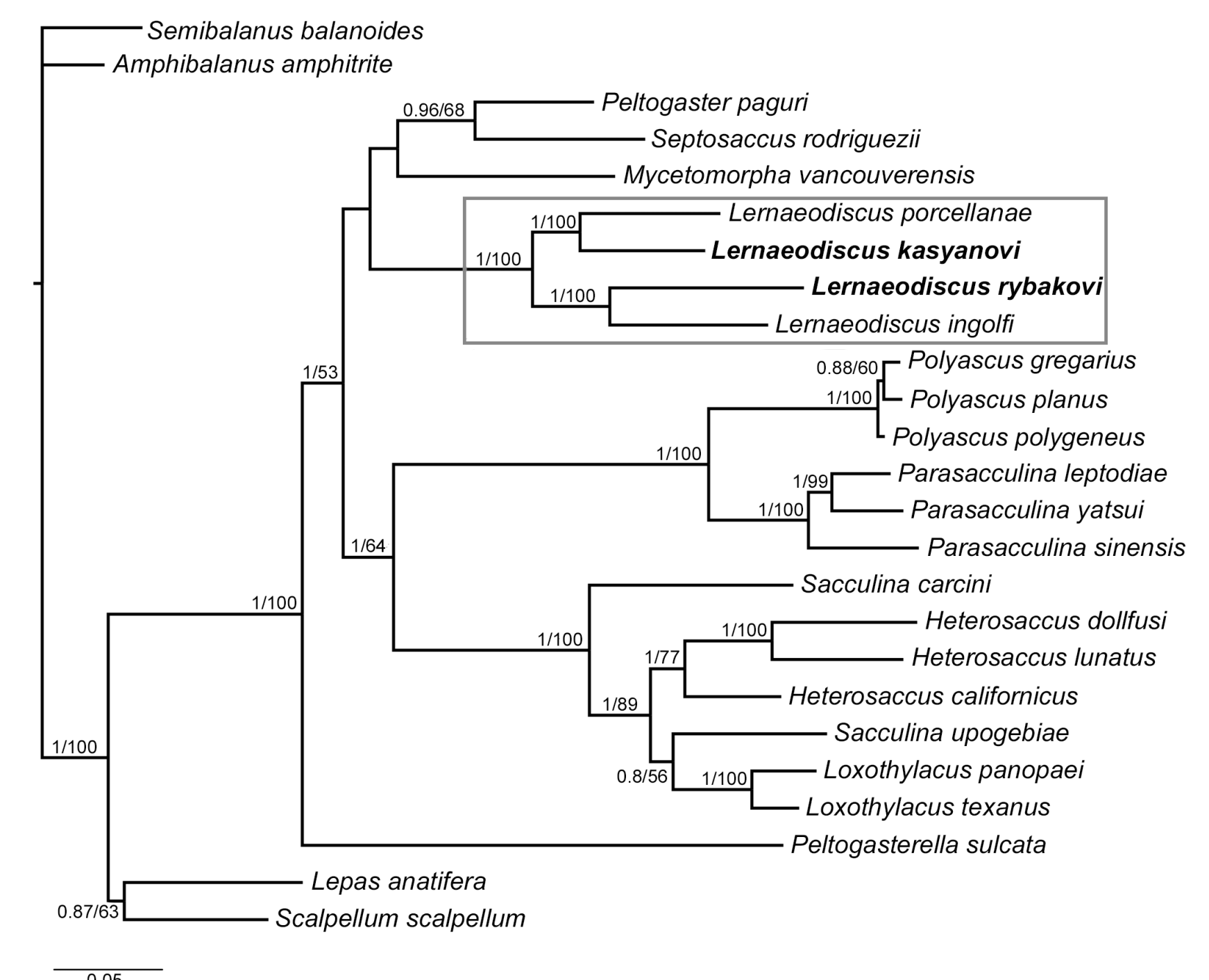


Schematic drawings of the mature externae of *Lernaediscus kasyanovi* (a), *L. rybakovi* (b), and immature externae of *L. kasyanovi* (c), *L. rybakovi* (d). mo, mantle opening; r, receptacle



Female *Pachycheles stevensii* that also carries a large brood of its own embryos attached to the pleopods

The presence of developing eggs in a number of *P. stevensii* infested females (an extremely rare phenomenon) and the absence of embryos in the *L. kasyanovi* infested females indicates that the degree of control of the parasite over the host can vary even within the same genus.



Bayesian phylogenetic tree for the three-gene concatenated dataset (18S, 28S and COI).

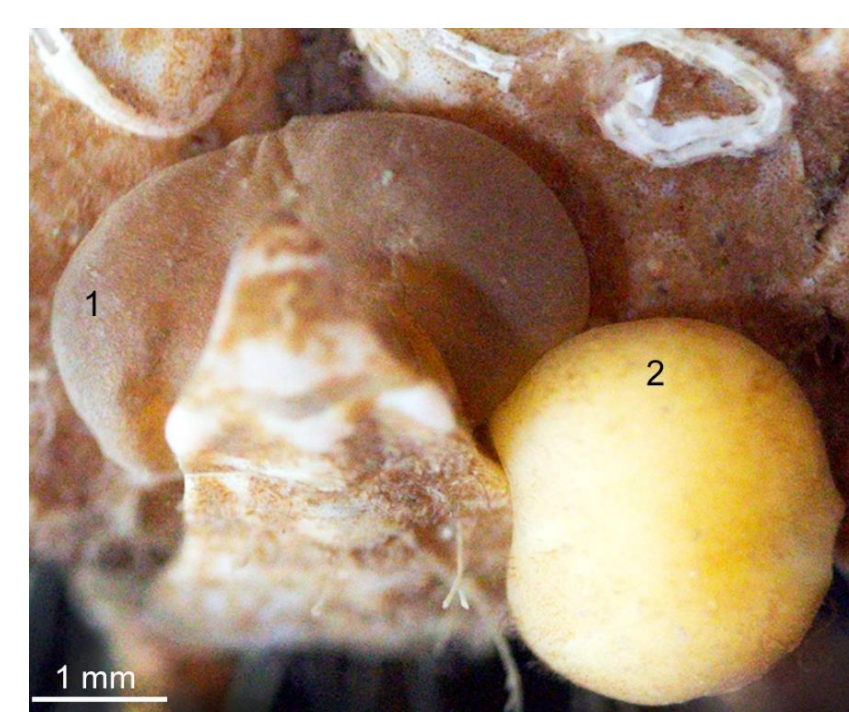
Molecular analysis confirmed the monophyly of the genus *Lernaediscus* and revealed that *L. rybakovi* is the sister taxon to *L. ingolffi*, while *L. kasyanovi* – the sister taxon to *L. porcellanae*.



*Pugettia aff. ferox* infested by *Parasacculina pilosella*



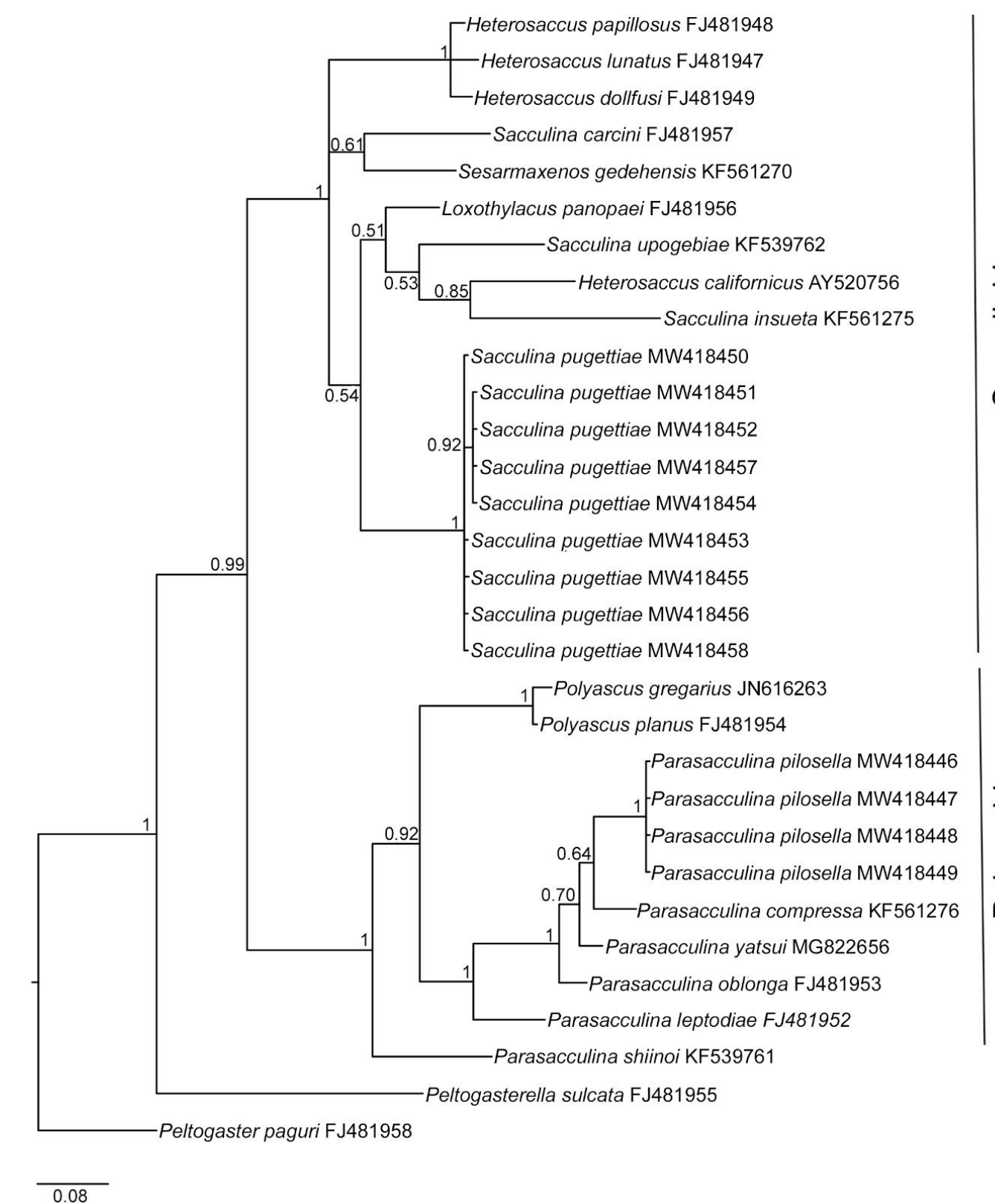
*Pugettia aff. ferox* infested by *Sacculina pugettiae*



*Pugettia aff. ferox* infested by both rhizocephalans. 1, *P. pilosella*, 2, *S. pugettiae*

It was found that the spider crab *Pugettia aff. ferox* from Peter the Great Bay is simultaneously infested by two rhizocephalans, *Parasacculina pilosella* and *Sacculina pugettiae*. The two rhizocephalans differ well by the morphology of the external cuticle, the shape and position of receptacles, and the structure of the colleteric glands. The intensity of two-species multiple infestations reached four externae per host.

It is the first finding of multi-species infestation of a single crab specimen.



Bayesian inference analysis of 16S rDNA sequences for the Sacculinidae and Polyascidae. Numerals above or below the branches are Bayesian posterior probabilities.

Molecular analysis revealed that these rhizocephalans are unrelated and should be placed in different genera and families. *Sacculina pugettiae* is clustered within the monophyletic clade of Sacculinidae, whereas the second parasite is nested in the genus *Parasacculina*, belonging to the family Polyascidae and thus should be named *Parasacculina pilosella*.

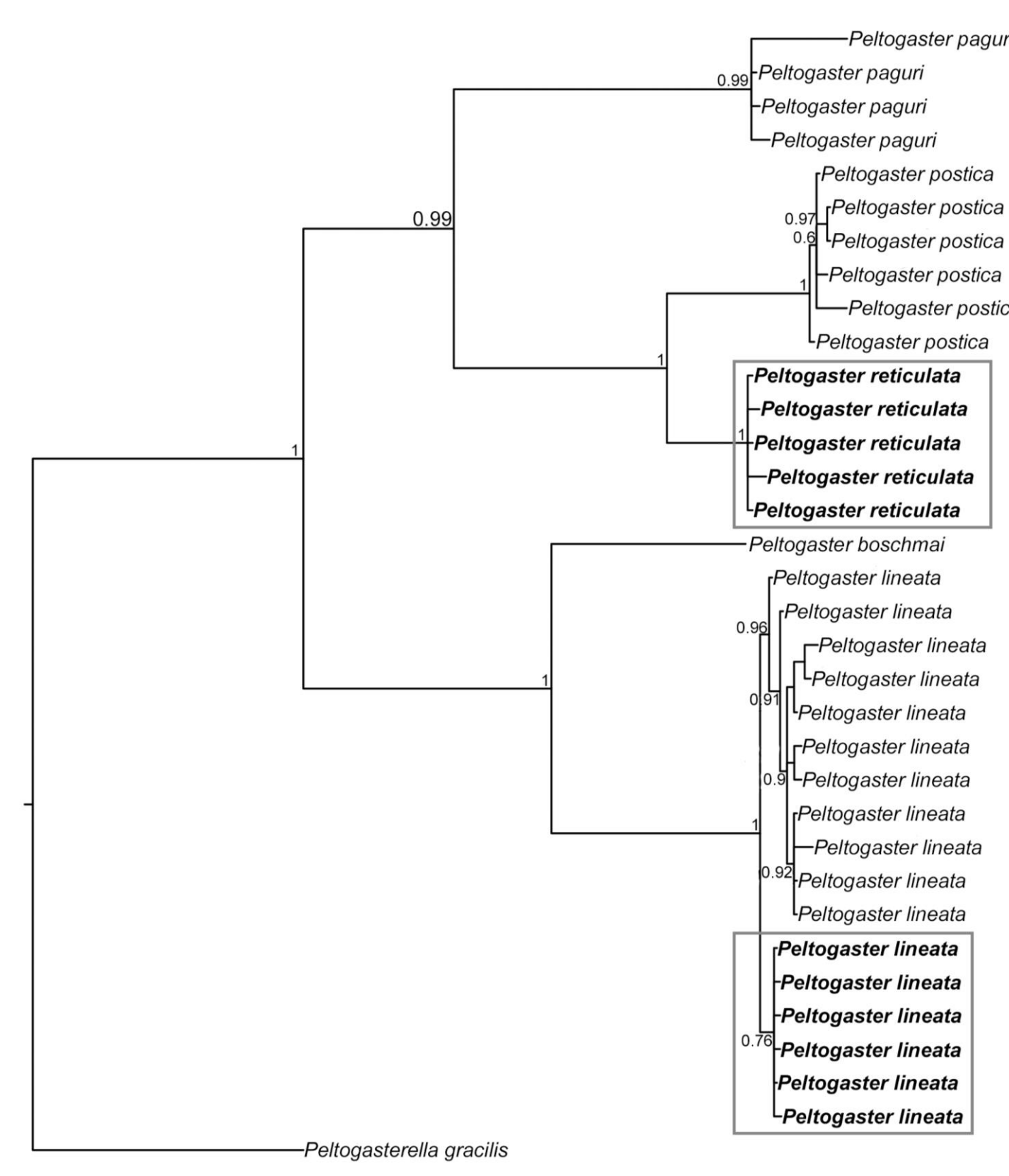


*Pagurus minutus* infested by *Peltogaster reticulata*



*Pagurus brachiomastus* infested by *Peltogaster lineata*

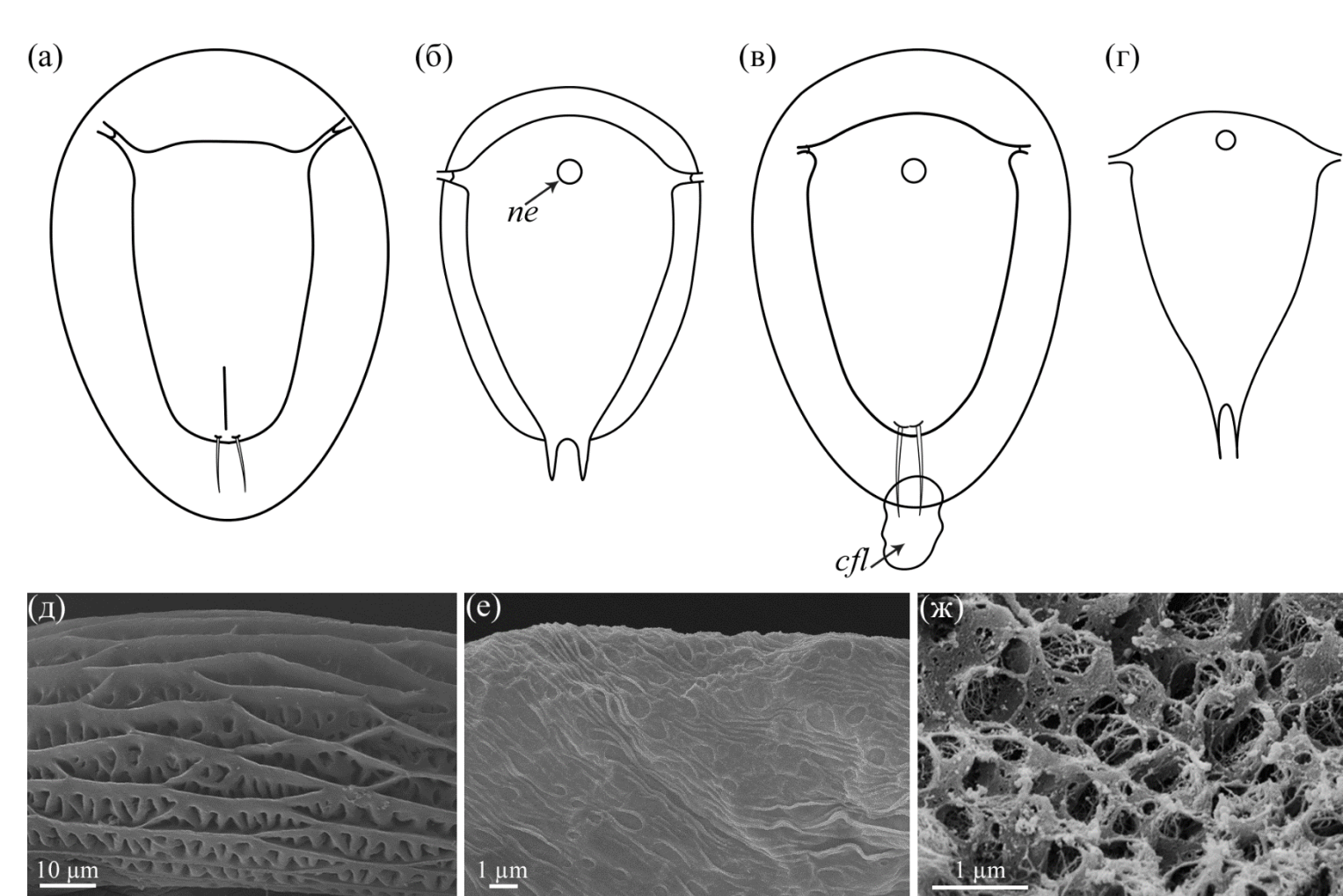
*Peltogaster reticulata* is parasite the hermit crab *Pagurus minutus*. *Peltogaster lineata* inhabiting hermit crab *Pagurus brachiomastus* is the second *Peltogaster* species from Peter the Great Bay. It differs well from *P. reticulata* by size and color of the externa. In *P. reticulata*, mature externa is green, olive or brown, but in *P. lineata*, it is white, yellow or pale. Moreover, externa of *P. reticulata* is considerably longer (length:width ratio is 3.5–4.1 in *P. reticulata* vs 2.5:1 in *P. lineata*).



Bayesian phylogenetic tree of *Peltogaster* for combined molecular data (concatenated sequences from COI and 16S mtDNA). Numerals at nodes are posterior probability values.

It was shown that *P. reticulata* is the sister taxon to *P. postica* infesting the same host in southwestern Japan.

The morphology of the externa of *P. lineata* from Japanese, Korean and Russian waters is very similar. The host preference of *P. lineata* is changing to the north from *P. filholi*+*P. nigrivittatus*+*P. maculosus* in Japan to *P. filholi*+*P. brachiomastus* in Korea, and to *P. brachiomastus* in Russia. It is likely that Peter the Great Bay is the northern border of *P. lineata* area.



The external appearance (a–d) and structure (e–g) of the flotation collar of the nauplii of *Peltogaster* (a, e), *Lernaediscus* (b, f), *Parthenopea* (c, g) (no: Рыбаков, Xēr, 2013, рис. 3d). Designations: cfl, caudal process of the flotation collar; ne, naupliar eye.

The complete larval development of all studied rhizocephalans with kentrogonid type of development includes five naupliar and one cypris stage. All nauplii can be divided into peltogastrid-type larvae (families Peltogastridae and Peltogasterellidae) or sacculinid-type larvae (families Sacculinidae and Polyascidae) differing by the presence or the absence of the flotation collar, the dorsal setae on the head shield, the structure of the frontolateral horns, appendages, and furca. The sexual dimorphism of cyprids is undoubtedly associated with different functions of male and female larvae.

Peter the Great Bay (the northwestern Sea of Japan) is characterized by significant fluctuations in water temperature throughout the year, reaching as low as  $-1.9^{\circ}\text{C}$  in winter, and the presence of ice-cover in December–May. The reproductive season of rhizocephalan parasites in this area coincides with the summer – the most favorable time for their embryonic development, larval release and settlement. Mature externae with developing embryos in the mantle cavity occur during summer – early autumn producing multiple larval generations per reproductive season. The report study was funded by RFBR, project number 20-04-00097.