A new species of the genus Coryphella (Gastropoda: Nudibranchia) from the Kuril Islands

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ABSTRACT. A new species of the family Coryphellidae, Coryphella alexanderi sp. nov. is described based on specimens collected in the Kuril Islands, North-West Pacific, from the upper sublittoral to 200 m depth. An integrative analysis was conducted, including a molecular phylogenetic analysis based on four markers (COI, 16S, H3, 28S), an automatic species delimitation method ABGD, and an analysis of the external and internal morphology using light and scanning electron microscopy. The distinctiveness of Coryphella alexanderi sp. nov. is well established both morphologically and genetically, and it differs from externally similar species in radular characters. Phylogenetically Coryphella alexanderi sp. nov. is closely related to Coryphella trophina, which occurs sympatrically in the same geographic and bathymetric ranges. Coryphella alexanderi sp. nov. appears to be restricted to the middle and northern Kuril Islands, which is consistent with the high numbers of endemic taxa in this area.

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Introduction

The Coryphellidae is a monophyletic nudibranch family, with most species described from boreal and polar regions in both hemispheres [Korshunova et al., 2017]. This family was recently resurrected following the integrative revision of the highly diverse and polymorphic family Flabellinidae s.l. [Korshunova et al., 2017]. The study by Korshunova et al. [2017] supported the validity of the family Coryphellidae and introduced nine genera for 27 coryphellid species. However, further research indicated that the taxonomical scheme for Flabellinidae s.l. proposed by Korshunova et al. [2017] might be excessively splitting [Furfaro et al., 2021; Ekimova et al., in press]. Because of this, Ekimova et al. [in press] suggested to unite the entire diversity of the family Coryphellidae within the single genus Coryphella. Nevertheless, several new Coryphellidae species were recently described from Norway and the North-West Pacific [Korshunova et al., 2017], and it is suspected that diversity of this group might be much higher than previously thought.

In the North-West Pacific only seven Coryphellidae species are currently known: Coryphella verrucosa (M. Sars, 1829), C. amabilis (Hirano et
Kuzirian, 1991), C. nobilis A. E. Verrill, 1900, C. sanamyanæ (Korshunova et al., 2017), C. trophina (Bergh, 1890), C. abei Baba, 1987, and C. athadona Bergh, 1875. All these species are well characterized and have clear distinctive morphological traits [Baba, 1987; Korshunova et al., 2017; Ekimova et al., in press]. Coryphella verrucosa and C. nobilis are amphiboreal species, C. amabilis and C. trophina display a wide geographic range in the North Pacific, and the other three species are restricted to the Sea of Japan and adjacent areas [Baba, 1987; Roginskaya, 1990; Hirano, Kuzirian, 1991; Jung, Park, 2015; Martynov et al., 2016; Korshunova et al., 2017; Ekimova et al., in press]. Most species are very abundant in shallow-water hydrozoan communities [Martynov et al., 2015; 2016; Chichvarkhin, 2016].

In this paper an additional North-West Pacific species of the genus Coryphella is described from the Kuril Islands, North-West Pacific, based on the integrative approach combining morphological and molecular data.

Material and methods

The material examined was collected in two localities: (1) one specimen was collected in the northern Kuril Islands (exact locality and coordinates are not available) during the “Aquatis Expedition” [Semenov, 2013], and four specimens were collected during the expedition of the R/V “Akademik Oparin” (cruise 56, Russia) at one site: Urup Is., 46°17.0 N 150°17.0 E. The holotype and paratypes are deposited in the collections of the National Scientific Center of Marine Biology, MIMB. One paratype is deposited in the collection of Zoological Museum of Lomonosov Moscow State University, White Sea Branch (ZMMU WS).

Molecular methods

Molecular methods included obtaining of the four molecular markers commonly used in Flabellinidae s.l. systematics: cytochrome c oxidase subunit I, 16S rRNA, histone H3 and 28S rRNA [Korshunova et al., 2017; Ekimova et al., in press]. DNA extraction, amplification, and sequencing followed methods described in Ekimova et al. [2019, 2020]. All newly obtained sequences were submitted to NCBI GenBank (Table S1). Raw reads for each gene were assembled and checked for ambiguities and low-quality data in Geneious R10 (Biomatters, Auckland, New Zealand). Edited sequences were verified for contamination using the BLAST-n algorithm run over the GenBank nr/nt database [Altschul et al., 1990]. For phylogenetic reconstruction, dataset obtained in previous comprehensive study on the family Coryphellidae was used for the analysis [Korshunova et al., 2017]. Indel-rich regions of the 16S alignment were identified and removed in Gblocks 0.91b [Talavera, Castresana, 2007] with the least stringent settings. Sequences were concatenated by a simple biopython script following Chaban et al. [2019]. Phylogenetic reconstruction was conducted for the concatenated multi-gene partitioned datasets. The best-fit nucleotide evolution models were tested in the MEGA7 [Kumar et al., 2016] based on the Bayesian Information Criterion (BIC) for each partition. The Bayesian phylogenetic analysis (BI) and estimation of posterior probabilities was performed in MrBayes 3.2 [Ronquist, Huelsenbeck, 2003]. Markov chains were sampled at intervals of 500 generations. The analysis was initiated with a random starting tree and ran for 10⁷ generations. Maximum likelihood phylogeny inference (ML) was performed in the HPC-PTHREADS-AVX option of RaxML HPC-PTHREADS 8.2.12 [Stamatakis, 2014] with 1000 pseudoreplicates under the GTRCAT model of nucleotide evolution. Bootstrap values were placed on the best tree found with SunTrees 3.3.1 from DendroPy Phylogenetic Computing Library 3.12.0 [Sukumaran, Holder, 2010]. Final phylogenetic tree images were rendered in FigTree 1.4.0 and further modified in Adobe illustrator CS 2015. For computational species delimitations methods, the COI alignment was used; in addition, the nuclear H3 alignment was analysed to identify species-specific polymorphisms. To confirm the status of clades recovered in our analysis as distinct species we used the Automatic Barcode Gap Discovery (ABGD) method [Puillandre et al., 2012] that detects breaks in the distribution of intra- and interspecific distances referred to as the “barcode gap” without any prior species hypothesis. The ABGD analysis was run on the online version of the program (http://wwwabi.snv.jussieu.fr/public/abgd/abgdweb.html) with the following settings: Pmin = 0.001, Pmax = 0.2, Steps = 10; X = 1.0; Nb bins = 20 and three proposed models – Jukes Cantor (JC69), Kimura (K80) and Simple distance. COI sequences were aligned for ABGD analysis with the MUSCLE [Edgar, 2004] algorithm in MEGA7 [Kumar et al., 2016]. P-distances for COI alignment were calculated in MEGA7 [Kumar et al., 2016].

Morphological studies

All collected specimens were used for the examination of their external morphology under a stereomicroscope. The internal morphology of three specimens was also examined, including the digestive and reproductive systems. The buccal mass of each specimen was extracted and soaked in proteinase K solution for 2 hours at 60 °C to dissolve connective and muscular tissues. The radula and the jaws were rinsed in distilled water, air-dried, mounted on an aluminium stub, and sputter-coated with gold for visualization under a JEOL JSM 6380 scanning electron microscope (SEM). Features of
the jaws were examined by optical stereomicroscopy and SEM. For study of the reproductive system specimens were dissected from the dorsum along the midline and examined under a stereomicroscope.

Results

Phylogenetic analysis

The concatenated analysis of four markers (COI+16S+H3+28S) revealed well-resolved and highly supported trees at the species level using both BI and ML (Fig. 1). The topology of BI and ML tree was similar, except for the deeper relationships and the position of Coryphella falklandica Eliot, 1907. The new species represented a highly supported clade [PP (posterior probability from BI) = 1; BS (bootstrap support from ML) = 100], which was recovered as sister to Coryphella trophina with moderate support (PP = 0.98; BS = 67). This clade was sister to a monophyletic group representing by the North-East Atlantic species: Coryphella lineata (Lovén, 1846), C. chriskauei (Korshunova et al., 2017), C. browni Picton, 1980, C. monicae (Korshunova et al., 2017), C. borealis Odhner, 1922, C. orjani (Korshunova et al., 2017), but these relationships were not supported.
Species delimitation

The ABGD analysis supported the initial species hypothesis with 15 species-level groups in the initial partition (Fig. 1), supporting the identity of all monophyletic species clades except *C. sanamyanae*, for which no COI data was available. All studied specimens from the Kuril Islands appeared in a single group. The lowest $p$-distance in COI marker (Table 1) was found between the new species and *Coryphella lineata* (6.74%). Maximum intraspecific distance within the new species was 2.07%. Analysis of the H3 dataset showed a high genetic divergence of the new species from other *Coryphella* species, with a minimum divergence of 3 substitutions between the new species and *Coryphella orjani*.

Taxonomic description

Order Nudibranchia de Blainville, 1814
Suborder Cladobranchia
William & Morton, 1984
Superfamily Fionoidea J. E. Gray, 1857
Family Coryphellidae Bergh, 1889
Genus *Coryphella* J. E. Gray, 1850

*Coryphella alexanderi* sp. nov. (Figs 2–4)

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**Type material.** Holotype MIMB42468, partly dissected, radula and jaws mounted on SEM stub. Paratypes: MIMB42469, three specimens, two dissected, same locality and collector as holotype. ZMMU WS14380, one specimen, dissected, northern Kuril Is., coordinates not available, 10–20 m depth, 04.08.2016, coll. Alexander Semenov.

**Type locality.** Sea of Okhotsk, Urup Is., 46°17.0'N, 150°17.0'E, 148–198 m depth, 05.07.2019, coll. Anastassya Maiorova.

**Description.** *External morphology* (Fig. 2). Body length up to 22 mm. Body narrow, tapering to tail. Rhinophores about two times longer than oral tentacles. Rhinophores smooth with thin wrinkles. Anterior foot corners present. Notal edge reduced, continuous. Cerata in continuous rows, not united in separated groups. Anus pleuroproct. Reproductive opening on right side under anterior ceratal rows. *Coloration* (Fig. 2). Background color of body translucent milky-white. Dorsal side of oral tentacles with opaque white pigment dots. Opaque white line on back side of rhinophores, becoming pigment white band on rhinophoral tip. Tail with white line. Digestive gland diverticula inside cerata from orange to pale brown. Cnidosac area white, with thin, white pigment band.

**Remarks.** Both morphological and molecular analyses support the distinctiveness of *Coryphella alexanderi* sp. nov. from other species in the genus. The external morphological characters of this new species shows the most resemblance to the North-West Pacific species *Coryphella sanamyanae*, the amphiboreal species *C. nobilis* A. E. Verrill, 1880, and the North-East Atlantic species *C. browni*, as it

<table>
<thead>
<tr>
<th>Species</th>
<th>$p$-distance value</th>
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<tbody>
<tr>
<td><em>Coryphella amabilis</em></td>
<td>12.61</td>
</tr>
<tr>
<td><em>Coryphella athadona</em></td>
<td>12.09</td>
</tr>
<tr>
<td><em>Coryphella borealis</em></td>
<td>7.94</td>
</tr>
<tr>
<td><em>Coryphella browni</em></td>
<td>7.77</td>
</tr>
<tr>
<td><em>Coryphella chriskaugei</em></td>
<td>9.15</td>
</tr>
<tr>
<td><em>Coryphella gracilis</em></td>
<td>12.44</td>
</tr>
<tr>
<td><em>Coryphella falklandica</em></td>
<td>16.75</td>
</tr>
<tr>
<td><em>Coryphella lineata</em></td>
<td>6.74</td>
</tr>
<tr>
<td><em>Coryphella monicae</em></td>
<td>10.71</td>
</tr>
<tr>
<td><em>Coryphella nobilis</em></td>
<td>10.36</td>
</tr>
<tr>
<td><em>Coryphella orjani</em></td>
<td>9.50</td>
</tr>
<tr>
<td><em>Coryphella sanamyanae</em></td>
<td>n/a</td>
</tr>
<tr>
<td><em>Coryphella trilineata</em></td>
<td>12.95</td>
</tr>
<tr>
<td><em>Coryphella trophina</em></td>
<td>9.33</td>
</tr>
<tr>
<td><em>Coryphella verrucosa</em></td>
<td>11.92</td>
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"I.A. Ekimova"
possesses continuous rows of cerata, reddish color of the digestive gland diverticula, and white rhinophores. However, *Coryphella alexanderi* sp. nov. differs from all these species by internal anatomical features. First of all, it differs in radular characters, since in *C. nobilis* and *C. sanamyanae* the radicular tooth possesses a very small central cusp that is usually compressed. *Coryphella browni* has narrower lateral teeth with small cusps and tiny denticles; this species also possesses a single row of denticles on the masticatory edge of the jaws, while in *C. alexanderi* sp. nov. there are at least 10 rows of serrated den-

articles. From other species of the genus *Coryphella* inhabiting the North-West Pacific, *C. alexanderi* sp. nov. differs by its external morphology. From *C. verrucosa* and *C. amabilis* it differs by having the cerata arranged in continuous rows (in groups in *C. verrucosa* and *C. amabilis*), from *C. trophina* by having almost smooth rhinophores (perfoliated in *C. trophina*); *Coryphella athadona* and *C. abei* possess a Y-marking on head, that is absent in *C. alexanderi*.

**Etymology.** This species is named after my beloved father Alexander V. Ekimov, who passed away during the preparation of this manuscript. Alexander V. Ekimov was a researcher in the field of high energy physics, his passion for his work inspired me to become a scientist and his support always encouraged me to be fully dedicated to my research. He will be greatly missed.

**Discussion**

The new species *Coryphella alexanderi* sp. nov. is well characterized both morphologically and genetically (Figs 1–3). Phylogenetically, *C. alexanderi* sp. nov. is close to another North-West Pacific species, *Coryphella trophina* (Fig. 1). But these species have extremely different external and internal morphology [Ekimova et al., in press] and have different ecological traits. While *Coryphella alexanderi* sp. nov. is supposed to be an exclusively cnidarian feeder, being found on *Rhizorhagium roseum* and *Eudendrium* sp. (Fig. 2A), *C. trophina* has a unique diet, which includes either various hydrozoans, or smaller nudibranch species inhabiting same cnidarian colonies [Roginskaya, 1990].

The discovery of *Coryphella alexanderi* sp. nov. indicates that the knowledge on nudibranch biodiversity in the North-West Pacific in general and in the Kuril Islands in particular, is far from being completely understood. Recently, several new species were described from this area, including representatives of the nudibranch genera *Akiodoris* Bergh, 1879 [Martynov, Korshunova, 2020], *Cadlina* Bergh, 1879 [Korshunova et al., 2020a], *Onchidoris* Blainville, 1816 [Martynov, Korshunova, 2017], *Cuthonella* Bergh, 1884 [Korshunova et al., 2020b], and *Dendronotus* Alder et Hancock, 1845 [Ekimova et al., 2015; Martynov et al., 2020]. Distributional ranges of most of these new taxa are restricted to the Kuril Islands waters, thus possibly indicating high degree of endemism in the area.

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