



**Тезисы докладов  
Международного семинара  
по биоразнообразию и эволюции моллюсков**

**26–27 сентября 2019  
Владивосток, Россия**



**Abstracts  
of the International Seminar  
on Biodiversity and Evolution of Mollusks**

**September 26–27, 2019  
Vladivostok, Russia**



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Far Eastern Branch of the Russian Academy of Sciences  
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OF MARINE BIOLOGY

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*on Biodiversity and Evolution of Mollusks*

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**Russian Far East Malacological Society  
and development of malacology in eastern Russia  
and the Asia-Pacific region**

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In the year of 2019, the Russian Far East Malacological Society (RFEMS) celebrates its 25<sup>th</sup> anniversary. The society was formed in October – November 1994, in the era of political and economic chaos in Russia, but the child born was lucky. Among achievements of the past 25 years, I would call successful publication of our journal, the *Bulletin of the Russian Far East Malacological Society* (23 volumes since 1996), one national and three international meetings (including 2019 seminar on biodiversity and evolution of mollusks), annual meetings of the society members, growing membership (68 members from Russia, China, Republic of Korea, Canada, Japan, Ukraine, Vietnam and USA), running the RFEMS website (<http://rfems.dvo.ru/index.php/en/>), and, most important, formation of a malaconetwork through activity of the RFEMS and contribution of our members to science of malacology. Personal contacts and sharing research findings during our international meetings lead to joint studies and further publications, dissemination of information via the website and the journal facilitate progress of our science, and, finally, increase malacological capacity building in the Asia-Pacific region. In this regard, I see the importance of non-profit and non-governmental science societies and international malacological cooperation in the Asia-Pacific. Involvement of the Russian, Chinese, US, Korean and Japanese malacologists in the RFEMS in various ways obviously facilitated growing number of joint projects and papers on mollusks. Thanks to the ForBio programs, we enjoy presentations of early-career scientists participating in the 2019 seminar and, taken together with contributions of resource persons of the ForBio and MEDUSA *Course on Systematics, Morphology and Evolution of Marine Molluscs* held at the Vostok Biological Station in September 2019, this seminar increases geographical coverage of international cooperation. The RFEMS is getting global!

Silver jubilee is a celebration held to mark a 25<sup>th</sup> anniversary, and that is kind of age of maturity, at least, for a science society. We celebrated 10<sup>th</sup>, 15<sup>th</sup> and 20<sup>th</sup> anniversaries, but they were “ordinary” birthdays while the silver jubilee allow us to consider some long-term results and outcomes of the RFEMS activity. Although the RFEMS was born as a regional malacological society in a turbulent post-Soviet time and in a remote Russian region (“eastern Siberia” = Russian Far East, a week train travel distance from Moscow to Vladivostok), it became *de facto* the first sustainable and active national mollusk’s society with profound international relations.

Below I present a brief historical timeline of the RFEMS to show how the child was born and grew up.

**1994** The RFEMS was founded by a group of scientists from Vladivostok with the headquarters in the Institute of Marine Biology, Far Eastern Branch of the Russian Academy of Sciences (IMB FEB RAS; at present, A.V. Zhirmunsky National Scientific Center of Marine Biology (NSCMB) FEB RAS); first President – Alexander I. Kafanov, Vice-President – Vladimir V. Gulbin, Secretary – Konstantin A. Lutaenko

**1997** First volume of the *Bulletin of the Russian Far East Malacological Society* was published (with “1996” date on cover)

**1998** First malacological meeting in the Russian Far East was held, organized jointly by the RFEMS and the IMB FEB RAS

**2003** New charter of the RFEMS was adopted at the annual meeting; new President (Konstantin A. Lutaenko), Vice-President (Larisa A. Prozorova) and Secretary (Olga Ya. Semenikhina) were elected

**2004** Second malacological meeting organized jointly by the RFEMS and the IMB FEB RAS with international participation; celebration of the 10<sup>th</sup> anniversary

**2006** The RFEMS website was launched

**2008** The *Bulletin of the RFEMS* went online

**2009** Celebration of the 15<sup>th</sup> anniversary

**2014** Third malacological meeting organized jointly by the RFEMS, IMB FEB RAS, Institute of Biology and Soil Science FEB RAS and the All-Russian Hydrobiological Society with international participation

**2019** Fourth malacological meeting (seminar) with international participation

**A study of the germ plasm granule formation  
during oogenesis of the bivalve mollusc  
*Ruditapes philippinarum***

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According to studies in various animal models, meiotic differentiation is connected with the presence of the so-called “germ plasm”, a cytoplasmic substance whose formation and transformation are generally conserved during animal life cycles. To date, it is known that germ plasm arise and is stored during oogenesis with the participation of the mitochondrial cloud, also known as Balbiani body. The germ plasm is called polar granules in *Drosophila*, germinal granules in *Xenopus*, P granules in *Caenorhabditis elegans*. Despite the obvious link between mitochondrial cloud and germ plasm granules, the precise mechanism of their formation is still unknown. The process of germ plasm formation has not been ultrastructurally investigated yet in any bivalve species. We took advantage of our work with *Ruditapes philippinarum* (Manila clam) to figure out how germ plasm arises in this bivalve representative.

Comparative study of maturing oocytes based on the ultrastructure of their cortical layer allowed us to track down consecutive stages of a complex mechanism that we call “germ plasm granule formation complex”, or GGFC. The initial GGFC morphologic pattern appears as a granular substance (GS) that arises in the cytoplasm by a mechanism that is not clear yet. A study of serial sections showed that GS typically has irregular 3D shape and is surrounded by several mitochondria situated in close vicinity to the GS periphery. Some mitochondria were seen deteriorated and mitochondrial material marked by anti-CYTB antibody labelled by colloidal gold was found around the GS and inside of this substance. iEM study showed that Vasa could be found in the cytoplasm close to these mitochondria and inside them. We suggest that Vasa contributes to the mechanism of mitochondrial disassembly and incorporation of mitochondrial material into the GS. Both Vasa and CYTB were also found inside the the initial form of GG that appears at the centre of GS. We suggest that, as a result of mitochondrial disassembly and incorporation of mitochondrial material in the GS, the following stage of GGFC arises: it lacks adjacent mitochondria, is bigger and the forming GG

is separated from GS by a thin cavity. The next stage is characterized by the development of a large cavity around the GG followed by the appearance of GG that lacked GS envelope.

The GGFC describes the process of GG formation that appears morphologically different in comparison to the Balbiani body that is usually connected with formation of multiple germ plasm granules. Indeed, each GGFC form only one GG and multiple GG are formed by correspondingly multiple GGFC. The novel pattern of germ plasm formation that we discovered may be specific of bivalve molluscs.

## **Bivalve mollusks of Pacific origin in the Barents Sea: distribution and ecology**

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The Barents Sea is a borderline sea that aggregates the warm waters of the Atlantic Ocean and the cold Arctic Ocean in its waters. Due to this feature, the fauna of bivalve mollusks has a number of characteristics: the presence of Arctic endemics, species with Pacific and Atlantic origin.

Atlantic species have greatest biodiversity in the Barents Sea. They make up 68% of the total number of bivalve mollusks found in this area. These species are quite rare and, mainly, in well-warmed zones (southwest of the Barents Sea). A total of 67 species of mollusks were noted. They include such species as *Spisula elliptica*, *Palliolium tigrinum*, *Bathyarca glacialis*, and others.

Bivalve mollusks with Pacific origin have a smaller number of species, but are found everywhere not only in the Barents Sea, but also in practically the entire shelf zone of the Arctic. For the Barents Sea, 29 species of bivalve mollusks are recorded. In most cases, these are boreal-arctic species creating large concentrations. The most prominent representatives of the Pacific species group are: *Chlamys islandica*, *Ciliatocardium ciliatum*, *Macoma calcarea* and others. In the Barents Sea, these species inhabit a wide range of temperatures, salinities and depths of the mollusks.

**Euopisthobranch mollusks of the order Cephalaspidea  
(Gastropoda: Heterobranchia) of the Kuril-Kamchatka Trench  
and the adjacent area of the Pacific abyssal plain**

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The first data on abyssal Cephalaspidea s.l. (including Ringiculidae) in the north-western Pacific Ocean were obtained during the expedition aboard R/V *Vityaz* in the 1950s–1960s. Extensive sampling in the abyssal and hadal areas, revealed a diverse and rich benthic fauna. During the expeditions aboard R/V *Vityaz*, abyssal and hadal zone of the Kuril-Kamchatka Trench (KKT) was studied, and cephalaspids were collected at the depth of 5070 to 8355 m. Several euopisthobranchs were consequently described from abyssal zone of the Pacific Ocean basin and KKT by Yu.S. Minichev, however data on diversity and taxonomy of Cephalaspidea s.str. in this region remains sparse.

Our study focuses on the distribution and diversity of different cephalaspidean species collected from different parts of the KKT and adjacent abyssal plain during the deep-sea expedition KuramBioII (2016). Additionally, material from adjacent abyssal area collected during the KuramBioI (2012) and the SokhoBio (2015) was studied.

Approximately 200 cephalaspidean specimens were collected from 32 stations. Our study recovered several undescribed taxa from different parts of the KKT and adjacent abyssal plain, belonging to five different families: Retusidae, Cylichnidae s.l., Philinidae, Scaphandridae and Aglajidae. Molecular phylogenetic and species delimitation analyses based on four gene markers (COI, 16S, H3, 28S) were conducted to determine species identity and phylogenetic relationships of several taxa. The morphological analysis included anatomical dissections and scanning electron microscopy.

A total number of 167 new cephalaspidean sequences were generated. Herein, we focus on the family Philinidae being the most common cephalaspidean taxon in

the sampling region, however general remarks on all groups are given. In the examined material, a total of 8 morphospecies, belonging to the family Philinidae were identified. Three species will be described as new for sciences: two from the KKT and one from the western slope of the KKT and Kuril Basin of the Sea of Okhotsk.

Preliminary data obtained for other families indicate a high cryptic diversity in the studied area, along with very complex evolutionary history, especially in the families Aglajidae and Cylichnidae. The level of divergence found within Cylichnidae indicates the presence of at least nine undescribed species from the KKT.

Scientific research was performed using the equipment of the “Taxon” Research Resource Center ([http://www.ckp-rf.ru/ckp/3038/?sphrase\\_id=8879024](http://www.ckp-rf.ru/ckp/3038/?sphrase_id=8879024)). Molecular study was partly conducted using the equipment of the Core Centrum of the N.K. Koltzov Institute of Developmental Biology RAS.

**The breeding pattern of abyssal mollusks  
of the genus *Ringiculoides* Minichev, 1966  
(Heterobranchia: Ringiculidae)**

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The genus *Ringiculoides* Minichev, 1966 (family Ringiculidae) includes two species that live in the Kuril-Kamchatka Trench at a depth of 4–6 thousand meters. An egg clutch was found for the first time in one of the individuals of *Ringiculoides vityazi* Chaban, Kano, Fukumori, Chernyshev, 2018 (shell height of 4.1 mm) collected from a depth of 5755 m. The representatives of both the order Cephalaspidea, to which Ringiculidae were previously thought to belong, and Nudibranchia, which are currently regarded as a sister to Ringiculidae (Kano et al., 2016), lay eggs in mucous clutches of various shapes attached to the substrate (Hurst, 1967; Thompson, 1976; etc.). The egg clutch of *R. vityazi*, however, was found in the mantle cavity. The aim of the present study was to describe the morphology of this egg clutch.

Individual fragments of the clutch containing 2–3 capsules were examined and photographed using a Leica DMLS-2 light microscope equipped with a video camera and a Quanta-250 scanning electron microscope. For light microscopy, the capsules were cleared in clove oil. For scanning electron microscopy, the fragments of the clutch were dehydrated in 70° and 96° ethanol, dried in hexamethyldisilazane, mounted onto the specimen stubs with graphite paste and coated with platinum.

The clutch is located antero-laterally on the bottom of the mantle cavity. It consists of 4 rows of egg capsules; the rows are oriented almost parallel to the anterior margin of the mantle. The clutch contains about 20 white capsules. The capsules are oval-cylindrical in shape, with thick walls, about 140 µm in diameter; they are tightly pressed against one another. The inner chamber of each capsule contains a dividing egg 110–120 µm in diameter. Each capsule has a widely conical operculum about 80 µm in diameter. The surface of the operculum is porous and irregularly covered with papillae 2–3 µm in length and about 0.5 µm in diameter. The central papilla is the largest, about 20 µm in height and about 15 µm in diameter; it is hollow inside, with two openings at the apex. The capsules are unequally developed: the largest are located closer to the hermaphroditic opening, at the right margin of the mantle cavity, the opercula in some of these capsules have fallen off.

Brooding of embryos in the mantle cavity is a rare phenomenon among the mollusks of the subclass Heterobranchia. The morphology of the clutch in the abyssal genus *Ringiculoides* is described for the first time. The distinct characteristics of this clutch is

*The breeding pattern of abyssal mollusks of the genus Ringiculoides*

the location inside the mantle cavity and the presence of individual dense egg capsules that are not enclosed in the common mucous matrix. Additional material is needed to study this unusual clutch using histological methods.

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**Gametes and reproduction of the chemosymbiotic  
bivalve mollusc *Calyptogena pacifica* Dall, 1891  
(Vesicomysiidae: Pliocardiinae)**

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Bivalve molluscs of the subfamily Pliocardiinae (Bivalvia: Vesicomysiidae) are a chemosymbiotrophic group, obligate for reducing sulphide-rich conditions. Some pliocardiiines have extensive transoceanic distribution patterns although their habitats are separated by vast areas unsuitable for the life of adult molluscs. For understanding the mechanisms of dispersal of obligate chemosymbiotrophic animals and formation of fauna of the fragmented reducing environments reproductive biology of pliocardiiines is especially important. Besides, it is known that characters of spermatozoa are among the most phylogenetically informative for bivalve molluscs. Nevertheless, information on pliocardiiine gamete morphology available in the literature is scarce.

We studied the gamete structure of *Calyptogena pacifica* from the Bering Sea in the area of the submarine Piip Volcano. The gonads of the studied molluscs are organized as a system of tubules and acini. Mature eggs reach 200 µm in diameter and are located in the ovaries in the central part of tubules. The relatively numerous growing oocytes are located adjacent to the tubule walls along the periphery. The germinal epithelium of testes includes gametogenic cells at different stages of development: spermatogonia, spermatocytes I and II and spermatids. The asynchronous growth of gametes indicates an annual reproductive cycle with multiple spawning or with continuous spawning.

Spermatozoa of *C. pacifica* have an elongated bullet-shaped head  $4.3 \pm 0.2$  µm long from the tip of the acrosome to the base of the mid-piece. The apical elongated-conical acrosome consists of two parts: an elongate dome-shaped acrosomal vesicle and a sub-acrosomal material adjacent to the nucleus. The mid-piece of the sperm is formed by a complex of four spherical mitochondria, interconnected via intermitochondrial junctions. A ring of electron-dense material located distal to the mitochondria was found. Mitochondria surround proximal and distal centrioles.

We compiled all information on spermatozoa of five different pliocardiiine species published earlier. The differences in the structure of the spermatozoa in molluscs of the genera *Calyptogena* and *Phreagena* support the earlier conclusions on the distant affinity of the genera, based on the morphological characteristics of shells and soft body, as well as on molecular markers. The data obtained in our work for the first time indicate that the ultrastructural features of spermatozoa can be used to diagnose genera within the subfamily Pliocardiinae.

**Brief description of bivalve mollusks  
of the Mayskaya Thermal Electric Plant  
marine techno-ecosystem in Zapadnaya Bay  
(Sovetskaya Gavan Bay, Tatar Strait)**

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Mayskaya Thermal Power Plant is located on the coast of the Tatar Strait in Zapadnaya Bay (48°56' N). This enterprise uses sea water for its cooling and has an impact on natural ecosystems.

The object of research was the section of the coast and the bottom of the Zapadnaya Bay, with slag soils mixed with shells and stones, within a radius of 20–60 m from the drain pipes of the cooling circuit of the power plant. The composition of the soil on the site is due to the proximity of the power plant ash dump. In addition, the surfaces of the dismantled drain pipes were explored. From inside the pipes were half filled with slag. The materials were collected on July 18 and September 17, 2018 from the intertidal zone to a depth of 0.6 m, 15 hydrobiological stations were completed. For quantitative accounting of mollusks, a specialized manual diving bottom grab with a capture area of 0.05 m<sup>2</sup> was used. Additionally, we used manual sampling by digging out accounting areas of 0.05 m<sup>2</sup>. The sampling on the surface of the pipes were performed from accounting areas of 100 cm<sup>2</sup>. The material was also collected during the survey of beach tanatocenoses in the study area. Additionally, we used data from surveys of the littoral zone of the site in September 2007 (10 stations).

Since the stones and the drain pipe were removed from the water before the study began, mussels located on the stones and on the outside of the pipe were dried, so it was impossible to determine their specific biomass. In addition, the shells of *Nuttallia* were with severely damaged periostracum, therefore, it was impossible to determine their age.

In the area of Zapadnaya Bay, adjacent to the Mayskaya Power Plant, 10 species of aquatic organisms were found (2 species of plants and 8 species of animals), 5 species of them were bivalve mollusks: *Macoma balthica*, *Ruditapes philippinarum*, *Mytilus trossulus*, *Nuttallia obscurata*, *Mya arenaria*. Massively, *R. philippinarum*, *N. obscurata*, and *M. trossulus* were noted. The two species: *R. philippinarum* and *M. trossulus* were found both in natural settlements and in the cooling system drain pipes of the power plant. Compared with the techno-ecosystem of the Vladivostok Thermal Power Plant, where, according to Zvyagintsev and Moshchenko (2010), 90 species of animals and 10 plant

species have been recorded, the species composition of hydrobionts of the technoecosystem of the surveyed site is extremely poor. Considering that local tanatocenoses composition coincides with the biocenoses composition, we can argue that the species composition is fully identified.

We give a brief description of the common species of bivalve mollusks in the study area.

***Ruditapes philippinarum***. A warm-water subtropical-low-boreal species. The mollusk in the bay lives in the littoral zone, forming aggregations near the drain pipe. The density of the mollusk in aggregations reached 8 ind./m<sup>2</sup> (average 4 ind./m<sup>2</sup>), biomass – 88 g/m<sup>2</sup> (average 44 g/m<sup>2</sup>). However, *R. philippinarum* was also found in the drain pipe fouling, where it is marked both in the slag, and attached to the pipe surface. Here the mollusks formed the highest density and specific biomass (up to 6600 ind./m<sup>2</sup> and 51 kg/m<sup>2</sup>, respectively).

The settlement of *R. philippinarum* in the drain pipe was represented by individuals whose shell length ranged from 6 to 51 mm, an average of 30 mm. The weight of mollusks varied from 0.4 to 18.6 g, with an average of 8.3 g. The proportion of juveniles (length less than 30 mm) was 48%. The age of the studied mollusks ranged from 1 to 7 years, with an average of 4 years. The largest specimens of the species were recorded in the littoral zone with a shell length of 36 to 53 mm, an average of 42.5 mm, and a weight of 6.8 to 23.7 g (on average, 11.5 g). The age of the *R. philippinarum* was from 4 to 7 years, on average – 5 years.

The high density and specific biomass of this species are not typical for the northwestern part of the Tatar Strait, where it is marked singly at the northern edge of its range. The mollusk distribution observed in the area adjacent to the Mayskaya Power Plant is uniquely associated with thermal pollution.

***Nuttallia obscurata***. Warm-water subtropical-low boreal species. Living specimens of *N. obscurata* in 2018 were not found. Living mollusks at the site were found in 2007 during foot surveys of the littoral zone. Their density was 0.1–2 ind./m<sup>2</sup>, on average about 0.5 ind./m<sup>2</sup>. In addition, live mollusks in this area are regularly dug from the ground by amateur anglers, using their meat as bait for fishing gear. In coastal emissions and on the littoral, a large number of clamshells are noted. The density of empty shells in the study area is from 0.1 to 5 specimens/m<sup>2</sup>. The length of the shells was from 33 to 60 mm, an average of 43.5 mm.

*N. obscurata*, being an estuary-lagoon-type warm-water species, in the northwestern Tatar Strait is noted only in Zapadnaya Bay. Its appearance on this site, apparently, should be explained by the same reasons as the appearance of *R. philippinarum*.

***Mytilus trossulus***. Warm-water subtropical-low boreal species. Mollusks live on separate stones in the immediate vicinity of the drain pipes (density up to 5400 ind./m<sup>2</sup>) and also in the fouling of the outer surface of the drain pipe. Here we found the highest density of mussels (16100 ind./m<sup>2</sup>). The densities of *M. trossulus* were the highest of all the species found. Mussels from the settlement on the rocks were with a shell length

from 7 to 27 mm, an average of 15 mm, and from fouling of the drain pipe – from 6 to 34 mm, an average of 19 mm.

The fouling of *M. trossulus* is noted in Sovetskaya Gavan' in different parts of the bay, which gives reason to talk about the introduction of this species, probably due to ship fouling, or with ballast waters. However, Zapadnaya Bay is the only place in the western part of the Tatar Strait where this species is able to settle not only in fouling, but also on natural grounds.

In Zapadnaya Bay at the maximum distance from the drain pipes, the cold-temperate species *Macoma balthica* and *Mya arenaria* were also singly recorded.

So, in general, mollusks are distributed relatively evenly on the slag ground of the surveyed area, with density from 0.1 to 2 ind./m<sup>2</sup>. At a distance closer than 20–30 from the drain pipes, mollusks aggregations with area about 1 square meter are found. The density within the agregations increases to 3–5 ind./m<sup>2</sup>. The bivalve mollusks form the highest density in the settlements on the outer surface of the drain pipes – up to 5400 ind./m<sup>2</sup> and up to 16000 ind./m<sup>2</sup> in the slag filling the pipe. The surface of the pipes is covered with mollusks by 10–30% from the inside to 70–90% from the outside. Settlements represent mosaic spots of species aggregations. The areas of aggregations are from 0.02 to 0.1 m<sup>2</sup>. Thus, the abundance of bivalve mollusks increases towards the source of thermal pollution. In the techno-ecosystem, bivalve mollusks show absolute quantitative dominance.

**Stripes matter: first attempts to resolve  
*Coryphellina rubrolineata* O'Donoghue, 1929 species complex  
(Gastropoda: Nudibranchia)**

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Nudibranch species *Coryphellina rubrolineata* O'Donoghue, 1929 is a well-known, brightly colored cladobranch sea slug, which shows an abnormally wide range from the Mediterranean and the Red seas to Philippines and Australia. The specific morphological identification traits are perfoliate rhinophores and a red, purple or violet stripe on the dorsal side, which normally continues from a head to a tail. Also, two additional stripes under the cerata are very common. However, different varieties may have discontinuous stripes, or several additional ones. The molecular study has been never conducted to confirm the species identity of molluscs with those color varieties.

We have studied several specimens collected from the Red Sea (type locality) and Vietnam. Our methods included traditional anatomical dissections, SEM studies of jaws and the radula and the molecular phylogenetic analysis of four genes, accompanying by modern species delimitations methods.

We have shown that the species *C. rubrolineata* is restricted to areas, which are close to the type locality (the Mediterranean Sea, the Red Sea). In Southern Vietnam *Coryphellina* species represent a species complex, with at least four distinct species found. Three of these species are new for science, while one species is identical to recently described *Coryphellina lotos* Korshunova et al., 2017 from Japan. All five species found, differ from each other in coloration pattern and arrangements of red bands and stripes. Also, we have found some differences in radular characters, while the morphology of the reproductive system was nearly identical in all species, including "typical" *C. rubrolineata*. Further revisions are needed for deeper understanding of *Coryphellina* diversity in the Indo-West Pacific region and to uncover processes, underlying cryptic and pseudo-cryptic speciation of these molluscs.

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## **Mollusks as proxies in environmental studies**

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Common, highly visible, ecological and commercially important on a global scale, mollusks are widely used as model systems and indicators in a multitude of areas ranging from archaeology through behavior and communication. They are ideal subjects to look into anthropogenic impacts and are used as models in calcification patterns studies to look at marine acidification effects. Terrestrial and freshwater forms are especially sensitive and good barometers of environmental changes.

Shells in general are important components of benthic communities, contributing to its structure and facilitating settlement of many other organisms. Bivalves in particular are important ecosystem engineers in coastal and estuarine areas and the knowledge of their life habits can provide valuable information on substrate types. Mollusk global distribution and long fossil record allows their use in environmental reconstruction and their sensitivity to changes can provide detailed information on both short-term habitat disturbance and long-term ecosystem's restructuring. Faunal composition, abundances and carbon dating of shells gives independent information that can be crossed with other type of archives such as oceanographic records and human historical recordings.

This presentation will give an overview of the use of mollusks as tools in environmental reconstruction in general.

**Distribution of some pelagic larvae of bivalve mollusks  
in the western part of Chukchi Sea**

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The plankton samples collected in September–October 2016 in the 48<sup>th</sup> cruise of R/V *Akademik Oparin* in Chukchi Sea with the NORPAC net (mesh 150 µm) in the horizon of 15–0 m have a plenty of pelagic larvae of bivalve mollusk. Their biggest quantity was found in the period from September 22 until October 1. The most yield area was situated on the opposite side of the Bering Strait and the second maximum of larvae quantity was found on the Herald's Rise. Most important factors influencing the distribution of larvae are the water surface temperature and concentration of Chl-*a*.

## **Microbial aspect of mollusc host species divergence: metabarcoding study in intertidal snails' cryptic species**

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The importance of gut symbionts for normal metabolic functioning, immunity, reproduction, etc. was demonstrated for both vertebrates and invertebrates. Gut microbiome is a host species-specific community of symbiotic microorganisms. The composition of this community mirrors niche partitioning between the hosts' species due to even faint differences in food quality. In respect to closely related host species, both fine spatial differentiation of populations and alimentary preferences affect digestive modes, spectrum of engulfed microorganisms and inevitably change species-specific microbial community. Moreover, specific and balanced microbial community formation can be regarded as significant attribute during speciation. Marine intertidal molluscs of the subgenus *Neritrema*, genus *Littorina* (*Littorina saxatilis*, *L. arcana*, *L. compressa*, *L. obtusata*, *L. fabalis*) are common inhabitants of intertidal zone in the North Atlantic region. They are consumers of bacterial biofilms and grazers of micro- and macroalgae. Intertidal zone is characterized by diverse factors/stressors with values gradually changing along vertical gradient and *Littorinas*, inhabiting different zones of intertidal area, are generally accepted as a reliable example of early stages of ecology-driven speciation. The analysis of this species group allows to compare the scale and nature of differences in microbial communities associated with ecotypes of one species and between related species with different evolutionary distances. Within this project we characterized typical environmental microbial communities of biotopes inhabited by periwinkles and the gut symbionts of different snails to make a conclusion about possible evolutionary effects on host-symbionts association as a whole system.

**Distinctivness of the sublittoral and upper bathyal  
vetigastropod fauna of the Ogasawara Islands  
(Mollusca: Gastropoda)**

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The Ogasawara (Bonin) Islands are an oceanic island group located ca. 1000 km south of the mainland Japan, and form the middle part of the Izu-Ogasawara-Mariana island arc. Although they fall in a subtropical climate zone within the same latitude range as the Okinawa-Amami (Ryukyu) Island groups, their marine faunas differ considerably because of the difference in the marine environments, especially the influence of the Kuroshio Current. In contrast to the extremely rich marine faunas of the Okinawa-Amami Islands, those of the Ogasawara and adjacent Northern Mariana Islands are known to be generally poor but distinctive.

The gastropod fauna of the Ogasawara Islands was relatively well documented, though mainly in the intertidal and upper sublittoral zones: a total of 1031 species of marine gastropods were recorded, including 21 “probably endemic” species. On the other hand, species in the sublittoral to bathyal zones have not been well surveyed, not only in the Ogasawara Islands but also in many other regions in Japanese waters, especially the Nansei Islands, making it difficult to carry out detailed biogeographical comparisons.

This being so, the gastropod fauna of the Ogasawara Islands is here surveyed in detail, with special emphasis on the sublittoral and upper bathyal zones, based on the material obtained by various dredging programs carried out by the National Museum of Nature and Science. Among gastropods, the clade Vetigastropoda was selected for analysis because of its lack of a widely dispersing teleplanic larval stage and relatively high local speciation in comparison to other subgroups.

For biogeographical comparison, specimens collected from the Nansei Islands (mainly around Amami-oshima Id.) at equivalent depths in previous surveys were also examined for the present study, as well as published results of previous studies in temperate Japanese waters including some in Sagami Bay.

As a result, more than 2700 vetigastropod specimens (including ca. 170 imperfect shells or identifiable fragments but excluding some poorly preserved ones) in 748 lots were classified into 92 morphospecies belonging to 17 families. Seventy-two among 92 species were identified as previously named species, and 20 were considered to be probably undescribed. Fifty species (54%) were recorded for the first time in the Ogasawara Islands, including 22 (24%) recorded for the first time in Japanese waters.

Twelve species (13%) were considered to be endemic to the Ogasawara Islands, and four are so far recorded only from this area.

Looking at a summary of the geographical distributions of species recognized in the present study, 73 species in total (79%) are distributed in the Nansei Islands (63 species) and/or TWP (61 species). Only two species are confined to temperate Japanese waters, besides the Ogasawara Islands. These numbers show the dominance of tropical/subtropical elements in the vetigastropod fauna in the Ogasawara Islands.

Marine faunas of southern Japanese waters are influenced by the Kuroshio Current to various extents. This is evident in the Okinawa and Amami Islands, and on the Pacific coasts of mainland Japan and in the Izu Islands, where a number of tropical/subtropical species are distributed. On the other hand, the Ogasawara Islands are located far south from the Kuroshio main stream, and are affected by various complicated ocean currents, sometimes coming directly from the TWP. In this context, it is noteworthy that at least six species are distributed both in the Ogasawara Islands and in the TWP, but probably not in the Nansei Islands. A typical example is *Astralium provisorium*, which was obtained at as many as 28 stations around the Ogasawara Islands but has not been found at all in the Nansei Islands during similar dredging surveys. However, because of the insufficiency of dredging surveys around the Nansei Islands, more detailed discussion is premature.

Turning to the comparison with the temperate Japanese fauna, comparable dredging surveys had been carried out in Sagami Bay, resulting in recording of 91 vetigastropods. The number is comparable to that of the Ogasawara Islands, but the two faunas (Sagami Bay and the Ogasawara Islands) share only 21 species. The mainstream of the Kuroshio Current flows between these two areas, and it could inhibit the southward transmission of temperate species.

The most important characteristic of the vetigastropod fauna in the Ogasawara Islands is the relative richness of endemic species. Twelve species (13% of the total) are regarded herein as endemic, with four additional species so far only recorded in this area. Besides them, three intertidal vetigastropods have been shown to be endemic to this area. Some further species differ morphologically to some extent from those in other populations, but are rather provisionally held not to be separable at the species level in the present study. If more detailed research is carried out in the future, some of these may also be found to be distinct species that are endemic to this area.

To some extent the high endemism found here stems from narrowing the target group to the vetigastropods. Species of the clade Vetigastropoda generally lack a widely dispersing larval stage and they tend to have a relatively narrow geographical distribution. Even so, in view of the fact that no distinct endemic vetigastropod species are recognized in the surrounding island areas, such as the Izu and Nansei Islands, the high degree of endemism around the Ogasawara Islands is exceptional. This is apparently related to a susceptibility to genetic isolation in this area, due to the complex ocean currents discussed above. It is conceivable that isolation of a temperate species that

*Kazunori Hasegawa*

migrated by some chance from the temperate zone to the Ogasawara Islands is one basis for speciation. However, possible sister species of some other endemic species are distributed in the TWP but not in other areas in Japanese waters, and more complex speciation mechanisms could thus also be involved in this region.

## New data on cladobranch diversity (Gastropoda: Nudibranchia) in Vietnam

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Nudibranchia is a very diverse group of marine invertebrates, encountering more than 4000 species. They are distributed worldwide, with the highest diversity in the Indo-West Pacific region. Despite there are numerous works dedicated to extensive studies of regional biodiversity, the most recent ones indicate an undervaluation of detected species composition. Also, some taxa require a comprehensive revision on different taxonomic levels and obtaining of extensive molecular data.

Studies on heterobranch fauna of southern Vietnam are very fragmented. To date, only two revisions of local species composition were conducted, however they were based on a sparse sampling in restricted areas. In total, 150 species of heterobranchs were registered, among which about 120 species were nudibranchs. Nevertheless, this amount is much lower than nudibranch diversity in closest areas (Thailand, Malaysia, Philippines). The main goal of our study was to observe and revise the species diversity of the suborder Cladobranchia, using molecular barcoding techniques and traditional morphological methods. The material was collected in 2012–2018 from Nha Trang Bay by the scuba diving and snorkeling.

A moderate species diversity of cladobranch nudibranchs were registered. However, among more than 25 species found, about 15 species were registered for Vietnam fauna for the first time. A new species of the genus *Eubranchus* was found and described. *Pteraeolidia semperi* (Bergh, 1870) is represented in the Indo-West Pacific by a species complex, with three species detected in Southern Vietnam. The same cryptic diversity was shown for *Coryphellina rubrolineata* O'Donoghue, 1929 species complex, with four pseudocryptic species found. Two species, *Melibe japonica* Eliot, 1913 and *Bornella adamsii* Gray, 1850, which are designated as minor synonyms of *Melibe viridis* (Kelaart, 1858) and *Bornella stellifer* (Adams et Reeve, 1848) correspondingly, was shown to be distinct species and their taxonomic status should be reinstated.

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## **Fauna and distribution of bivalves of the Kuril-Kamchatka Trench (Pacific Ocean)**

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The Kuril-Kamchatka Trench with a maximum depth of 9600 m is one of the deepest trenches of the World Ocean and has the second (after the Izu-Bonin Trench) largest area of hadal habitat. It is located in an eutrophic area of the Pacific Ocean with one of the highest levels of primary production and has an abundant and diverse hadal bottom fauna. Investigation of the materials collected by the KuramBio II German-Russian expedition (RV *Sonne*, 2016) (8 stations), and three Russian expeditions (RV *Vityaz*, 1949, 1953, 1966) (16 stations) from the hadal zone of the Kuril-Kamchatka Trench (6000–9583 m depth) revealed a rich fauna of bivalves including 33 species and morphospecies belonging to 15 families, of which 18 species (54.0%) were determined to the species level and 14 species and morphospecies (42.4%) were first records for the Kuril-Kamchatka Trench. The richest families were Thyasiridae (8 species) and Lyonsiellidae (5 species). For estimation of the relationships between the hadal fauna of the Kuril-Kamchatka Trench and the abyssal fauna of the Pacific Ocean, the KuramBio II expedition performed 3 stations (5101–5752 m depth) on the Pacific slope of the Kuril Islands and the abyssal plain adjacent to the Kuril-Kamchatka Trench. Of the 33 species and morphospecies found in the Kuril-Kamchatka Trench, 15 species and morphospecies (45.5%) were recorded outside the trench at depths of less than 6000 m. *Vesicomya sergeevi* Filatova, 1971 and *Parayoldiella ultraabyssalis* (Filatova, 1971) formed the most abundant populations in the trench (72.9% of all specimens of bivalves collected by the KuramBio II expedition) and were predominant in samples from the trench bottom. In the hadal zone of the Kuril-Kamchatka Trench, 3 depth ranges (6000–6999, 7000–8999, 9000–9583 m) were distinguished that differ in the species composition of bivalves. The species richness decreases with increasing depth. The bivalve fauna of the upper hadal zone of the Kuril-Kamchatka Trench (depths 6000–6999 m) is characterized by the highest species richness (23 species and morphospecies) and is represented mainly (65.2%) by bathyal-hadal and abyssal-hadal species. This depth range is considered as a zone of transition between the abyssal and hadal zones. The fauna of this transitional zone is most closely connected with the fauna of the abyssal plains adjacent to the trench. For many abyssal species, the above-mentioned depth range is the lower limit of vertical distribution, while for a number of hadal species it is the upper limit of their vertical distribution in the hadal zone. Thirteen species and morphospecies (39.4%) do not penetrate deeper than 7000 m. At depths of more than 7000 m,

the proportion of hadal endemics significantly increases (more than 66%), more than one third of them were recorded only at these depths. At the maximum depth of the trench (over 9500 m), only 5 species and morphospecies were recorded. Fourteen species and morphospecies (42.4%) are endemic for the hadal zone of the Kuril-Kamchatka Trench. Of the 18 species identified, only 6 species (33.3%) were found exclusively in the hadal zone of the trench. The high species richness and quantitative abundance of bivalves in the Kuril-Kamchatka Trench is apparently associated with large amounts of organic matter supplied to the slopes and bottom of the trench, thus creating favorable conditions for the existence of bivalve populations. Taxonomic decisions herein: *Bathyspinula vityazi* (Filatova, 1964) is a distinct species and not a synonym of *Bathyspinula calcarella* (Dall, 1908); *Ledella spinuliformis* F.R. Bernard, 1989 is synonymized with *Ledellina formabile* Filatova et Schileyko, 1984. This research was supported by the Russian Foundation for Basic Research (grant no. 19-04-00281-a).

## **Current malacology: historic impediments and advanced technology**

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The molecular revolution in the marine biodiversity studies significantly changed the attitude to taxonomical research. It became generally recognized by scientific community that molecular data can be essential in taxonomic decisions, particularly useful in assessments of intraspecific variability and interspecific differences. Nevertheless the using of molecular data still did not become a routine procedure in taxonomy. Only about 3% of molluscs described in last decade are associated with a DNA sequence (based on collections of Muséum National d'Histoire Naturelle, most dynamic world museum housing the type specimens). Around 57% of molluscs (in 2000–2014) were described by citizen scientists, extremely rare or never using new advanced techniques. Majority of shelled molluscs were and still are described on the basis of empty shell alone, sometimes lacking important diagnostic characters (eg. protoconch) or of poor preservation.

Routine usage of the molecular barcoding of marine gastropod molluscs allowed us to re-evaluate the species boundaries and reveal numerous cryptic and pseudocryptic species (e.g., in *Gemmuloborsonia* and *Crassispira*, Conoidea), and also to break apart the species complexes, which were previously synonymized with “lumping” approach due to incorrect assessment of the intraspecific variability. Recent examples are recognition of six species within broadly distributed “species” *Lophiotoma acuta* (Perry, 1811), of which four can be readily distinguished on the basis of shell morphology, while existence of two was proved by molecular data; or the recognition of four species within the *Bursa granularis* (Röding, 1798) complex, previously treated as single broadly distributed throughout the Indo-Pacific and tropical western Atlantic species. Recently we discovered the presence of at least two morphologically indistinguishable molecular species within *Exilia vagrans* Kantor et Bouchet, 2001, the species originally based on significant well preserved and anatomically studied material.

Nearly 15 years ago Benoit Dayrat (2005) introduced the concept of integrative taxonomy and proposed seven guidelines “to help integrative taxonomists recognize cases when species are supported by broad biological evidence and therefore are deserving of an official name”. Among those was the suggestion that “No new species names should be created if type specimens deposited in a museum collection are preserved in a way that prevents any further molecular study.” Still this remains merely a declaration, particularly for molluscs.

Bearing in mind more and more refined molecular techniques, that already (although with difficulties) allow extraction and sequencing DNA from old specimens and even shells we should propose the concept of “concerned taxonomy practice”.

1. When new species is described with using molecular methods, the holotype should be selected from sequence specimens, even if it is incomplete, damaged or in poorer condition than other available specimens.

2. The formal species description based on empty worn shells should be avoided by all means.

3. When the new species is described based on material not immediately suitable or planned to be sequenced, the preference for selection of the holotype should be given to most recently collected specimens, the specimens that contains preserved or dried body inside.

4. If the description of the species is based on empty shells alone the preference for selection of the holotype should be given to shells that were not processed by chemical means (e.g., bleached).

**The diversity of neurotransmission-related molecules  
in the central nervous system  
of the mussel *Crenomytilus grayanus***

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The currently held opinion is that the central nervous systems of molluscs are simple, especially in sedentary animals, such as bivalves. Here, we examined the distribution of neurotransmitters, neuropeptides, and enzymes for neurotransmitter synthesis (5-hydroxytryptamine, FMRFamide,  $\gamma$ -aminobutyric acid, choline acetyltransferase, galanin, and dopamine) and the transcriptional factor GCM-1 in the central nervous system of the mussel *Crenomytilus grayanus*. We detected FMRFamide, choline acetyltransferase, galanin, alpha-acetylated tubulin, dopamine, and GCM-1 in all ganglia. Neurons immunopositive for 5-hydroxytryptamine were found in the cerebropleural and pedal ganglia, but unexpectedly no 5-hydroxytryptamine-immunopositive somata were observed in the visceral ganglia. We demonstrate that alpha-acetylated tubulin is expressed in all mussel neurons and may be a potential pan-neuronal marker for bivalves. Further, we investigated the proliferative status of neurons in mussel ganglia and describe a small population of proliferating cell nuclear antigen (PCNA)-immunopositive cells, which do not express any of the tested neurotransmitters. We conclude that adult mussel neurons do not proliferate; the PCNA-like-immunopositive cells might be glia. These new results suggest that, although the nervous system of bivalves is secondarily reduced as a result of their immobile or sedentary lifestyle, the molecular heterogeneity of neurons in ganglia is the necessary condition for chemical sensitivity and motor regulation in bivalves.

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## ***Parathyasira* cf. *dunbari* in Arctic waters**

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Currently the genus *Parathyasira* Iredale, 1930 typified by *P. resupina* Iredale, 1930 from the Tasman Sea consists of 10 accepted species. *Parathyasira dunbari* (Lubinsky, 1976) was originally described from shelf depths of the northwestern region of the Canadian archipelago. It was considered as a shallow-water high-Arctic species, which is endemic for the Canadian-Greenlandic region. Later, this species was reported from other localities, such as off the Svalbard Archipelago (Kedra et al., 2010) and the Haakon Mosby Volcano, Norwegian Sea (Gebruk et al., 2003). Morphologically, *P. dunbari* is very similar to *P. equalis* differing in less defined posterior area with narrowly rounding posterior margin. We believe that because of this morphological similarity, *P. dunbari* has often been mistaken as *P. equalis*. On the basis of our data, *P. dunbari* is widely distributed in the Kara and Laptev seas, suggesting a much wider geographical range than currently recognized. Shell morphology and soft part anatomy were studied for specimens collected in different localities using scanning and transmission electron microscopy. Study of gill anatomy showed the absence of abfrontal extensions of gill filaments consisting of bacteriocytes with chemosymbiotic bacteria. However, numerous bacteria of unidentified phylotype were observed at inner surface of mantle margins. Despite the strong morphological similarity between *P. dunbari* and *P. equalis*, preliminary molecular results based on sequences of nuclear gene 28S suggest only distant relationships between these species. Obviously, the genus *Parathyasira* in current understanding needs a revision.

**Vasa-positive germ plasm granules as a factor  
in the shift from mitosis to meiosis in gametogenic cells  
of the bivalve mollusc *Ruditapes philippinarum***

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Survival of the living organisms is provided by the reproductive function of germ cells which are uniquely capable of undergoing meiosis. The Vasa-positive germ plasm granules (GG) are known to accompany meiotic division but their role during this process is still poorly understood. Our study concerns the GG transformation during the shift from mitosis to meiosis in both oogenesis and spermatogenesis of the bivalve mollusc *Ruditapes philippinarum* (Manila clam).

Using specific markers such as anti-Vasa antibodies and anti-CYTB antibodies we analyzed the ultrastructural mechanisms of GG transformation during the shift from mitosis to meiosis. It was found that both oogonia and spermatogonia have Vasa-positive GG that are located in the vicinity of mitochondria. Since no signs of GG formation were observed, we suggested that these structures are inherited by premeiotic cells from primordial germ cells following a preformative way of germ plasm inheritance. GG were recorded in both compact and dispersed forms and, based on quantification, we conclude that during the mitosis-to-meiosis shift the compact Vasa-positive GG undergo dispersal. Since cytoplasmic Vasa was not found in oogonia and spermatogonia before GG dispersion, we suggest that GG are a unique source of Vasa at this stage.

Vasa labelling was registered close to the nuclei and inside of these organelles. In primary spermatocytes and primary oocytes this protein was observed in synaptonemal complexes. Presumably, the GG-originated Vasa participates in the pro-meiotic chromatin transformation occurring during meiosis onset.

Vasa was also found in contact with some mitochondria and inside mitochondria. Using the anti-CYTB antibody we noticed that the mitochondria adjacent to the Vasa-positive GG substance are disassembled. Actually, premeiotic disassembly of mitochondria induced by the GG substance was previously described by transmission electron microscopy. Being confirmed here using a specific mitochondrial marker, this fact may provide new clues for the mechanism of extramitochondrial localization of mitochondrial material. It was previously proposed that mitochondrial ribosomes could translate

nuclear RNAs during germ-line cells differentiation. Based on our study, it seems likely that the mitochondria may release their ribosomes by demolishing their own structure and a role of Vasa in mitochondrial disassembling could be supposed.

We wanted to figure out if premeiotic events are similar or different between females and males. Our comparative study showed that the meiotic onset is similar in both sexes. The dispersion of Vasa-positive GG followed by intramitochondrial and intranuclear Vasa invasion is a single event of germ plasm related structures (GPRS) action. Any other patterns of the GPRS transformation do not exist during the mitosis-meiosis-shift in both oogenesis and spermatogenesis in the clam *R. philippinarum*.

## Skeneidae and Anatomidae in the seas of the Eurasian Arctic

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Representatives of the families Skeneidae and Anatomidae are micromolluscs with adult size usually not exceeded 3 mm. These snails are the only tiny microvetigastropods known from the Arctic waters. Like many others small organisms, they remain poorly studied and their taxonomical and morphological diversity is underestimated in many regions of the world. In the Arctic and subarctic regions, species composition of microvetigastropods is satisfactory described by Warén (1991, 1993) for Skeneidae and by Høisæter and Geiger (2011) for Anatomidae only in the waters around Greenland, Iceland, and Norway. Morphological characters of shell and radula are still remaining the only useful tools in their taxonomy.

Little known about microvetigastropods from the Arctic regions East to the Norwegian Seas. The numerous faunistic studies often do not try to identify tiny organisms and reduce diversity of Anatomidae to *Anatoma crispata* (Flemming, 1828) and recognize only two skeneids – *Skenea trochoides* (Friele, 1876) and *Skenea basistriata* (Jeffreys, 1877). Gorbunov (1946) described one more species – *Ganesa bujnitzkii* (Gorbunov, 1946), from the region between the Laptev and the East Siberian seas. Its taxonomical status remains unclear so far. In addition, Nekhaev (2014) mentioned a possibly undescribed *Skenea* cf. *trochoides* from the Kola Peninsula.

We studied collections of the Zoological Institute of Russian Academy of Sciences (St. Petersburg, Russia) and the Laboratory of Macroecology and Biogeography of Invertebrates, St. Petersburg State University (St. Petersburg, Russia). Material was collected in the Eurasian Arctic seas: Barents, Kara, Laptev, and East Siberian, while samples from the White and Chuckchi seas were absent in the studied material. Also, we had seen reference collections (including type specimens) stored in the Swedish Museum of Natural History (Sweden) and Zoological Museum of Bergen University (Norway). All specimens of microvetigastropods were studied under the stereomicroscope, after that 35 shells (15 for Anatomidae, 20 for Skeneidae) and 16 radulae (7 for Anatomidae, 9 for Skeneidae) of selected specimens were studied with a scanning electronic microscope.

As a result, we found three species of the family Anatomidae instead of one living in the region. There are *Anatoma crispata*, *Anatoma schanderi* Høisæter et Geiger, 2011 and *Anatoma* sp. n. The new species from the northwestern Barents Sea can be recognized

from all other representatives of the family by its flat spire. We also suggest that *Anatoma schioettei* Høisæter et Geiger, 2011 described from the upper slope of Greenland is actually a conchological variety of *Anatoma crispata*. Both species differ by a sutsel (space between the start of selenizone and suture of the subsequent whorl in *Anatoma schioettei* relatively larger) and number of axials (e.g. 22 in *Anatoma crispata* vs 15 in *Anatoma schioettei* on teleoconch I). However, these characters were quite variable in our material and we found intermediate forms. Moreover, studied specimens do not have distinct differences in shell shape, radular morphology and size of protoconch.

We found two empty shells of *Anatoma schanderi* from the waters around New-Siberian Islands. The species was previously known only from Nova Scotia to the Norwegian Sea. This species is the only representative of the family known from the waters east to the Barents Sea.

According to our preliminary analysis, at least five species of the family Skeneidae live in the regions: *Skenea rugulosa* (Sars G.O., 1878), *Skenea trochoides*, *Skenea basis-triata*, *Skenea ossiansarsi* (Warén, 1991) and “*Rugulina*” *bujnitzkii* (Gorbunov, 1946). *Skenea* cf. *trochoides* noted by Nekhaev (2014) was considered as a form of *Skenea trochoides*.

We studied two syntypes of *Ganesa bujnitzkii* which are the only known representatives of the species. Due to the similar pattern of the shell sculpture we suggest that this species is related with the genus *Rugulina* Palazzi, 1988.

In addition, with a high probability, two more species *Skenea polita* and *Skenea ferruginea* are also inhabit the Barents and Kara seas.

The study was supported by the Russian Scientific Foundation under the grant no. 18-74-00010 (examination of ZIN collection and morphological studies). Study of the recent material from the Barents Sea was able to support of grant Council of the President of the Russia under grant no. MK-4797.2018.4. Work with the scanning electronic microscope was performed at the Research park of St. Petersburg State University Interdisciplinary Center for Nanotechnology.

**On the intertidal bivalve fauna  
in the Far Eastern Marine Biosphere Nature Reserve  
(Peter the Great Bay, Sea of Japan)**

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Bivalve mollusks are one of the most common groups of marine benthos. Their role becomes especially significant in the intertidal and subtidal zones. The aim of this study was to determine the present species composition of bivalve mollusks in the intertidal zone of the Far Eastern Marine Biosphere Reserve (FEMBR), which is most vulnerable to anthropogenic impacts.

The material was the samples of macrobenthos collected in the intertidal zone of the reserve. In the eastern sector, studies were conducted in June 2012 in Srednyaya Bay (continental coast) and in Molchansky Bay (Bolshoy Pelis Island). In the southern sector, studies were conducted in July–August 2012 in Pemzovaya and Kalevala bays, as well as in Zapadnaya Bay (Furugelm Island) and off Cape Ostrovok Falshivy. In July–August 2014, samples were collected in the Western (Minonosok Bay) and southern sectors (Cape Ostrovok Falshivy) of the reserve.

A total 15 bivalve species and one bivalve hybrid were found. Three species – *Arca boucardi*, *Mactra chinensis*, and *Mercenaria stimpsoni* – and one hybrid of the bivalves *Mytilus galloprovincialis* and *M. trossulus* with traits of the latter proved to be new to the intertidal fauna of the reserve. Most of the species were found in the Southern sector. The 17 bivalve species referred to intertidal zone of the reserve according to data published in literature, were not registered in this study.

Below is a list of intertidal bivalve mollusks of the Reserve. The species were identified using the keys of A.N. Golikov, G.N. Volova, O.A. Scarlato, A.V. Martynov and K.A. Lutaenko. The bivalves are deposited in the collection of the FEMBR (Vladivostok). The species new to the intertidal fauna of the reserve are indicated by asterisk.

**Order MYTILIDA  
Family Mytilidae**

1. *Crenomytilus grayanus* (Dunker, 1853)

Found in Srednyaya Bay. Previously, it was indicated for the intertidal zone of the coast and islands of the eastern and southern parts of the Reserve. In the intertidal zone, it inhabits hard sediments.

2. *Musculista senhousia* (Benson, 1842)

Found in Pemzovaya Bay and off Cape Ostrovok Falshivy. Distributed everywhere.

3. *Mytilus trossulus* A.A. Gould, 1850

Found in Pemzovaya Bay and off Cape Ostrovok Falshiviy. Distributed everywhere.

4.\*Hybrid of *M. galloprovincialis* and *M. trossulus* with traits of *M. trossulus*

Found in the intertidal zone off Cape Ostrovok Falshiviy. Earlier, the subtropical-low-boreal invasive species of *M. galloprovincialis* did not produce hybrids with indigenous mussels and was registered in the southern part of the reserve in fouling of driftwood.

5. *Septifer keenae* Nomura, 1936

Found in Zapadnaya Bay. Previously, it was common off the coast in the eastern and southern parts of the reserve. Inhabits hard and mixed sediments.

**Order ARCIDA**

**Family Arcidae**

6. \**Arca boucardi* Jousseume, 1894

Found in intertidal zone of Zapadnaya Bay, southern sector. Inhabits hard sediments.

**Order CARDIIDA**

**Family Tellinidae**

7. *Macoma incongrua* (Martens, 1865)

Found in Kalevala Bay and off Cape Ostrovok Falshiviy. Previously, it was also registered in the southern part of the reserve. Inhabits mixed and soft sediments.

8. *M. contabulata* (Deshayes, 1855)

Found in the intertidal off Cape Ostrovok Falshiviy. Previously, it was registered in the southern part of the reserve in the upper subtidal. Inhabits mixed and soft sediments.

**Family Psammobiidae**

9. *Nuttallia obscurata* (Reeve, 1857)

Found off Cape Ostrovok Falshiviy. Previously, it was registered in Sivuchya Bay. Inhabits mixed and soft sediments.

**Family Mactridae**

10.\**Mactra chinensis* Philippi, 1846

First found in the intertidal zone of Cape Ostrovok Falshiviy. Previously, it was registered on soft sediments at a depth of 5–30 m.

11. *Spisula sachalinensis* (Schrenck, 1861)

Found in the intertidal zone off Cape Ostrovok Falshiviy. Previously, it was registered on soft sediments from the intertidal zone to a depth of 22 m.

### **Family Veneridae**

12. *Protothaca euglypta* (Sowerby III, 1914)

Found in Zapadnaya Bay and off Cape Ostrovok Falshiv. Previously, it was registered in the intertidal zone of the southern and eastern parts of the reserve. Inhabits mixed sediments among seagrass rhizomes.

13. \**Mercenaria stimpsoni* (A.A. Gould, 1861)

First found in the intertidal zone of Cape Ostrovok Falshiv. Inhabits soft sediments in the upper subtidal zone.

14. *Ruditapes philippinarum* (A. Adams et Reeve, 1850)

Found off Cape Ostrovok Falshiv. Previously, it was registered in the Southern and eastern parts of the reserve. Inhabits mixed and soft sediments.

### **Order PHOLADIDA**

#### **Family Myidae**

15. *Mya japonica* Jay, 1857

Found in the intertidal zone of Cape Ostrovok Falshiv. Inhabits soft sediments of intertidal and upper subtidal zones.

### **Order PANDORIDA**

#### **Family Laternulidae**

16. *Laternula marilina* (Reeve, 1860)

Found in the intertidal zone of Cape Ostrovok Falshiv. Previously, it was registered in the southern part of the reserve.

In the present intertidal bivalve fauna of the reserve, five families have the highest species richness: Mytilidae, 6 species from 5 genera; Veneridae, 5 species from 4 genera; Mactridae, 3 species from 3 genera; Myidae, 3 species from 2 genera; and Psammobiidae, 2 species from 2 genera. Other 11 families are represented by one genus each. Rare species have not been registered.

The invasive species, *M. galloprovincialis*, was previously encountered only in fouling of driftwood washed on the coast of the southern part of the reserve. However, according to our data, the typical form of the indigenous species *M. trossulus* is currently registered only in the eastern part of the reserve, and its hybrids with *M. galloprovincialis* have been found in the southern part. Thus, an Atlantic species is introduced into the benthic communities of the Far Eastern Marine Reserve through hybridization with native species.

## **Growth and distribution of the bivalve mollusc *Macoma calcarea* in the Kara Sea**

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The work is devoted to the analysis of the patterns of distribution and linear growth of *Macoma calcarea* in the southwestern part of the Kara Sea based on the results of observations (119 stations at depths of 4–415 m) in August–September, 2012–2013. Former studies have shown that, at least, for almost 100 years *M. calcarea* has been a characteristic component of coastal (at depths less than 50 m) benthic communities in the southwestern part of the Kara Sea, sometimes dominating by biomass. The densest beds of molluscs were confined to silty biotopes at depths less than 50 m. In this depth range, it was possible to relate the characteristics of the distribution of *M. calcarea* only to the characteristics of bottom sediments. The greatest molluscs abundance (biomass and density of up to 90 g/m<sup>2</sup> and 120 ind./m<sup>2</sup>, respectively) were found in habitats with a high proportion of silt (more than 80%), which is well explained by trophic characteristics of this species. No effect of inter-specific interactions on the distribution of *M. calcarea* was revealed. The maximum size and age of *M. calcarea* (37 mm and 21 years, respectively) in the southwestern part of the Kara Sea, as well as the average annual growth rate (about 2 mm/year) were close to that in other areas of distribution. A comparison of results of mollusc age determination using outer and inner (along the shell sawing) rings, showed that the inner marks almost completely duplicated the outer rings. Both individual and group growth were characterized by weak heterogeneity within the studied area. The variation of individual growth rate was, apparently, determined by the characteristics of the initial period of mollusc growth.

## Going in depth into the evolutionary relationships between two species of the *Gibbula* genus

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In recent years, studies on the systematics and phylogenetic relationships among top-shells of the family Trochidae have proliferated, although a clear and consensual image of the evolutionary relationships within the group is still lacking. The genus *Gibbula* Risso, 1826 (Trochidae, Cantharidinae) is a good example of this. The taxonomy of the group, traditionally shell-based, together with vague original descriptions of some of the taxa, have led to problems with identifications of species in the field. Furthermore, the genus was recently characterized as paraphyletic and split (Williams et al., 2010; Uribe et al., 2017), and some of the species are now assigned to *Steromphala* Gray, 1847. However, this taxonomic reassignment lacks a strong phylogenetic support, and more complete genetic and morphological analyses are still needed to characterize the patterns of variability of taxa within this group. Therefore, we decided to maintain the original genus name. Here, we have studied two species, *Gibbula divaricata* and *G. rarilineata*, species with overlapping morphological characteristics and variability, making them hard to differentiate. Both species share the same habitat and distribution in the Mediterranean Sea. We have analyzed the patterns of COI genetic variability in more than 700 specimens sampled throughout the Mediterranean and Black Sea, and a nuclear gene (ITS) for individuals of the main mitochondrial lineages revealed for *G. divaricata* and *G. rarilineata*. We found that the evolutionary scenario is more complex than previous studies have shown. At least two well-differentiated mitochondrial lineages, supported by nuclear information, are observed in *G. divaricata*, corresponding to the eastern and western basins of the Mediterranean. Furthermore, phylogenetic analyses suggest that one of these lineages seems more closely related to *G. varia*, a third species of atlantic-mediterranean top-shells. In *G. rarilineata*, two lineages are also identified for the same basins and the Black Sea. We also identify some individuals as putative hybrids between *G. divaricata* and *G. rarilineata*. We will discuss the evolutionary, systematics, and taxonomic implications of these results, together with the historical dynamics of the two species, *G. divaricata* and *G. rarilineata*, their demographic trends, patterns of gene flow, and the establishment of a potential hybrid zone between them.

## **Cephalaspidea systematics: state of the art and future challenges**

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The concept of Cephalaspidea has changed tremendously since first introduced by Paul Fischer in 1887. Nevertheless, the idea of a group united by the shared presence of a shield-like head remained until the mid 1980's and was only with the work by Paula Mikkelsen in 1996 based on cladistic analyses of morphological characters that sound evidence for the removal of certain groups (e.g. Acteonidae, Aplustridae, Ringiculidae) from the Cephalaspidea was produced. During the beginning of the XXI century the generalization of molecular phylogenetics helped to define the limits of Cephalaspidea, and as a consequence runcinids were showed not to be members of the clade. As the limits of Cephalaspidea become well established, attention was re-focused towards the definition, composition and relationships of genera and families. DNA-based phylogenetic studies have challenged the traditional definition of many families and genera and our understanding of their relationships. Paradigmatic examples are the families Aglajidae, Haminoeidae, Philinidae among others. Recent research on meiofaunal gastropods have also generated new insights about the systematics of “cephalaspidean-like” slugs.

In this presentation I will provide an historical overview of the evolution of the concept and composition of Cephalaspidea and will discuss the latest finding and their implications to our understanding of the systematics of the group. I will conclude highlighting the present challenges and open questions that in my point of view shall drive systematics research in these molluscs during the forthcoming years.

**Hybrid zone between two mussel species,  
*Mytilus trossulus* and *Mytilus galloprovincialis*,  
in north-western Japan Sea:  
genetic and morphologic variability**

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Hybrid zone of Pacific mussel, *Mytilus trossulus* and the Mediterranean mussel, *M. galloprovincialis*, was studied in the north-western part of the Sea of Japan, Peter the Great Bay. The analysis was based on 8 polymorphic enzyme loci, 2 nuclear DNA markers (Me-5 and ITS-1, 2) and morphometry (11 traits and 10 indices). Using all types of genetic markers, the prevalence of local species *M. trossulus* was detected in the settlements. The fraction of the introduced species *M. galloprovincialis* in the whole material is relatively low. However, in some settlements (Posyet Bay, Kievka Bay and Lazurnaya Bay) quite a significant proportion of hybrids (Fh, %):  $24.2 \pm 8.0\%$ ,  $14.6 \pm 5.1\%$  and  $9.0 \pm 3.2\%$  was revealed. Main outcome is made on the continuation of the invasion of *M. galloprovincialis* in north-western part of the Sea of Japan; it is stated that in the Posyet Bay and Kievka Bay even permanent settlements of this mussel are already available which never recorded here before. Second conclusion is that genetic introgression still present between two taxa of *Mytilus* ex. gr. *edulis* investigated from the area in Peter the Great Bay and its vicinities. Although it keeps at low level, which vary across years judging on obvious differences in amount of all types of hybrid occurrence in samples 2011 vs. 2012–2013 and from other records in the Vostok Bay area for 14 years interval: 2012–2013: 0%, 2011: 0%, 2003:  $1.60 \pm 0.90\%$ , and 1999:  $8.95 \pm 1.68\%$ .

## **Pteropods make thinner shells along a natural ocean acidification gradient**

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Rising atmospheric carbon dioxide concentrations lead to ocean acidification (OA), which affects marine calcifying organisms. Shelled pteropods, a group of calcifying zooplankton, are widely regarded as bio-indicators of OA. Their delicate aragonitic shell can be damaged or even partially dissolve in waters with low aragonite saturation levels. However, recent studies indicated that they have a potential repair mechanism and may be protected from dissolution by an impermeable organic layer around the crystal structures of the shell, the periostracum. Whether this implies a possible resilience to the effects of ocean acidification is currently under debate. This study examines the impact of ocean acidification gradients on net calcification of *Limacina helicina* pteropods in the northern California Current Ecosystem. Shell thickness was assessed to quantify the amount of precipitated shell by means of Micro-CT scanning, whereas the loss of shell was determined by the extent of dissolution on the outer surface of the same shells through Scanning Electron Microscopy (SEM). Additionally, since at least two *L. helicina* subspecies are described from the CCE, we examined variability in DNA sequences of the mitochondrial Cytochrome Oxidase 1 gene to assess whether individuals belong to the same gene pool. Integrating these results provided valuable insight into the debate about vulnerability of pteropods to ocean acidification. We show that shell thickness of *L. helicina* declines by 33.7% along a natural OA gradient generated by the combined effects of acidification and upwelling in the northern California Current Ecosystem. Low levels of genetic diversity ( $\pi=0.26\%$ ) were found based on partial sequences of the mitochondrial COI gene, indicating a homogenous population. Shell thickness was decoupled from individual size, and thinner shells did not show more dissolution on the outer shell surface. Based on these results, we infer that the decrease in shell thickness is not so much the result of dissolution, but rather a decline in calcification, perhaps on account of an adaptive response.

**A tool equipment for a tiny driller:  
first look at the morphology  
of the buccal complex of *Doto coronata***

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*Doto coronata* (Gmelin, 1971) is a well-known species of nudibranchs, feeding on hydroid polyps of the genus *Obelia*. It is known, that the individuals of *Doto* penetrate the stolon the polyp during feeding process and have a kind of drilling feeding mode. However, the feeding mechanism and the morphology of the buccal complex of this species have not been described before, which makes any comparison to other drilling nudibranchs and other drilling gastropods impossible.

In the present study we performed a detailed description of the general and fine morphology of the buccal complex using methods of light microscopy, SEM and TEM. The computer-based 3D-reconstruction of the buccal complex of *D. coronata* was constructed as well.

According to our data the buccal complex of *D. coronata* is relatively small and takes less than one third of the head volume. The length of the buccal complex in the specimen 11 mm long is about 270  $\mu\text{m}$ . The length of the radular tooth is 7  $\mu\text{m}$  in the specimen of length 7 mm. The jaws are quite thin and not visible without magnification. However, the buccal musculature and the buccal armature are well-developed and resemble the buccal structures found in *Dendronotus frondosus* (Ascanius, 1774), which belongs to the same superfamily Dendronotoidea.

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## Disentangling the taxonomy of *Mercuria* Boeters, 1971 using an integrative approach

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The western Palearctic freshwater snail genus *Mercuria* Boeters, 1971, composed of 28 species, is primarily distributed in lowland localities of the Mediterranean basin, occurring at the Iberian and Italian peninsulas, France, Morocco, Algeria, and Tunisia. The low variability in the male reproductive system of some close-related species has led in many cases to wrong classifications. In general, shell shape shows a great intra and interspecific variation, while in a few other cases some studied populations are hardly differentiable from others and could be almost categorized as cryptic species. This fact and also the problem of the missing type material in many old collections actually make the systematics of the group a challenging task.

For this purpose, we carried out a revision of the genus, focusing on different types of data. We analyzed a total of 79 localities according to the distribution of this genus. Firstly, 2–8 specimens per population were dissected in order to check the validity of the described species. For molecular data, three molecular markers mtCOI, 16S rRNA, and 28S rRNA were amplified and sequenced from 209 specimens of 11 recognized species, covering the 40% of the species richness for this genus. Phylogenetic relationships among species were inferred using both maximum likelihood (ML) and Bayesian inference (BI), measuring the robustness by parametric bootstrap (ML) and Bayesian posterior probability (BPP). Three methods of species delimitation were tested, the distance-based automatic gap discovery (ABGD), the Poisson Tree Processes (PTP) method, and the single-threshold generalized mixed Yule-coalescent method (ST-GMYC). The ABGD delimitation was the nearest to the species delimited by classical taxonomy using morphology, although in all methods the number of identified species was overestimated.

Geometric morphometric (GM) analysis was conducted in order to record intra- and interspecific variability of shell shape, analyzing a total of 1014 pictures corresponding to 44 localities. Shell variables were scored through a total of 21 landmarks and semilandmarks per specimen. All the data were subjected to a Procrustes superposition analysis, the consensus of each population and each species was obtained and analyzed individually for both levels using a Principal Component Analysis. A warp analysis was conducted in order to obtain the thin-spline plot per species to visualize the variation of the shell within species.

After analyzing molecular, morphological, and GM data, we conclude that *Mercuria bayonnensis* (Locard, 1894), the populations of *Mercuria anatina* (Poiret, 1801)

from the Netherlands (Boeters, Falkner, 2017: *Zoosystema*, 39 (2): 227–261), and *Mercuria edmundi* Boeters, 1986 are junior synonyms of *Mercuria tachoensis* (Frauenfeld, 1865), with an Atlantic distribution in Europe, whereas *Mercuria meridionalis* (Risso, 1826) and *Mercuria emiliana* (Paladilhe, 1869) are junior synonyms of *Mercuria similis* (Draparnaud, 1805), occurring along the Mediterranean coast in southern Europe. The rest of the analyzed species were considered as valid. Despite this decrease in diversity, our integrative approach revealed an unexpected species richness of the genus in the Mediterranean region, with morphological and molecular data indicating five new species for science.

## **Recent and rapid radiation of freshwater snails in the ancient Lake Biwa**

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Ancient lakes often harbor a variety of endemic species which have been radiated within the lakes. Several hundreds of cichlid fishes were rapidly evolved in the Great East African Lakes and there is also a great diversity of amphipods in the Siberian Lake Baikal. Lake Biwa, located at the central Japan, is an ancient lake and also harbors a suit of endemic snails in the subgenus *Biwamelania*. We studied the diversification history of the *Biwamelania* snails with respect to the geological history of Lake Biwa. Using genome-wide DNA analyses, we found that two genetically distinct snail lineages independently colonized Lake Biwa and they concurrently and rapidly radiated into 15 extant *Biwamelania* species. A combination of paleontological evidence and molecular dating technique demonstrated that the radiation of *Biwamelania* was tightly linked to the latest enlargement of the lake about 0.4 million years ago and suggested that increased ecological opportunity associated with the lake expansion drove the rapid adaptive radiation. The clear geology and abundant fossils in Lake Biwa can provide a framework for determining the evolutionary factors that have facilitated rapid species diversifications within ancient lakes.

## **Locomotion of *Chaetoderma nitidulum* (Mollusca: Aplacophora)**

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The progress of technology over the past decades has allowed us to observe animals in natural surroundings not only on the land, but also under the water. Due to the spread of amateur and professional photography and video, the secrets of relationships within the populations, between different species of animals have been discovered, the process of reproduction, hunting, feeding and many others have been visualized. Most often, underwater photography and video are used in popular-science films, however, the visualization of some processes can be key for understanding the biology of various species.

There are many invertebrates that spend most of their life inside marine sediments – in sand and silt. This way of life is also led by the wormlike mollusk *Chaetoderma nitidulum*, whose biology remains poorly understood. *Ch. nitidulum* were collected in the vicinity of the WSBS named after N.A. Pertsov in June 2018 using a grab dredge at a depth of 70 m. A typical way of moving worm-like animals in sandy and silt soils is a wave-like movement and direct peristalsis, respectively. The purpose of this work is to visualize the process of burrowing and to compare the stages of the movement of these mollusks with the dynamics of the circulatory and muscular system.

A convenient visualization method is the replacement of muddy soil with gelatin and the subsequent observation of *Ch. nitidulum* in the laboratory. *Ch. nitidulum* is slow-moving animal and for a good visualization of the movements of this mollusk it is best to use a TimeLapse, which allows you to take photos within a specified period. On the basis of the data obtained, the main stages of the movement of *Ch. nitidulum* were identified, and the circulation pattern and muscle contraction were also described. In the front end, the longitudinal, diagonal and circular muscles are well developed, they are washed by the extensive cerebral sinus, and the musculature of the posterior part of the body is much less developed, there mainly happens oxygenation of the blood, because there are located the gills. Thus, the anterior end of the body is mainly involved in peristalsis, and it pulls up the posterior one.

## **Morphology of Onchidorididae's (Mollusca: Nudibranchia: Doridoidei) spicules networks**

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Onchidorididae (Doridoidei, Nudibranchia) is the family of nudibranch mollusks with spicules in their integuments which bear supporting and protective functions. Despite that general morphology and arrangements of the spicules well described, data of localization, internal morphology and correlations between spicules morphology and strength are fragmentary. This study describes in detail the spicules of three dorids *Acanthodoris pilosa* (Abildgaard in Müller, 1789), *Adalaria proxima* (Alder et Hancock, 1854), *Onchidoris muricata* (Muller, 1776) inhabiting in the White Sea.

The collection of material was carried out in the summer period 2016–2019 in the Kandalaksha Bay of the White Sea in the vicinity of the N.A. Pertsov White Sea Biological Station. During the research classical methods of light and scanning electron microscopy were used.

As a result of the study of spicular morphology there were described four types spicules (monoactine, diactine, triactine, tetractine) for all studied species, as well as spherules, which have been found only in papillae of *A. pilosa*. The external and internal morphology of spicules have been studied by scanning electron microscopy. Three types of internal organization of the spicules have been identified – monolithic, mixed, and concentric. There was no find reveal correlation between the type of the spicules and their internal structure.

For the first time have been proposed the correlation between the general morphology, thickness of the tracts and thickness of the integuments: the density of the integuments does not depend only on the number of the spicules, but also on the character of their colocalization and the thickness of the layer in which they are located. The specula networks of body *A. pilosa* has thick covers and doesn't have elongated spicules in the papilla, but *O. muricata* and *A. proxima* have vertical tracts with elongated spicules in the papilla with thin cover.

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**Mollusks associated with *Ulva* spp.  
along the coasts of Jeju Island, South Korea**

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The genus *Ulva* comprises a small genus of edible green algae, often known as “sea lettuce”, that is distributed worldwide. It grows mainly in marine and brackish water. The various species exhibit a wide range of variation in response to environmental conditions, and can be difficult to identify; thus, molecular analysis has often been used to separate the species. Mollusks, usually small grazers and the carnivorous species that prey on them are among the dominant organisms inhabiting the algae. The algal mats also provide shelters that protect the animals from physical stressors (such as desiccation and the impact of waves) and against predators.

Specimens of *Ulva* spp. were collected from the intertidal and subtidal zones at five localities around the island, from Aug. 7 – Sept. 17, 2017. Large collections were made at 4 localities, Jongdal, Jocheon, Keumdeung, and Pyoseon, with a smaller one at Sinyang. All invertebrates found on the algal mats were collected, placed in 70% ethanol, and the mollusks, being the most numerous phylum, were identified first. Numbers of specimens for each species, their maturity, and living or dead specimens were recorded. Species were also grouped according to families. Only the smaller species, known to be actively associated with seaweed were studied. Larger species that also occurred on the *Ulva*, mainly those living in different environments, such as rock and sand dwellers, were regarded as advectitious and excluded from this study.

A total of 79 species belonging to 3 classes and 30 families was recorded, mainly gastropods (70), with 6 bivalves and 3 chitons. Five specimens were identified only to class or family. The most speciose families in Gastropods were the Trochidae (16), and Columbelloidea and Nassariidae with 6 species each, followed by the other families having 1–3 species each; in the Bivalvia the Mytilidae had 5 species. Jocheon had the largest number of species (46), followed by Keumdeung and Jongdal, and Pyoseon with 45, 31, and 30, respectively. Sinyang, which were not as intensively sampled, had 7 species.

Many larger species were obtained, mostly rock or sand dwellers which may live adjacent to, or at the base of, the alga mats and are regarded as advectitious. As mentioned above, the smaller species known to feed on seaweed were the objects of a more intensive study. A total of 23 small species belonging to 8 families was recorded, mainly gastropods, with three bivalves. They were mainly species of Trochidae, Columbelloidea, and Nassariidae, with several other families with 1–3 species each. Apart from

the carnivorous nassariids, they were mainly grazers and deposit feeders. Most intertidal grazing gastropods are apparently generalist herbivores and are not associated with any particular species of algae. Smaller species and juveniles of larger species usually inhabit algal mats. The great majority of specimens obtained belonged to the smaller species that were grazers or detritus feeders; however, two species of Nassariidae, which are carnivorous or scavenging, were also common. Although the Nassariidae are mainly carnivorous or scavenging sand dwellers, two small species, *Nassarius fraterculus* (Dunker, 1860) and *N. multigranosus* (Dunker, 1847) were common at several localities, and this indicates that those nassariids may inhabit the algae while feeding on the small grazers, especially the trochids, two of which were abundant at most localities.

Of the 23 small species inhabiting the *Ulva*, six, both herbivores and carnivores, were abundant at several localities. Two species of Trochidae, *Cantharidus bisbalteatus* (Pilsbry, 1901) and *C. japonicus* (A. Adams, 1853) were dominant at Jongdal-ri, Jocheon, and Keumdeung; a species of Cerithiidae, *Cerithium dialeucum* Philippi, 1849 at Jongdal-ri, and *Anachis miser* (Sowerby I, 1844), in the Columbelloidea, at Jocheon. The carnivores, two species of Nassariidae, *N. fraterculus* and *N. multigranosus*, were dominant at Jocheon and Keumdeung. All four herbivores and *N. fraterculus*, are quite common in the intertidal and shallow zones around the island, and *N. multigranosus*, is locally common.

The two *Cantharidus* species, reported from Korea and Japan, usually inhabit various species of seaweed and coralline algae in the intertidal and subtidal zones. *C. dialeucum*, ranging from Japan to Indo-West Pacific, is mainly a detritus feeder but is also known to feed on algae, often found on algae-covered rocks. *A. miser*, ranging from Korea to the western Pacific and Australia, is a grazer occurring among seaweed on rocks, and also living as an epizooite on *Ulva*. *N. fraterculus* and *N. multigranosus* inhabit rocky and gravelly shores, and also mudflats, being commonly found on muddy sand.

Each species was assigned a zonal-geographical grouping based on its known distribution range: tropical-subtropical (southward to Southeast Asia), subtropical (southward to Taiwan and the northern part of the South China Sea), and subtropical-low boreal (subtropical areas and the Yellow and East Seas). A zonal-geographical examination of the small species actively associated with *Ulva* spp., based on their distribution, reveals that they were divided almost equally between tropical-subtropical species, preferring warm water, and subtropical or subtropical-low boreal, preferring moderately warm to somewhat cooler water. Of the more speciose families, the small trochid species were mainly subtropical/low-boreal in distribution, most columbellids and all the Cerithiidae were completely tropical-subtropical.

This analysis revealed that, overall, the mollusk species associated with *Ulva* spp. were divided almost equally between subtropical and subtropical-low boreal species, (52%), and tropical-subtropical species (48%). The gastropods exhibited a similar division, 48% to 52%, respectively, and most bivalves (52%) were subtropical in distribution.

Two of the three chitons were also subtropical, and one was classified as subtropical-low boreal. Two previous faunal studies established that the mollusk fauna studied was divided almost equally as above. However, more recent studies indicate that, overall, the bivalve fauna is beginning to exhibit a more tropical-subtropical nature.

Of the 78 species obtained from the *Ulva* spp., most of the larger species inhabited other types of substrates, and may be regarded as advectitious, coincidentally occurring on, or near, the algae at the time of sampling. The smaller species obtained were those that feed on seaweed and the epibioties found there, and were much more numerous, especially the Trochidae and Columbelloidea. Several species of Nassariidae, which may be feeding on the grazers, were also quite common. An analysis of the distribution patterns demonstrates that the species associated with *Ulva* spp. are almost equally subtropical to warm-temperate (low boreal) and tropical-subtropical in nature. However, with climate change, especially ocean warming, more warmer water species may find their way to Jeju Island, eventually giving the mollusk fauna a more tropical-subtropical character.

**An assemblage of mollusks associated  
and with the high latitude scleractinian coral  
*Alveopora japonica* (Eguchi, 1968)  
in Jeju Island, off the south coast of Korea**

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The scleractinian coral *Alveopora japonica* Eguchi, 1968 is a high-latitude coral ranging from southern Japan and Jeju Island, Korea to eastern China and Taiwan. This species is expanding its range along the coastline of Jeju Island. In 2012, a survey was conducted on the northwest coast, to examine the extent and density of the population there. Colonies were obtained by SCUBA diving from a 1 m x 10 m area at a depth of 10 m, and a large number of invertebrates were obtained from the coral. A total of 27 mollusk species, bivalves and gastropods, were identified. A boring mytilid, *Lithophaga curta*, and an arcid, *Barbatia stearnsi*, were the dominant species. Several grazing gastropods, such as *Turbo cornutus*, and encrusting bivalves, such as the Spondylidae and Chamidae, also use the coral as a habitat. It is proposed that the presence of these mollusk species in the coral suggests its use as a “nursery” for juvenile species, a ready food supply of organic detritus, and a refuge from predators. A zonal-geographical examination of the distribution ranges of these mollusks reveals a majority of warmer water species.

**Land snail fauna of islands  
in the Peter the Great Bay (Sea of Japan):  
biodiversity and distributional patterns**

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Species composition and distributional patterns of land snail fauna of 27 islands located in central and southern parts of the Peter the Great Bay were studied. The mollusks on the islands were collected by authors and some other collectors for over the past 20 years, mainly in the period of 2016–2018. All specimens are deposited in L.A. Prozorova's malacological collection housed in the FCS Northeast Asian Biodiversity FEB RAS (Vladivostok).

Terrestrial malacofauna of 11 islands belonging to Far Eastern Marine Reserve was studied before and as a result taxonomic list including 33 species in 18 genera and 14 families was published (Prozorova, 2004; Prozorova et al., 2018). Malacological survey conducted on other 16 islands of central and southern parts of the Peter the Great Bay, enlarges insular species list significantly. Up to date, land snail fauna on 27 studied islands counts 41 species in 21 genera and 17 families, including two species new for the region. That is about 62% of the total diversity in a whole Primorye Territory where terrestrial fauna counts 66 species in 31 genera and 21 families. However, insular fauna differs from continental that not only in number of species. The islands are inhabited by many more species distributed to south adjacent territories of East Asia. There are no endemics on any islands in the Peter the Great Bay because of not enough period of isolation from the continent approximately estimated as 8–10 thousand years. Nevertheless, five mollusk species are known in Primorye Territory only on islands – *Pupilla* cf. *muscorum* on Bolshoi Pelis Is., *Columella* cf. *acicularis* on Moiseev Is., “*Karaftohelix*” *diversita* found on 6 islands as well as new for the region *Punctum* sp. on Furugelm Is. and *Cochlicopa* cf. *hachijoensis* found on 4 islands. Probably these five species are more common on Korean Peninsula, which northern part is nearly unstudied malacologically.

The dependence of the diversity of terrestrial malacofauna on the area of the studied site (island) is often discussed in the literature. In general, the islands of Peter the Great Bay are also characterized by an increase in biodiversity to a certain level with increasing area. However, this dependence is clearer when comparing not separate islands, but groups of closely spaced islands that were probably united in some periods of

the past. Table demonstrates decrease in consecutive order of diversity from 33 to 5 mollusc species from the largest in area island group I to the smallest group VII.

**Number of land snails on 27 islands separated in 7 group of closely spaced islands in the dependence on total square of group**

No of group	Islands united in the group	Total square (km <sup>2</sup> )	Number of species
I	Russky Is., Elena Is., Engelm Is., Ponov Is., Reineke Is., Naumov Is., Klykov Is.	116.46	33
II	Bolshoi Pelis Is., Matveyev Is., Stenin Is., De-Livron Is., Durnovo Is., Gilgdebrandt Is.	8.61	30
III	Ricord Is., Pakhtusov Islands, Kamen' L'va Is.	5.19	23
IV	Furugelm Is., Vera Is.	2.65	22
V	Moiseev Is., Zheltukhin Is., Krotov Is., Karamzin Is., Sergeyeu Is., Tsyvol'ko Is.	0.80	19
VI	Dva Brata Is., Kozlov Is.	0.0624	14
VII	Verkhovsky Islands	0.0206	5

Land snail diversity on each island also depends on the availability of fresh water and livable biotopes, especially forest, which usually provides the most diverse habitats for molluscs. Wetlands, meadows, bushes and overgrown debris on rocky shores also represent unique land snail habitats with high biodiversity. The most high mollusk diversity is revealed on the biggest Russky Island (97.6 km<sup>2</sup>) where 29 from 41 recorded species occur on. There are several streams and ponds, lake and river on that island mainly covered by oak forest. Landscapes of the island are very diverse, but disturbed in part by military bases activity bases during Soviet time. Other 25 studied islands are much smaller in area, less populated or unpopulated at all. Four islands Popov, Reineke, Polshoi Pelis and Furugelm with fresh water, which square not less 3 km<sup>2</sup> are inhabited by 21–26 land snail species. On waterless small islands with square less than 1 km<sup>2</sup> only 5–17 species were found. However, two small islands Pakhtusov (0.18 km<sup>2</sup>) and Klykov (0.15 km<sup>2</sup>) are inhabited by 22 and 26 land snail species respectively, which is connected with presence of fresh water sources. It was found that the smallest group VII, which includes the Verkhovsky Islands, represented by the tops of the rocks, is inhabited only by 5 land snail species, including 2 common eurytopic species and 3 specialized inhabitants of the rocks along the coast of the Sea of Japan and the Yellow Sea.

**Aquatic and terrestrial molluscs  
of the Con Dao Archipelago (southern Vietnam):  
first results of malacological survey in March 2019**

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Con Dao Archipelago located about 80 km from mainland Vietnam includes 16 mountainous islands and islets with the total land area 76 km<sup>2</sup>. The islands are composed of magmatic rocks of different ages partially covered by Quaternary marine sediments. Despite of isolation from the continent, surface marine waters around islands are influenced by the Mekong River freshwater yield decreasing salinity significantly during rainy season. Many of islands and part of the biggest Con Dao are under protection since 1984 as part of the National Park enlarged in 1998. Main protected ecosystems include mangrove, coral reefs, sea-grass meadow and dry tropical forest dwelling by rich and scanty studied tropical malacofauna. In early March 2019, malacological survey was conducted on two largest islands Con Son and Bay Canh. Terrestrial, freshwater, littoral, mangrove and some coral reef habitats were examined in co-operation with staff of the national park. Totally more than 100 mollusc species were recorded, including new for the archipelago and probably new for science.

In leaf litter of dry forest on hills, under wet stones and dead wood, on and under trees on sea coast and lowland inside Con Dao island we found 14 land snail species in 8 families – southeast Asian *Myxostoma petiverianum*, *Giardia siamensis*, *Amphidromus* cf. *inversus annamiticus*, *Macrochlamys* sp., *Megaustenia* sp., giant African snail *Achatina fulica* and probably undescribed endemic species in *Cyclophorus*, *Cyclotus*, *Perrottetia*, *Hemiplecta*.

In two shallow lakes and artificial channels on the Con Dao Island 8 freshwater species were found Mekong Delta clam *Pilsbryconcha compressa* and southeast Asian gastropods *Filopaludina* cf. *martensi*, *Pila polita*, *Melanoides tuberculata*, *Sermula riqueti*, *Digoniostoma siamensis*, *Lymnaea swinhoei*, *Indoplanorbis exustus*.

Littoral with sand, rocks and stones was examined. Inside sand bivalves prevail including hammer oyster *Malleus* sp. abundant in Con Son Bay. Large horse mussel *Atrina* sp. was recorded in strongly protected Dam Tre Bay as well. On sandy flat cerithioideans *Cerithium corallium* and *Rhinoclavis* species are common. On rocks and stones gastropods of genera *Nerita*, *Cellana*, *Planaxis* are abundant, while species in *Monodonta*, *Neripteron*, *Littoraria*, *Tenguella* are sporadic. Oysters including *Pinctada* sp. are

common on hard substrates as well. Oyster shells are often used by chitons as a shelter. Before our study 17 small chiton species were known on Con Dao Islands (Sirenko, 2015). We found new for the archipelago larger *Liolophura japonica* dwelling big stones on supralittoral of Con Son Bay.

In intertidal of Con Son Bay near destroyed coral reef large opisthobranch *Aplysia* feeding on sea grass bed was found. Its olive-yellowish body is covered by reticulated black lines and distinct rings like that in *A. dactylomela*. Based on molecular data (Alexander, Valdes, 2013) the population from the Indo-Pacific region should be recognized as a separate species, *A. argus*.

Two places with unique sandy mangroves on Bay Canh Is. and on Dam Tre Bay of Con Dao Is. located in the vicinity of coral reefs with many *Tridacna* were examined as well. Mangrove forest of Bay Canh Is. is more developed and dwelled by 23 gastropod species in genera *Littoraria*, *Cassidula*, *Terebraria*, *Monodonta*, *Clypeomorus*, *Nerita*, *Haminoea*. Under deadwood on sand with mud *Melampus* cf. *adansianus* and small *Neripteron* sp. were found. Lower mangrove forest in Dam Tre Bay is inhabited mainly by *Clypeomorus pellucida* with sporadic *Littoraria*, *Monodonta*, *Tenguella*, *Nerita* and *Cellana* species.

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## **Species composition and distribution of pelagic larvae of bivalves in Nevelski Strait**

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The main part of the studies on species composition and distribution of pelagic larvae of bivalves, inhabiting the Far Eastern seas of Russia, was carried out earlier in the northwestern part of Sea of Japan (Korn, Kulikova, 1997). Considerably less attention is devoted to the larval plankton of the Okhotsk and Bering Seas, and Kamchatka waters (Kulikova, Korn, 1999). As for the Sakhalin shelf waters, there is information on taxonomic composition of bivalve larvae only in meroplankton of coastal part of the Aniva Bay (Kulikova, Kolotukhina, Omelyanenko, 2017), as well as data on distribution of adult bivalve mollusks in the northwestern part of Tatar Strait and Amurskiy estuary (Dulenina, 2018). Information on species composition and distribution of bivalve larvae in plankton of Nevelski Strait, the northern part of Tatar Strait, is absent in literature. The aim of the work was to study species composition and distribution of pelagic larvae of bivalves in Nevelski Strait, the northern part of Tatar Strait.

The work was carried out in Nevelski Strait at the end of July – beginning of August 2018. Plankton samples were taken totally from bottom to surface water layers by Jedi plankton net in four horizontal sections, each of which had 10 stations. The sections were located along the line connecting Pogibi village and Sredniy Cape, as well as to the south (1 section) and to the north (2 sections) of the line. Depth of the sampling varied from 0.6 m in shallow western and eastern parts of the strait to 22 m in the deep-water central part. A total of 40 plankton samples were collected and processed. At the same time measurements of temperature of surface and bottom water layers were conducted. The material was processed by standard method using MBS-10 binocular and an Ergaval microscope (Kulikova et al., 2013, 2014). The samples of plankton viewed in Bogorov's chamber. The earliest bivalve larvae at the veliger stage were not identified due to their small size. In identifying of the larvae at a later stage (the “velikonch stage”), identification guides and literature data were used (Kasyanov et al., 1983; Semenikhina et al., 2006; Evseev, Kolotukhina, 2008).

During time of observation pelagic larvae of 20 taxa of bivalve mollusks were identified in Nevelski Strait. Among them 10 taxa were identified to the species, 4 taxa – to the genus, and 6 taxa – only to the family. The total number of larvae varied from

3.06 ind./m<sup>3</sup> at station 19, located at Pogibi Cape, to 222 ind./m<sup>3</sup> at station 22, located to the south of the Sredniy Cape. Depth of sampling at the stations was 0.9 and 2.2 m, respectively, temperature of the surface and bottom layers of water at both stations was about 17.0 °C. Most of all were representatives of family Astartidae – up to 111 ind./m<sup>3</sup>. The taxonomical composition was dominated by representatives of the family Veneridae: *Ruditapes philippinarum* (Adams et Reeve, 1850), *Callista brevisiphonata* (Carpenter, 1864), *Mercenaria stimpsoni* (Gould, 1861) and members of the family not identified to the species. Their number was: 0.76–8 ind./m<sup>3</sup> for *R. philippinarum*, 1.6–4 ind./m<sup>3</sup> for *C. brevisiphonata*, 0.76–1.63 ind./m<sup>3</sup> for *M. stimpsoni* and for other representatives of the family. At the same time, most of the larvae were recorded at stations 16 and 35, located in the central part of the strait at a depth of 15 and 23 m, respectively, at temperature of the surface and bottom water layers 17.9 and 16.5–17.4°C. The next in terms of species diversity was family Mytilidae: *Mytilus trossulus* (A.A. Gould, 1850), *Crenomytilus grayanus* (Dunker) and representatives of the family not identified to the species. Their numbers were: 0.76–5.4 ind./m<sup>3</sup> for *M. trossulus* larvae, 1–2 ind./m<sup>3</sup> for *C. grayanus* larvae and for other representatives of the family. A greater number of Mytilidae larvae were also recorded at the central stations of the strait – 17 and 38, located at a depth of 10 and 15 m, respectively, at a temperature of the surface and bottom waters layers 17–17.8 and 16.8 °C. Family Mactridae included two representatives in plankton: *Mactra chinensis* Philippi and members of the genus *Spisula*, not identified to species level. The density of *M. chinensis* larvae was 1.74–5 ind./m<sup>3</sup>, *Spisula* sp. – 2 ind./m<sup>3</sup> at the central stations of the strait – 14, 35–36, located at a depth of 10 and 15–22 m, respectively, at a temperature of the surface and bottom water layers 17.8 and 16.1–17.4 °C.

Mollusks of the families Hiattellidae, Cyrenidae, Ostreidae and Pharidae each included only one representative in plankton: *Hiattella arctica* (Linne), *Corbicula japonica* (Prime, 1867), *Crassostrea gigas* (Thunberg, 1793), and *Siliqua alta* (Broderip et Sowerby, 1829). The number of them was 1–2 ind./m<sup>3</sup> for *H. arctica*, *C. gigas*, and *S. alta*, 2–10 ind./m<sup>3</sup> for *C. japonica*. The larvae of these species were found at the central stations of the strait – 17, 35, and 36, with the depths and temperature indicated above. Other representatives of bivalve mollusks, such as the families Cardiidae, Lasaeidae (*Mysella* sp.), Tellinidae, Pholadidae, Lyonsiidae (*Lyonsia* sp.) and Myidae (*Mya* sp.) were registered in plankton in small numbers (1–5 ind./m<sup>3</sup>) mainly at the central stations 16–17, 36 and 38.

Due to desalination caused by neighborhood with the Amur Estuary, a decrease in the species composition of Bivalvia in plankton was noted in the study area, as compared with the neighboring marine waters of Tatar Strait (Dulenina, 2018). The fauna of Nevelski Strait is represented both by the marine (families Astartidae, Veneridae, Mytilidae, Mactridae, Hiattellidae, Ostreidae, Pharidae, Cardiidae, Lasaeidae, Tellinidae, Pholadidae, Lyonsiidae) and also by the mesohalobiotic species (families Cyrenidae and Tellinidae). The predominance of larvae of family Astartidae – relatively deep-sea mollusks, whose larvae were rarely found in plankton and therefore poorly studied,

was apparently due to the presence of massive concentrations of adult individuals of the family in the study area (Dulenina, 2013). The predominant concentration of pelagic larvae of bivalves by the western coast of the strait near the Sredniy Cape, as well as in its central deepwater part, was apparently due to the work of currents that captured plankters off the coast near the Pogibi Cape and other shallow parts of the strait, took it to depth to the central part and concentrated them off the opposite shore.

The distribution of bivalve larvae that we observed in the area is in good agreement with the researchers' data on the distribution of plankton in other areas of the Sea of Japan (Mileykovsky, 1981; Mileykovsky, 1985; Morozova, 2005; and others).

## **Glochidia of *Inversiunio johogamensis* from Nagara River basin (Honshu Island, Gifu Prefecture, Japan)**

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One of the first significant works on the morphology of glochidia of Unionidae in Japan was made by S. Inaba (1941, 1964), where in addition to pictures and descriptions of the larvae, the author attempts to analyze interpopulation variability of glochidial shells, and also gives a species identification characters for the unionids of Japan. Another research devoting to glochidia of 10 species of anodontins (attributed to the genera *Anodonta* Lamarck, 1799 and *Cristaria* Schumacher, 1817) and naiads (defined as *Unio* Phillipsson, 1788 and *Inversidens* Simpson, 1900) from the Biwa Lake (Shiga Prefecture) was made (Higashi, Hayashi, 1964). Next step was a taxonomic revision of the genus *Inversidens* from Okayama Prefecture, Honshu Island, based partly on glochidia features (Kondo, 1982). A monographic report with brief descriptions of larval shells for 16 unionids species inhabiting Honshu, as well as photographs taken on a light microscope was later published (Kondo, 2008). As a result of glochidia morphology investigation, new genus *Inversiunio* Habe, 1991 was separated from the genus *Inversidens* based on differences of glochidia morphology: hooked subtriangular glochidia of *Inversiunio* versus hookless semi-circular or semi-oval glochidia of *Inversidens* (Habe, 1991). Recently, the validity of the genus *Inversiunio* was confirmed by molecular genetic studies (Sano et al., 2017).

Measurements of glochidial shells exhibit remarkable variation among habitats in freshwater mussels and often do not give appropriate features for identification on genus level. On the contrary, differences in external glochidial valve sculpture received by scanning electron microscopy is thought to help with taxonomic revisions and can be used as diagnostic characters (Panha, Eongprakornkeaw, 1995; Hoggarth, 1999; Sayenko, 2016). Most of investigations on glochidia morphology of unionids in Japan were observed by using light microscopy, while glochidia features received by scanning electron microscope were practically absent, what defined a purpose of this work.

The species status of mature glochidia was established by the adult individuals, from the demibranches of which they were extracted. Specimens of *Inversiunio jokohamensis* from an agricultural irrigation ditch at Seki City belonging to the Nagara River basin (Honshu Is., Gifu Pref., Japan), collected in summer 2018 was used for the study. The samples were collected by Kengo Ito (Gifu University, Japan) and provided by Dr. Yoshihiro B. Akiyama (National Institute for Land and Infrastructure Management, Kanagawa, Japan).

To prepare for work on a scanning electron microscope, the glochidia were cleaned from the soft tissues in a 5% KOH solution. The cleaned shells were washed several times in distilled water and a series of alcohols (80, 90, and 96%), after which they were mounted on a stub. Chromium was sputtered immediately after drying the samples on a stub to exclude the possibility of deformation of the hooks and outer shell layer.

The microsculpture of each larval shell was examined at least at four points: closer to the ventral end (i.e., to the hook), at the center of the valve (the adductor region), at the valve rim, and at the ligament. The fine structure of the exterior surface of glochidial valves was viewed with more than 10000 magnification. The description of the exterior valve microsculpture follows Panha, Eongprakornkeaw (1995) and Hoggarth (1999). The photographs of glochidia were obtained on a Zeiss MERLIN scanning electron microscope at the Biology and Genetic Engineering Center for Collective Use of the Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch of the Russian Academy of Sciences.

Glochidia of *Inversiunio jokohamensis* belong to *Anodonta*-type, having subtriangular shells with large styliform hook on the ventral margin of each valve. Glochidium slightly asymmetrical, height 167 to 173  $\mu\text{m}$  ( $170\pm 3$   $\mu\text{m}$ ), length 183 to 202  $\mu\text{m}$  ( $196\pm 6$   $\mu\text{m}$ ), valve height is always more than valve length with height/length ratio 0.86–0.91 ( $0.89\pm 0.03$ ). Hinge length (144–163  $\mu\text{m}$ ,  $152\pm 7$   $\mu\text{m}$ ) is at least 70% of the valve length.

Each hook is covered by at least 25 lanceolate, sharply pointed microspines arranged in 3–4 diagonal rows. Maximal height of the hook spines is 6.2  $\mu\text{m}$ .

Exterior microsculpture of glochidia of *Inversiunio jokohamensis* is beaded. Microsculpture covers not only the exterior valve surfaces but also the hook microspines, where sculpture becomes more sparse.

In contrast to many anodontins with different variations of net (looped) exterior surface sculpture (for, example, Asian genera *Cristaria*, *Sinanodonta* Modell, 1945, *Anemina* Haas, 1969), glochidia of some other naiads (whose adult shells with hinge teeth) can be described as rough, beaded or globular (Hoggarth, 1999; Sayenko, 2013, 2014, 2015, 2016; and others). In addition to already mentioned *Inversiunio jokohamensis*, glochidia of naiads *Middendorffinaia mongolica* (Middendorff, 1851) from southern Primorye and *Lanceolaria grayana* (Lea, 1834) from Japan have similar globular or beaded exterior surface sculpturing. From the other hand, glochidia of Asian mussel *Nodularia douglasiae* Griffith et Pidgeon, 1833 differ from the mentioned naiads by looped exterior valve microsculpture. If the validity of the mentioned Asian mussels is proved (Sano et al., 2017; Klishko et al., 2019), taxonomic compositions of these 4 genera so as the relationship between them are not completely understood. A taxonomic uncertainty with affiliation of *Inversiunio* and *Nodularia* Conrad, 1853 to Unioninae as well as unexpected affiliation of *Lanceolaria* Conrad, 1853 to Anodontinae (Lopes-Lima et al., 2017) indicates the need for further systematic revision, and data on glochidia morphology can give some additional features.

## Phylogenetic position of the limpet genus *Erginus* (Patellogastropoda)

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Genus *Erginus* includes five limpet species (WoRMS) and four of them inhabit the Far Eastern seas. Analysis of sequences of the gene COI of *E. sybariticus* and *E. puniceus* doesn't reveal differences between these two species (Sharina et al., 2014). The genus includes two subgenera (or genera, according to Chernyshev, 2018), *Erginus* and *Problacmaea*, which have different morphology of the soft body and teleoconch. All species of *Erginus* have enlarged nuchal cavity functioned as brooding pouch to accommodate embryos and juveniles.

Currently *Erginus* belongs to the family Lottiidae (WoRMS), but recently Chernyshev (2018) has established Erginidae, a new monotypic family for this genus. To confirm or refute this supposition and to resolve phylogenetic position of the genus *Erginus* on Patellogastropoda tree five molecular markers of *E. sybaritica* and two markers of *E. moskalevi* were used: two nuclear (18S and 28S rDNA) and three mitochondrial (COI, 12S and 16S rDNA) markers. All markers show that genus *Erginus* have isolated position from other Lottiidae genera, but branch topologies show differences. So exact position of *Erginus* relative to other families in the order Patellogastropoda is still unclear. Genetic distances also let us to suppose that species of genus *Erginus* more likely belong to the separate family. For complete conclusions phylogenetic analysis with the implication of the other species of the genus *Erginus* is required.

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**First global phylogeny of the deep-sea gastropod  
genus *Scaphander* reveals higher diversity,  
a possible need for generic revision  
and polyphyly across oceans**

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*Scaphander* (Gastropoda, Cephalaspidea) is a genus of deep-sea, soft-bottom snails currently comprised of about 17 valid extant species distributed worldwide. The traditional use of shell-based systematics and the difficulties to sample deep-sea species have led to rather confusing systematics of the genus. Valdés (2008, 2015) studied in detail several species of *Scaphander* from the tropical southwest and Eastern Pacific, and Eilertsen and Malaquias (2013) revised the systematics of the Atlantic species, providing the first molecular phylogeny for *Scaphander*.

To revise the systematics of the worldwide *Scaphander* and infer species relationships, we extended the taxon sampling including so far about 70% of the recognized diversity plus several other members of the family Scaphandridae and have used an integrative taxonomy approach, combining morphological characters, multi-locus molecular phylogenetics (COI, 16S rRNA, 18S, 28S rRNA), and molecular species delimitation methods (ABGD, GMYC, PTP).

We present the first global phylogeny of the genus, which points to greater species diversity and the possible need to reassess the taxonomic status of genera commonly confused with *Scaphander*, such as *Meloscaphander* and *Sabatia*. The results suggest that the biogeography and cladogenesis of *Scaphander* do not conform with a pattern of separate radiations in the Indo-West Pacific and the Atlantic + Eastern Pacific.

## **Sexual size dimorphism in the scallop *Mizuhopecten yessoensis***

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The phenomenon referred to as sexual size dimorphism (SSD), in which males and females differ in their body size, is common in many animal taxa and is usually interpreted as the result of sexual selection. SSD follows from two adaptive hypotheses: (1) the intrasexual selection hypothesis, by which the competition between males leads to selection for a larger body size, and (2) the fecundity advantage hypothesis, according to which natural selection drives females to have larger bodies in order to produce a larger brood size. SSD is predicted to vary across mating systems. Mating systems with males larger than females occur when males compete for female access or guard territories, while mating systems with group mating tend to occur in species where females are the same size or larger than males. To date, it is known that at a given age, females are larger than males in *Mytilus edulis* and *Calypptogena gallardoi*, both having group mating systems. Studies on sexual size dimorphism in Pectinidae are limited. In natural populations bivalve growth rates vary under different environments; therefore same-sized bivalves may have different ages, and this circumstance, in the absence of a method to determine individual age, hampers the comparison of results for the same aged mollusks. No data on sexual size monomorphism or dimorphism have been reported for the mobile long-lived scallop *Mizuhopecten yessoensis* (Jay, 1857), a common (fished and cultured) species in the subtidal benthos of the Sea of Japan. It is a group spawner; therefore, it is expected that the species will have female-biased SSD or both sexes have the same size. Because this scallop is important to marine aquaculture and provides a food source for humans, it is important to understand the ability to differentiate males from females of this species to ensure proper management policies.

The goal of this study was a comparison of the shell characteristics, tissue weights and growth patterns of the same aged males and females in a wild population *M. yessoensis* for a research of its SSD. The comparisons of parameters of the same aged individuals in population of *M. yessoensis* can be studied as the method for age and shell growth determination of each scallop during its lifetime was devised by author.

The scallops, 209 specimens, were collected by SCUBA divers from wild population at Ozero Vtoroe Bay of Peter the Great Bay in the northwestern Sea of Japan (42°72'38" N, 133°03'05" E). Individuals were sampled just before the spawning, when their gonads were mature (at the beginning of June) and the sex of the scallops can

be ascertained visually by noting the color of the gonad: female gonad is pink, while the male gonad is creamy.

It was found that both the males and females display the somatic growth during all their life. Gonad weight (in the pre-spawning period) increased with age too, until a threshold age was attained; and then gonad weight remained virtually unchanged. The threshold age coincides with the beginning of the senile period of scallop ontogenesis development. In the population studied, the threshold age was found for individuals of 6 years of age.

The shell height of the same aged scallops was not sexually dimorphic. The adductor muscle weight of both scallop genders of the same age do not differ. The shell growth rates in the studied bivalve species were similar for both genders. The von Bertalanffy equations of the shell height ( $H$ , mm) growth for males ( $H_m$ ) and females ( $H_f$ ) were respectively:

$$H_m = 144.7 (1 - \exp(-0.6079 (t - 0.6929))),$$

$$H_f = 142.4 (1 - \exp(-0.6308 (t - 0.6053))),$$

where  $t$  is scallop age, years.

Due to insignificant difference between the mean shell sizes of male and female scallops, it is impossible to visually identify the sex of live specimens during non-breeding periods.

The differences become significant with scallop age increase for such parameters as the total and shell weights (male-biased, >5 years old). A similar tendency was found for the adductor muscle weight of the scallops from the wild population. The results in terms of reproductive effort were illustrated by the gonad weight. Statistical analyses showed female-biased dimorphism in the gonad weight for age groups >4 years old.

The fecundity advantage hypothesis for *M. yessoensis* with group mating and external fertilization is at least partly realized by physiological mechanisms, the larger gonads of older females than older males in a population, in order to produce a larger brood. Gregarious settlement of this bivalve contributes to the reproductive success of the population so that the energetically costly ovaries may all be fertilized.

## ***Apodomenia* – a solenogaster without a foot**

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*Apodomenia enigmatica* is a bizarre recently described species of worm-shaped mollusk that appears to be a morphological intermediate between the two major aplacophoran groups, the Solenogastres (Neomeniomorpha) and Caudofoveata (Chaetodermomorpha). However, this animal is not a missing link; molecular and morphological studies show that it is a derived solenogaster that lacks a foot, mantle cavity, and radula. The phylogenetic placement of the species is based on analyses of new transcriptomes from 25 aculiferans (Solenogastres, Caudofoveata and Polyplacophora) together with earlier published data. Phylogenomic analyses included datasets with up to 525 genes. Our results indicate that aplacophoran taxonomy is in need of revision as several traditionally recognized groups are non-monophyletic. Most notably, Cavi-belonia, the solenogaster taxon defined by hollow sclerites, is not monophyletic. Taken together, these results shed light on the evolutionary history of Aplacophora and reveal a surprising degree of morphological plasticity within the group.

## **The mytilid's connecting system: from the bivalve mollusks to a substratum**

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There are many adult bivalve mollusks with attached mode of life on marine bottom, such superfamilies as Mytiloidea, Pinnoidea, Arcoidea, Ostreoidea and other. Among of them, species of family Mytilidae often dominate on rocky intertidal shores, largely due to their ability to form a strong byssus. Also, mytilids play an important role in the structure and functioning of marine ecosystems and are used in mariculture. The morphology of the byssus can significantly vary within the superfamily of bivalve mollusks and plays a very important role in their survival at the bottom. Also, byssus has historical significance in everyday life of southern areas of Italy and Portugal, where byssus of *Pinna nobilis* was collected for the production of “marine silk” from ancient times until now. Sardinia and Puglia were the main centers for the processing of extremely fine golden “marine silk” that was used for the production of a very light and warm knitwear, such as gloves, hats, socks, berets, ties, also decorative fabrics, and tapestries. Chiara Vigo from Sardinia is one of the few women, who are still manufacturing byssal cloth today. The Ethnographic Museum of Antioquia in Sardinia (Italy) and approximately ten other museums around the world house less than 100 examples of cloth of the legendary sea silk and other related artworks. It is interesting to note that as Latinized zoological term “byssus” as derived from the Greek word “bussus” that signified the sea silk. As a rule, textiles woven from byssal threads were highly praised in all ancient cultures where sea silk was known. For example, byssal threads were identified in a Roman Centurion's cloak, the raiment of King Tutankhamen, and might have been the of the Golden Fleece sought by the mythical Jason and Argonauts.

At present, morphology of byssus and its mechanical properties has been studied most extensively for *Mytilus edulis* (Linnaeus, 1758). The aim of this investigation was to study the morphology and structure of byssus apparatus and byssal threads, their mechanical properties and strength of byssal attachment in the common species of mytilids of the Sea of Japan: *Crenomytilus grayanus* (Dunker, 1853), *Mytilus coruscus* Gould, 1861 and *Modiolus modiolus* (Linnaeus, 1758).

The morphology of byssus apparatus and byssal threads were studied by the methods of light microscopy and scanning electron microscopy in the “Center of Collective Use” in A.V. Zhirmunsky National Scientific Center of Marine Biology FEB RAS. The mechanical properties and strength of attachment in the investigated mussels were measured using the dynamometers of different size.

After I conducted this study, I can conclude the following:

1. All investigated species have the general structure of collagenous byssus, which consist of three distinct parts: a root that deeply embedded in the tissue of mollusk foot; a compressed laterally stem; and the byssal threads with the oval adhesive disks at the distal ends of threads. The byssal threads are attached to a stem of byssus by the cuffs, which look like the rings.

2. The unattached mollusks can produce new byssus during all life. The newly produced byssal threads are colored in the light yellow color. The sizes of attachment adhesive disks depend on a diameter of distal tip of foot groove in the mytilids.

3. These mytilids are attached by the byssal threads hardly to any surface of biological and an anthropogenic substrates. The mechanical properties of byssal threads depend on the strength of cohesion of collagenous molecules in these mytilids. The proximal end of byssal threads more weak and extensible than the hard distal end of byssal threads. The hardest byssal threads has *M. coruscus*. The weakest byssal threads has *M. modiolus*. *C. grayanus* is characterized an average value of this parameter.

4. The strength of byssal attachment of these mytilids depends on a season and the number of functional active byssal threads. The strengtest byssal attachment and a little number of byssal threads has *M. coruscus* in independence of a season. The most weakly byssal strength and a large number of byssal threads has *M. modiolus*. In *C. grayanus* these parameters have average value.

5. The spacial distribution of bivalve mollusks *M. coruscus*, *C. grayanus* and *M. modiolus* is correlated clearly with the features of structure of byssus and its mechanical properties.

6. Thus, *C. grayanus* lives on rocks, boulders, and rocky bottom mixed with pebbles and shells at a depth of 1 to 60 m. In the bays and coves that are protected from the high tide, it is found in large numbers at a small depth of 0.5 to 1.0 m, whereas on open coasts, its preferred depth is from 3–5 to 20–30 m, giving way to another species, *M. coruscus*. This species successfully colonizes the open, relatively surf-beaten rocky areas of the coast, at a depth of 0.2–3 m. *M. modiolus* is usually found in protected bays on soft silty or sandy-silt substrates, among boulders and large pebbles, at a depth not exceeding 15 m.

**Collection of mollusks from Korea  
in the Zoological Museum, Science and Educational Museum,  
Far Eastern Federal University (Vladivostok)**

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The Zoological Museum (a part of the Science and Educational Museum, Far Eastern Federal University, Vladivostok) possesses the largest in Russia collection of mollusks from the Korean Peninsula and this valuable collection continues to grow. The Korean fauna is generally well-known but its regional aspects and taxonomy of many species, especially when we compare same species from China and Japan, is much in need of revision and verification. The collection comprises mostly marine gastropod (819 lots) and bivalve (1635 lots) mollusks. Freshwater fauna is also represented, but very poor: 2 lots (7 specimens) of gastropods and the only specimen of *Lamprotula leai* (Bivalvia: Unionidae); land snails have only 8 lots (26 specimens). Collections of other classes of mollusks are small: chitons – 11 lots, scaphopods – 4 lots.

Most of the collection consists of dry shells. All samples are labeled and listed in a systematic card catalog containing detailed information on each lot. All information is also in an electronic database created in the Access software. This allows to quickly sort, group and select objects in accordance with the user's request.

Gastropods are represented by more than 235 species from more than 60 families (exact numbers here and below are unknown since not all lots are identified to species level, therefore the number of families and species may be somewhat higher). The most diverse are the following families: Trochidae (more than 28 species); Lottiidae (21), Naticidae (14), Buccinidae (13), Muricidae (13), other gastropod families have less than 10 species.

The collection of marine bivalve mollusks is undoubtedly the most valuable as it is larger and more detailed studied scientifically. Currently, there are about 260 species of bivalves from nearly 50 families (in total, Korean marine bivalve fauna contains more than 450 species). The most diverse are the following families: Veneridae (over 30 species), Mytilidae (25), Tellinidae (24), Arcidae (19), Pectinidae (12). The remaining families are represented by less than ten species. Most part of the bivalve collection consists of samples from the eastern side of the Korean Peninsula, its southern tip and Jeju Island; major part is from South Korea but we have small collection from North Korea (97 lots out of 1635). North Korean samples were taken in 1990 (a Soviet expedition) and in 2011–2013 (students). South Korean samples originate from Gangwon Province (1997 and 2016), Ulsan and Yeongil bays (1997), other localities in Gyeongbuk,

Gyeongnam, along the Yellow Sea coast (Garolim Bay). A small but interesting collection is from Busan seashore (2009–2014); the large collection was taken in 2018 on tidal flats and beaches of Jeollanamdo (south-western Korea), however, these two collections are not identified yet. Most samples were donated to the Zoological Museum by K.A. Lutaenko (A.V. Zhirmunsky National Scientific Center of Marine Biology FEB RAS) and R.G. Noseworthy (formerly, Jeju National University) who published a number of papers on the Korean molluscan fauna; other collectors are scientists and amateurs from Vladivostok travelled to Korea on various occasions. Many photographic images of bivalves were taken and published during last two decades, so, digital archive of bivalve collection is available. Korea, Japan and China are three countries lie next to the eastern Russia and molluscan samples from these regions are very important for Russian scientists for taxonomic and biogeographic intercomparisons.

**How does the nervous system develop:  
the role of peripheral neurons in formation  
of a tetraneurous nervous system**

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Bivalve development data have changed the accepted neurogenetic scenario and revealed a role for peripheral sensory elements in the basic organization of the larval nervous system. We show data of the main neurogenetic events in bivalve mollusks, emphasize a new role for early neurons in establishing larval nervous systems, and apical organ morphogenetic functions. We demonstrate that peripheral neurons are expressed of different transmitter nature appear before the apical organ emerges, have varied locations in the larval body, and serve as scaffolds for larval central nervous system development in the several trochozoa groups. The visceral (lateral) nervous system (cerebropleural and visceral ganglia with connectives) forms in an anterior-to-posterior direction in bivalve larvae and the emerging processes of neurons with different transmitter phenotypes create a neuronal scaffold organized by pioneer axons. We presented new data on the development of the pedal/ventral nervous system that begins to develop much later, after settlement and during metamorphosis of bivalve larvae. The disruption of the serotonin gradient in larvae leads to pioneer axon navigation disturbance, resulting in nerve bundle malformation. These data indicate that serotonin plays a vital role in neurodevelopment. Novel comparative morphological data relating to neurogenetic events in bivalve mollusks cast new light on nervous system origins, cellular mechanisms of development, and the formation of the tetraneurous nervous system. Further, they contribute to understanding the morphology of the ground pattern of the larval neural architecture of Bivalvia and Mollusca.

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## **Radula fine morphology of the Conoidea**

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Conoidea is the superfamily of marine gastropods, with over 5.000 valid species (WoRMS, <http://www.marinespecies.org>). The foregut of various members of the Conoidea is characterized by the presence of a retractile proboscis, one or two pairs of salivary glands, the radula sac is positioned at the base of the proboscis (i.e. at an angle to the axis of the proboscis) rather than on the tip, a venom gland which produces the cocktail of various physiologically active toxin peptides used both for attacking prey and as defiance against predators (Dutertre et al., 2014; Puillandre, Fedosov, Kantor, 2016), and the distinctive feeding mechanism. Radula is variable in Conoidea in the number of teeth in a transverse row (from five to two) and in the morphology of the teeth. General radula morphology has been studied in detail (Taylor et al., 1993; Kantor, Taylor, 2002; Bouchet et al., 2011). Based on the teeth number in a row and the morphology of the marginal teeth several types of radula have been described.

The main role in the feeding is taken by the marginal teeth. The morphology of the marginal teeth is not correlated with the number of teeth in a row. The two most commonly found types of marginal teeth are duplex and hypodermic (both are used on the proboscis tip). A duplex tooth consists of a robust, pointed, major element and a smaller, more slender, accessory limb. The major elements of the marginal teeth are attached to the radular membrane along most of their length and only the tips are free. Another, unique for the conoideans type, is characterised by ‘hypodermic’ marginal radular tooth, which is a hollow harpoon with holes at the base and tip. The poison is injected into the pray through the central tooth cavity like syringe needles. This represent the most intricate radular morphology among gastropods and is found in several independent lineages of Conoidea, including the best-known family Conidae (cone snails). In this family the vestigial radular membrane may be present or absent and only a pair of hypodermic marginal teeth occurs in each transverse row. The hypodermic marginal tooth, as can be seen from transverse sections, is formed from a plate that is rolled to create a tube with overlapping edges. In the Conidae, the wall of the tooth is formed by several layers, but in other families of conoideans with hypodermic teeth the overlap is less marked and the wall is formed by no more than two layers (see Bouchet et al., 2011, fig. 3C).

The teeth of the conoidean taxa are considerably altered during tooth maturation in contrast to other gastropods (Kantor, Taylor, 2000). Duplex marginal tooth develops

from a “flat plate through thickening of the tooth edges and elevation of the posterior edge from the radular membrane as opposed to or the lengthwise folding of a nearly-flat plate once it progresses half way along the radula” (*Sibogasyrinx* Powell, 1969, Cochlespiridae; Kantor, Fedosov, Puillandre, 2018). The process of tooth formation and maturation is much less studied.

We studied the radula of three species of different families of Conoidea (*Lophiotoma* (Turridae), *Clavus* (Drillidae), *Conus pulicarius* (Conidae)) by light and electron microscopy. The radula of these species is significantly different from each other. *Conus pulicarius* have a hypodermic tooth without radular membrane. The radula of *Lophiotoma* consists of the two dagger-like marginal teeth attached to the radular membrane. The radula of *Clavus* consist of small central tooth, paired marginal denticulated teeth and pair of dagger-like marginal teeth. The radular membrane is well developed. Despite of the differences in the radula general morphology, there are lot of similarities in radular formation of studied species: ultrathin morphology of odontoblasts; odontoblasts number which form one marginal tooth; localization of the odontoblasts in the blind end of the radular sac; position of the marginal teeth; V-shaped configuration of the membrane in the radular sac. The main difference is the ultrathin morphology of the subradular epithelium which contains numerous tonofilaments in *Conus* while this epithelium is electron-transparent in *Clavus* and *Lophiotoma*. This morphology could be explained by functions of these cells. The epithelium of *Conus* participates in the enrolling of the tooth while other two species have radula membrane and the subradular epithelium probably strengthen the membrane. Thus, we may conclude that despite large differences in radular morphology the main patterns of the radula formation are similar throughout the superfamily.

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## Morphotypes and phylogeny of the marine gastropod genus *Tegula* in East Asia

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Marine organisms often show high levels of morphological variation within and among species as a result of adaptation to the habitat heterogeneity in coastal environments. In East Asian coastal area, marine gastropod genus *Tegula* show two contrasting shell-surface morphotypes (smooth vs ribbed) within species. In addition, *Tegula xanthostigma* (smooth shell species) have two shell-colour morphotypes (black vs light brown). However, geographical patterns of the shell morphotypes remain unclear. Thus, we investigated the relationship between the geographical patterns of two contrasting shell-surface morphotypes (smooth vs ribbed) and genetic diversity in East Asian members of *Tegula*. We also investigated the geographical and genetic patterns of two shell-colour morphotypes (black vs light brown) in *T. xanthostigma*.

Our results show that two morphotypes (smooth vs ribbed), distinguished by different sculpture, appeared sympatrically (*T. lischkei*) or allopatrically (*T. pfeifferi* and *T. rustica*) and could not be distinguished genetically. In addition, although the black morphotype of *T. xanthostigma* (a smooth-shelled species) and individuals of *T. argyrostoma* (a ribbed species) have been traditionally classified as separate species, the inferred phylogeny suggests that they belong in same clade. We also conducted a statistical analysis to determine whether shell morphology (colour or sculpture) varies with the degree of coastal exposure. We found no association between exposure and surface morphology. However, in *T. xanthostigma* the light brown morphotype preferred sheltered sites when compared with the black morphotype. Molecular methods and statistical analyses showed that smooth *T. xanthostigma* were divided into two species, with shell colour and habitat preference differing among species. While our findings show that shell sculpture is not a reliable character to use for species delimitation in *Tegula*, they suggest that differences in habitat preferences may potentially drive the genetic divergences seen in this group.

## **The use of aplacophoran comparative and evolutionary genomics to study molluscan biomineralization**

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Sclerites, calcareous scales and spicules, are a synapomorphy for the clade Aculifera (aplacophorans and chitons). This character distinguishes aculiferans from the shell-bearing conchiferans. While much is known about the genes and developmental processes behind shell formation in conchiferans, there have been no studies investigating the formation of aculiferan sclerites or whether or not sclerites are homologous to conchiferan shells. To address these questions, we are sequencing genomes from five species spanning the diversity of Aplacophora. These genomic resources will enable us to investigate the evolution of hypothesized “biomineralization genes” and other transcription factors and conduct *in situ* hybridization to explore the function of these genes in the emerging model aplacophoran *Wirenia argentea*. Here, we present a draft genome assembly for *Neomenia megatrapezata* (Solenogastres). Our sequencing strategy combined one lane of Illumina HiSeq X paired-end data (90X coverage) and one flowcell of Oxford Nanopore GirdION long reads (7X coverage). MaSuRCA yielded an N50 of 31 kbp with 26,726 contigs. Through increased nanopore sequencing and optical mapping, we plan to improve this assembly to near chromosome-level. Subsequently, we will produce high-quality annotations and use established orthology- and machine learning-based approaches to identify genes likely involved in biomineralization. High quality aplacophoran genomes along with the identification of biomineralization genes will shed light on the formation of aculiferan sclerites, their homology to conchiferan shells, and the biomineralized state of the last common ancestor of Mollusca. This research will provide valuable resources that will be broadly useful within the molluscan research community.

## **Morphological aspects of spermatogenesis in *Calyptogena pacifica***

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The organization of testes in the deep-sea bivalve mollusk *Calyptogena pacifica* was studied by light and electron microscopy. The specimens were collected from the southern summit of the Piip submarine volcano, Bering Sea, at a depth of 460 m.

Acinus is a structural unit of gonad where germ cells develop. The space between acini is filled by tightly packed hemocytes. The acinus wall is very thin and consists of a basal lamina covered from the outside by a thin layer of myoepithelial cells. In males, two populations of cells – somatic (accessory) and spermatogenic – are well detectable within an acinus.

The accessory cells can reach 80  $\mu\text{m}$  in length. Each accessory cell has the basally located nucleus with a single nucleolus. Cytoplasm is filled with numerous glycogen particles and a moderate amount of lipid droplets. Remnants of spermatogenic cells are found in the cytoplasm of accessory cells.

Spermatogonia, spermatocytes, and spermatids are located along the body of accessory cell, forming spermatogenous columns. Spermatogenesis in *C. pacifica* is generally typical for bivalve mollusks: the early appearance of proacrosomal vesicles in spermatogonia, the active accumulation of vesicles in spermatids, and the formation of compartments of spermatozoa (acrosomal complex, nucleus, midpiece, and flagellum) during spermiogenesis. A special feature of spermatogenesis in *C. pacifica* is the intracellular localization of axoneme. Although this phenomenon has already been described from bivalve mollusks, it should be stressed that spermatogenic cells with the inner axoneme are always found in minor proportion. However, in *C. pacifica* we did not observe any spermatogenic cells with the outer flagellum, except for late spermatids and spermatozoa.

Spermatozoa have an acrosomal complex that consists of a dome-shaped acrosomal vesicle up to 0.7  $\mu\text{m}$  in length and a deep subacrosomic canal extending from the base to the apical part of the acrosomal vesicle and filled with flacked substance of moderate electron density. The barrel-shaped nucleus is 3.5–4.0  $\mu\text{m}$  in length, filled with electron-dense chromatin having electron-lucent lacunae. Spermatozoa with four or five rounded mitochondria in midpiece are found. Unfortunately, there is very little data on the gamete structure in the family Vesicomysidae available in literature to date. As a rule, descriptions of sperm morphology are limited to size, shape of head, and number of mitochondria. In other *Calyptogena* species (such as *C. soyoeae* and *C. gallardoii*)

the number of mitochondria in the midpiece is always 4. The nucleus of *C. pacifica* is much larger and has a greater number of electron-lucent lacunae as compared to that in *C. soyoae*. The *Calyptogena* species are reported to have a continuous gametogenesis and multiple spawns during the year, which can be induced by a change of water flow. Large size of spermatozoa can limit their swimming capacity and, hence, the size of sperm and the water flow during spawning can be related.

The identified species-specific traits may be useful for further phylogenetic and taxonomic studies, since sperm morphology is still an important tool for solving taxonomic problems. This study was supported by the Russian Foundation for Basic Research (Projects nos. 19-04-00792 and 18-34-20064).

## **Evolutionary novelty: symbiosis of nudibranch mollusk with denitrifying and diazotrophic prokaryotes and actinomycetes**

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The symbiotic associations between microbes and invertebrates have resulted in some of the most unusual physiological, morphological and biochemical adaptations that have evolved in the animal world. Symbiosis has an environmental impact on animal habitat and ecosystems. Endosymbioses involving invertebrates and bacteria play critical roles in sustaining the high productivity of many marine environments. The discovery of new symbiosis expands our understanding of symbiotic diversity, their evolution, and the relationship between the host and the symbiotic microbes.

Of particular interest are nudibranch mollusks (Mollusca: Gastropoda: Opisthobranchia: Nudibranchia), unprotected by the shell and feeding mainly on sponges. They inhabit the bottom communities of almost all seas, in all latitudes, from the littoral zone to the depths of the oceans. Many species have unusual lipids that are rich in branched and odd fatty acids specific to bacteria, suggesting that symbiotic bacteria can be their source. Based on a comparison of biochemical, genetic and morphological data, a hypothesis was proved about the existence of symbiosis of nudibranch mollusks with heterotrophic bacteria. The mollusks epithelium has been found to include a renewable, self-replicating population of heterotrophic Gram-negative bacteria, cyanobacteria and actinobacteria that contribute significantly to the metabolism of the host fatty acids. The participation of symbiotic bacteria in metabolism is indicated by the abundance of odd and branched fatty acids.

New symbiosis between *Rostanga alisae* and the members of the bacterial groups Cyanobacteria, Rhizobiales, Rhodobacterales and Xanthomonadales, known as diazotrophs, is reported to be able to get energy by reducing nitrates and oxidizing carbon monoxide. Mollusk tissue sequencing revealed 74 bacterial phylotypes. The identified bacteria were first described as symbionts of marine invertebrates. Different tissues (notum, foot and intestine) have different symbiotic microbes suggesting the spatial organization of the microbial community of the mollusk. According to microscopy with fluorescent *in situ* hybridization (FISH analysis) with specific labeled oligonucleotide probes to 16S bacterial RNA, bacteriocytes were formed by representatives of the genera

*Labrenzia*, *Maritalea*, *Burkholderia*, *Achromobacter* and *Stenotrophomonas*. In addition to bacteriocytes surrounded by the membrane, including dozens of individuals, there were isolated bacteria in the epithelial cells of the epithelium. Also in the cytoplasm of intestinal epithelium cells and less commonly of epithelial epithelium, cyanobacteria were found, identified as *Synechococcus*, dominant in the number of clones. Thus, the mollusk *R. alisae* is an association with phylogenetically and physiologically diverse prokaryotic organisms. Fatty acid analysis indicates a trophic relationship between symbiotic microbes and the nudibranch mollusk. The obtained results expand the understanding of the biological role of symbiosis of Metazoa with bacteria.

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