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Fifty shades of white: morphological and molecular diversity of the *Cadlina laevis* species complex (Gastropoda: Nudibranchia) in the North-West Pacific

Irina A. EKIMOVA[1,6], Darya Yu. GRISHINA[1], Ángel VALDÉS[2], Tatiana I. ANTOKHINA[3], Olga V. CHICHVARKHINA[4], Dimitry M. SCHEPETOV[5]

[1]*Lomonosov Moscow State University, 1-12 Leninskie Gory, Moscow 119234, RUSSIAN FEDERATION;*
[2]*Department of Biological Sciences, California State Polytechnic University, 3801 West Temple Ave., Pomona, CA 91768, USA*
[3]*A.N. Severtsov Institute of Ecology and Evolution, Russian Academy of Sciences, 33 Leninskiy pr., Moscow 119071, RUSSIAN FEDERATION;*
[4]*National Scientific Center of Marine Biology, Far Eastern Branch RAS, 17 Palchevskogo St., Vladivostok 690041, RUSSIAN FEDERATION;*
[5]*Biological Faculty, Shenzhen MSU-BIT University, Shenzhen 518172, CHINA.*
[6]*Corresponding author; E-mail: irenekimova@gmail.com*

ABSTRACT. We provide a morphological examination and a barcoding study to investigate the species identity and variation limits within the *Cadlina laevis* species complex. Our molecular analysis based on the COI marker revealed seven new clades in the North-West Pacific *Cadlina* diversity. The distances between these clades are low in some cases (2.08–7.51% overall), and the species delimitation tests gave controversial results (1, 2, 13, 14 groups, depending on the analysis method). This does not allow to conclusively classify this diversity as interspecific or intraspecific. Morphological analysis showed a significant similarity of all examined groups, with minor differences found in the morphology of the central tooth of the radula and the reproductive system. However, these variations fit into the morphological variability of the North Atlantic species *Cadlina laevis s.str.* and cannot serve as evidence of the isolation of these identified groups. The discovered diversity may represent both a complex of at least 11 very close and cryptic species with not well-established species boundaries, or be a part of a single amphiboreal species *Cadlina laevis s.l.* This indicates an extremely complex evolutionary history of *Cadlina laevis* species complex, making this group is an interesting model object for studying speciation in boreal and Arctic communities.

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Пятьдесят оттенков белого: морфологическое и молекулярное разнообразие комплекса видов *Cadlina laevis* (Gastropoda: Nudibranchia) в северо-западной Пацифике

Ирина А. ЕКИМОВА[1,6], Дарья Ю. ГРИШИНА[1], Анхель ВАЛЬДЕС[2], Татьяна И. АНТОХИНА[3], Ольга В. ЧИЧВАРХИНА[4], Дмитрий М. ЩЕПЕТОВ[5]

[1]*Московский государственный университет им. М.В. Ломоносова, Ленинские горы 1-12, 119234 Москва, РОССИЙСКАЯ ФЕДЕРАЦИЯ;*
[2]*Department of Biological Sciences, California State Polytechnic University, 3801 West Temple Ave., Pomona, CA 91768, USA*
[3]*Институт проблем экологии и эволюции им. А.Н.Северцова РАН, Ленинский пр. 33, 119071 Москва, РОССИЙСКАЯ ФЕДЕРАЦИЯ;*
[4]*Национальный научный центр морской биологии, Дальневосточное отделение РАН, ул. Пальчевского 17, Владивосток 690041, РОССИЙСКАЯ ФЕДЕРАЦИЯ;*
[5]*Биологический факультет, Университет МГУ-ППИ в Шэньчжэне, Шэньчжэнь 518172, КИТАЙ.*
[6]*Автор-корреспондент; E-mail: irenekimova@gmail.com*

РЕЗЮМЕ. В данной работе представлены анализ морфологии и баркодинг с целью изучения видовой идентичности и пределов изменчивости видового комплекса *Cadlina laevis*. Наш молекулярный анализ, основанный на маркере COI, выявил семь новых клад рода *Cadlina* в северо-западной части Тихого океана. Попарные генетические дистанции между этими кладами в ряде случаев невелики (в целом 2,08–7,51%), а тесты определения видовых границ дали противоречивые результаты (1, 2, 13, 14 групп в зависимости от метода анализа). Полученные результаты не позволяют однозначно отнести выявленное разнообразие к межвидовому или внутривидовому уровню. Морфологический анализ показал значительное сходство всех изученных групп, при этом обнаружено несколько незначительных различий в морфологии центрального зубца радулы и половой системы. Однако эти вариации

укладываются в морфологическую изменчивость североатлантического вида *Cadlina laevis s.str.* и не могут служить доказательством обособленности выявленных групп. Обнаруженное разнообразие может представлять собой как комплекс из не менее 11 очень близких, криптических видов с не четко установленными видовыми границами, либо входить в состав единого амфибореального вида *Cadlina laevis s.l.* Это свидетельствует о чрезвычайно сложной истории эволюции видового комплекса *Cadlina laevis*, что делает эту группу интересным модельным объектом для изучения видообразования в бореальных и арктических сообществах.

Introduction

The nudibranch genus *Cadlina* Bergh, 1879 (Gastropoda: Nudibranchia) is widely distributed, but more speciose in temperate and cold oceans [Schrödl, 2000; Do *et al.*, 2020]. Of the approximate 30 species of *Cadlina* currently considered as valid [Schrödl, 2000; Do *et al.*, 2020; Korshunova *et al.*, 2020] only a handful are found exclusively in the tropics [Camacho-García *et al.*, 2005; Valdés *et al.*, 2006] and this group appears to be completely absent from the tropical Indo-Pacific [Gosliner *et al*., 2018]. The systematics of *Cadlina* has been problematic, the genus was historically classified as a basal Chromodorididae because of its radular morphology with denticulate teeth and the presence of conspicuous mantle glands [Rudman, 1984]. However, Rudman [1984] noted some differences with other Chromodorididae, including a complex spicule network and the seminal receptable connecting to a duct instead of directly to the vagina. More recently, *Cadlina* was transferred back to the family Cadlinidae along with genus *Aldisa* Bergh, 1878 [Johnson, 2011], based on molecular phylogenetic analyses. Species of *Cadlina* display euryphagy on several sponge taxa, from which they obtain terpenoids [Cimino, Ghiselin, 2009]. At least one species is able to synthesize terpenoids *de novo* and accumulate them in their tissue and egg masses [Dumdei *et al.*, 1997], suggesting these chemicals play important defensive roles in *Cadlina*.

Among dorid nudibranchs only two sponge feeders have been able to colonize subarctic and Arctic waters (the Barents Sea, the White Sea): *Cadlina laevis* (Linnaeus, 1767) and *Doris pseudoargus* Rapp, 1827 [Martynov *et al.*, 2006; Laakkonen *et al.*, 2021]. However, recent studies have shown that *C. laevis* is a species complex, represented by at least four species [Korshunova *et al.*, 2020], with the nominal species *C. laevis* restricted to the North Atlantic and Arctic, and other three species found in the Pacific. This may be indicative of the allopatric nature of the speciation of the *C. laevis* complex, possibly resulting from the Pleistocene climatic fluctuations [Ekimova *et al.*, 2019; Laakkonen *et al.*, 2021]. However, material from only a few areas in the North Pacific has been studied, and further investigation of the *C. laevis* species complex is needed to determine what drivers shaped the diversification of this group.

The main goal of this study is the comparative morphological analysis of putative species of *C. laevis* species complex in the North-West Pacific, supported by mtDNA data (barcoding) and species delimitation analyses.

Material and methods

Collection data

Specimens for this study (n = 111) were collected during various expeditions and field trips in the 2019–2022 period (Table S1, Fig. 1). Specimens from the White Sea were collected near the White Sea Biological Station (66°33’29.2”N, 33°06’19.6”E) by scuba diving from 5–15 m depth. A single sample from the Barents Sea was collected in Teriberka Bay (69°11.172’N, 035°07.964’E) by scuba diving from 12–14 m in depth. Specimens from the Sea of Japan were collected by scuba diving at depths of 0.5–18 m, in three localities: (1) near Vladivostok (43°03’25.9”N, 131°50’24.3”E), (2) Rudnaya Bay (44°20.057’N, 135°50.373’E) and (3) Oprichnik Bay (44°26’31.4”N, 135°59’40.6”E). Twenty-four specimens were collected during an expedition on board the R/V “*Akademik Oparin*” (Russia) to the Sea of Japan in July 2021 using an Agassiz trawl (AGT), at depths of 56–212 m. Finally, four specimens were collected during an expedition on same vessel to the Sea of Okhotsk near Urup, Iturup and Shikotan Is., July-August 2019, of them, three specimens were collected by scuba diving from depths of 5–16 m and one specimen by AGT, at depths of 263–273 m.

Most specimens were photographed alive in the laboratory and then preserved individually in collection vials, except those collected during expedition on the R/V “*Akademik Oparin*” these specimens were initially identified on board by external morphology (mainly by coloration: presence of yellow margin, yellow dots on notum, etc.), and preserved according to the initial identifications (many specimens of similar coloration in the same collection vial, photographs were taken only for a single specimen in each lot). All specimens were preserved in 96% EtOH and stored at -20°C to prevent DNA degradation. Voucher specimens are deposited in the collections of the National Scientific Center of Marine Biology (MIMB). Detailed sampling localities and voucher numbers for each specimen are given in Table S1.

DNA extraction, amplification, sequencing

Total genomic DNA was extracted from tissue samples preserved in 96% EtOH (Table S1) follow-

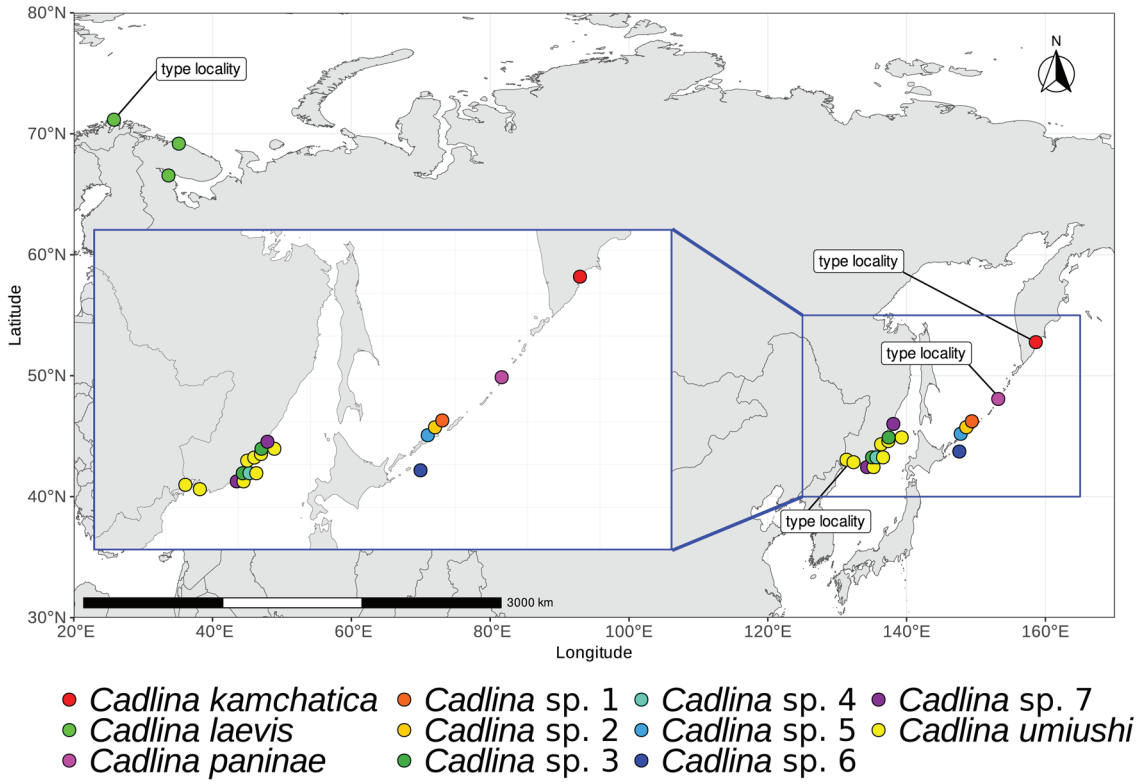

FIG. 1. Map of the North-West Pacific and Russian Arctic representing collection sites and type localities of described species of the *Cadlina laevis* species complex.

РИС. 1. Карта Северо-Западной части Тихого океана и Российской Арктики, с указанием точек сбора и типовых местонахождений описанных видов из комплекса *Cadlina laevis.*

ing the invertebrate protocol of the Canadian Center for DNA Barcoding [Ivanova *et al.*, 2006]. We performed an amplification of partial mitochondrial *cytochrome c oxidase* subunit I (COI) following methods described in Ekimova *et al.* [2019, 2021]. For sequencing, 1–2 μL of amplicons were purified by EtOH + Ammonium acetate precipitation [Osterburg *et al.*, 1975]. Sequencing was performed with a NovaDye Terminator Cycle Sequencing Kit by GeneQuest (Moscow, Russia). Sequencing reactions were analyzed using an ABI 3500 Genetic Analyser (Applied Biosystems) at N.K. Koltsov Institute of Developmental Biology (Moscow, Russia). All novel sequences were submitted to NCBI GenBank (Table S2).

Molecular phylogenetic analysis

All sequences obtained were assembled and checked for erroneous base-calling using Geneious R10. Assembled sequences were compared to the publicly available *Cadlina* sequences using the BLAST-n algorithm over the GenBank nr/nt database for verification of possible contamination. For the phylogenetic analysis a previously published dataset from Korshunova *et al.* [2020] was used, the full list is presented in Table S2. Original data and published sequences were aligned with the MUSCLE [Edgar, 2004] algorithm in MEGA 7 [Kumar *et al.*, 2016]. Due to the high number of identical sequences in original data (many specimens of *C. umiushi* and *C. laevis*, see Results section), many of them were removed from the final alignment as they are not phylogenetically informative. Saturation was checked by plotting the total number of pairwise differences (transitions and transversions) for all specimens (including the outgroup) against uncorrected *p*-distances, saturation was further examined separately for the first, second, and third codon positions. The best-fit nucleotide evolution model for reconstructions was selected in MEGA7 [Kumar *et al.*, 2016], GTR+G+I model was chosen. The final alignment included 645 bp. The Bayesian phylogenetic analysis with estimation of posterior probabilities was performed in MrBayes 3.2 [Ronquist, Huelsenbeck, 2003]. Markov chains were sampled at intervals of 500 generations. Two runs of 10^7 generations with four chains (one cold and three heated) were performed. The convergence was checked using TRACER v1.7.1 [Rambaut *et al.*, 2018]. Maximum likelihood phylogeny inference was performed in the HPC-PTHREADS-AVX option of RaxML HPC-PTHREADS 8.2.12 [Stamatakis, 2014] with number of pseudoreplicates inferred by

autoMRE option. Final phylogenetic tree images were rendered in FigTree 1.4.0 and further modified in Adobe Illustrator CS 2015.

Species delimitation

We used three molecular species delimitation methods (ASAP, GMYC, PTP) to confirm the status of recovered clades as putative candidate species. ASAP analysis [Puillandre *et al.*, 2021] was run using the online version of the program (https://bioinfo.mnhn.fr/abi/public/asap/asapweb.html, accessed on 10 June 2023) with the Kimura 2-parameter model and other settings remained default. Also, two separate analyses of Poisson Tree Processes (PTP) method based on the Maximum likelihood (mPTP) and Bayesian inference (bPTP) were conducted [Zhang *et al.*, 2013]. These tests were run using the PTP Server http://species.h-its.org/ptp/ (accessed on 10 June 2023) with 500000 generations and with other settings (thinning, burn-in and seed) set as default. The Bayesian phylogenetic trees inferred using single-gene COI dataset was used as an input tree. Additionally, we ran the General Mixed Yule-Coalescent (GMYC) test proposed by Pons *et al.* [2006] and implemented by Fujisawa and Barraclough [2013]. COI-based tree was calculated using BEAST 2.7 [Bouckaert *et al.*, 2019] and then analyzed in the R environment package *splits*, following Fujisawa and Barraclough [2013]. Uncorrected *p*-distances were calculated in MEGA 7 [Kumar *et al.*, 2016].

Population genetic analysis

Haplotype networks based on the COI dataset were constructed using PopART 1.7 (http://popart.otago.ac.nz, assessed on 23 June, 2023) with the TCS network algorithm [Clement *et al.*, 2002]. For the analysis, all sequenced specimens were included and also all available GB or BOLD sequences were added to the alignment [total number of specimens = 134 (Table S2)]. Resulting networks were edited in Adobe illustrator CS 2015 to highlight certain features.

Morphological analysis

The number of specimens used for each analysis is summarized in the Table 1. External features were examined under an Olympus SZ51 stereomicroscope (Olympus Corporation, Japan) and by examining photographs of live animals. The buccal armature (radula and labial cuticle) was extracted by dissection of the head region. Firstly, the buccal mass with the radula, odontophore, and labial cuticle was removed and dehydrated in a rising series of ethanol and acetone, critically-point dried, mounted on an aluminum stub, and sputter-coated with gold or a mixture of platinum and palladium. Examinations of intact odontophores and labial cuticle were performed with the scanning electron microscopes (SEM) JEOL JSM 6380LA or JEOL JSM 7000 (JEOL, USA). After examinations dried radulae, odontophores, and labial cuticles were again placed in distilled water for 1–2 days until full rehydration, and then placed in a 10% sodium hypochlorite solution to fully dissolve soft tissues. Afterwards, the radula was washed with distilled water 5 times, 15 minutes each, air-dried, mounted on an aluminum stub, sputter-coated with gold or a mixture of platinum and palladium and examined under the SEMs.

The reproductive organs were examined and sketched under an Olympus SZ51 stereomicroscope (Olympus Corporation, Japan). For species in which the penis was everted, we performed examination of the penial spine morphology using SEM. Penises were removed from the reproductive systems, critically point dried using the same procedure described above, mounted on an aluminum stub, sputter-coated with gold or a mixture of platinum and palladium and examined with the SEMs.

Morphological and molecular data for *Cadlina* sp. MIMB42230 have been already published in Ekimova *et al*. [2021], but several features (*i.e*., denticulation of first lateral teeth) were re-examined in this study.

Abbreviations

EA – Eastern Atlantic; NEP – North-East Pacific; NWP – North-West Pacific TWA – Tropical Western Atlantic; TWP – Temperate Western Pacific.

Results

Phylogenetic analysis

Single-gene COI tree revealed high support of most clades of low taxonomical levels (= candidate species), but the phylogenetic relationships between them were not resolved or supported in some cases. Trees based on both Maximum Likelihood (ML) and Bayesian Inference (BI) displayed similar topologies, resolutions, and nodal supports (Fig. 2, Fig. S1). The genus *Cadlina* was recovered as monophyletic with high nodal support (posterior probabilities from BI – PP = 1; bootstrap values from ML – BS = 96). Within this genus, several major monophyletic groups were recovered: (1) a clade grouping *C. luarna* (Er. Marcus et Ev. Marcus, 1967) (NEP), *C. rumia* Er. Marcus, 1955 (TWA), *C. flavomaculata* MacFarland, 1905 (NEP), *C. sparsa* (Odhner, 1922) (Chile), *C. modesta* MacFarland, 1966 (NEP) (PP = 1; BS = 72), (2) *C. luteomarginata* MacFarland, 1966 (NEP) and *C. sylviaearleae* Korshunova *et. al*., 2020 (NEP) (PP = 1; BS = 100), (3) *C. jannanicholsae* Korshunova *et. al*., 2020 (NEP), *C. japonica* Baba, 1937 (TWP) and *C. klasmalmbergi* Korshunova *et. al.*, 2020 (NEP) (PP = 0.99; BS = 100) and (4) *C. laevis* species complex, including *C. laevis s.str.*, *C.*

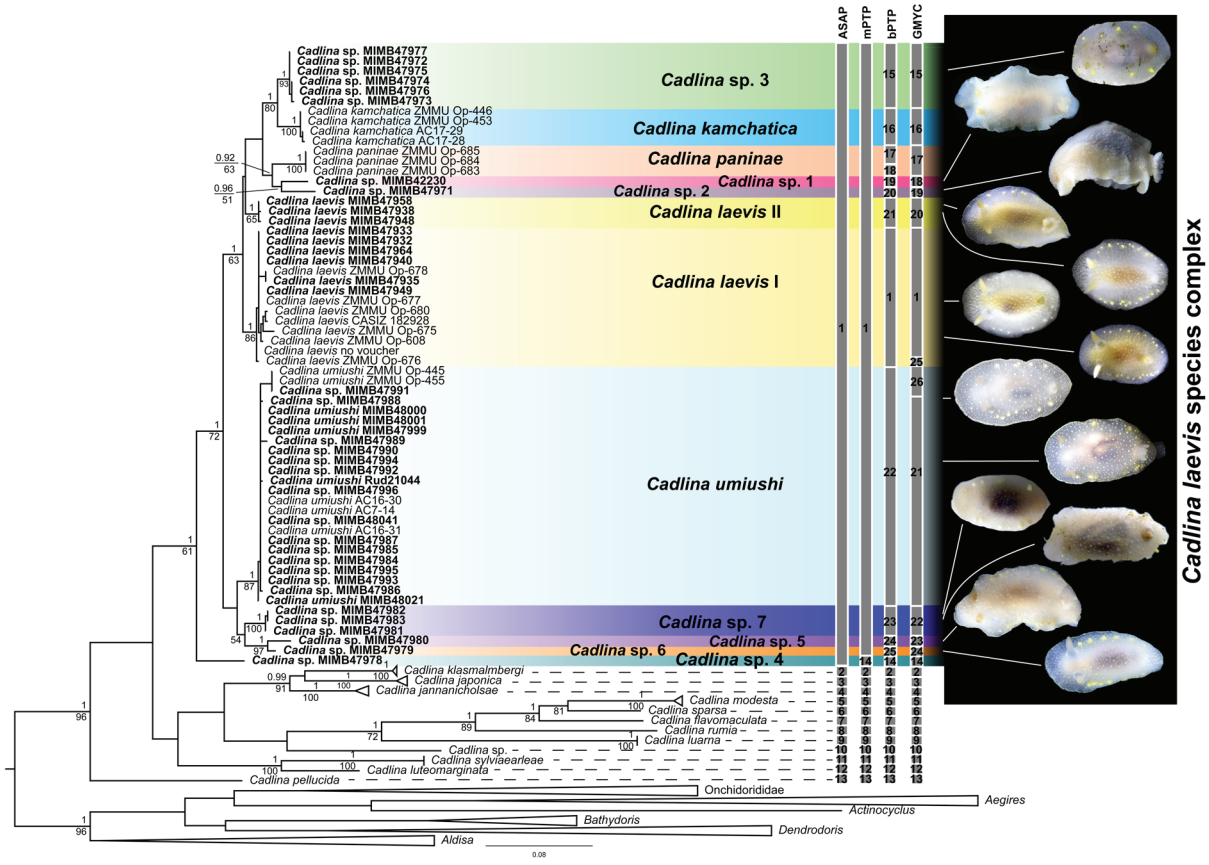

FIG. 2. Maximum Likelihood phylogenetic tree based on the COI-based dataset, species-level clades and outgroups are collapsed to a single branch, except representatives of the *Cadlina laevis* species complex. Specimens studied in this work are highlighted in bold. Numbers above branches indicate posterior probabilities from Bayesian inference, numbers bellow branches show bootstrap supports from Maximum likelihood analysis. Blocks on the right indicate species delimitation results, number refers to respective operational taxonomical unit. Respective photographs of studied specimens are given on the right.

РИС. 2. Филогенетическое дерево, построенное методом максимального правдоподобия, основанное на выравнивании по гену COI, клады и внешние группы на уровне вида сколлапсированы в одну ветвь, за исключением представителей видового комплекса *Cadlina laevis*. Образцы, изученные в данной работе, выделены жирным шрифтом. Числа над ветвями обозначают апостериорные вероятности байесовского анализа, числа под ветвями показывают бутстреп-поддержку анализа максимального правдоподобия. Блоки справа обозначают результаты тестов на определение видовых границ, номер относится к соответствующей оперативной таксономической единице. Справа приведены фотографии изученных экземпляров.

kamchatica Korshunova, Picton, Sanamyan et Martynov, 2015, *C. paninae* Korshunova *et. al*., 2020, *C. umiushi* Korshunova, Picton, Sanamyan et Martynov, 2015 and several *Cadlina* spp. from the North-West Pacific. Also, *Cadlina* sp. CASIZ175547 and *C. pellucida* (Risso, 1826) (EA) were recovered as derived singletons and unresolved deep relationships with major clades described above. Within the *C. laevis* species complex, *C. umiushi* was monophyletic (PP = 1, BS = 87), and *C. laevis* was monophyletic in the BI analysis (PP = 1), but contained two distinct monophyletic units (*C. laevis* I and *C. laevis* II, PP = 1 in both cases, Fig. S1). In the ML tree the relationships between *C. laevis* I and *C. laevis* II were unresolved. North-West Pacific species *C. paninae* and *C. kamchatica* formed two monophyletic and highly supported clades (PP = 1, BS = 100). Several specimens from the Sea of Japan (named *Cadlina* sp. 3 herein, Fig. 2) formed a monophyletic group with high support (PP = 1, BS = 93), which was recovered sister to *C. kamchatica* (PP = 1, BS = 80). Another specimen from the Sea of Japan, *Cadlina* sp. MIMB47978 (named *Cadlina* sp. 4 herein, Fig. 2) was recovered as a derived singleton, sister to all species from the *C. laevis* species complex (PP = 1, BS = 72). Four specimens from the Sea of Okhotsk (Kurile Islands) formed four derived singletons. *Cadlina* sp. MIMB47971 (named *Cadlina* sp. 1 herein, Fig. 2) together with *Cadlina* sp. MIMB42230 (named *Cadlina* sp. 2 herein, Fig. 2) (PP = 0.96, BS = 51) were recovered as sister to *C. paninae* (PP = 0.92, BS = 63). *Cadlina* sp. MIMB47979 (named *Cadlina* sp. 5

Table 1. Intra- and interspecific uncorrected p-distances (%) based on the COI gene.

Табл. 1. Внутри- и межвидовые нескорректированные попарные дистанции (в %), посчитанные по гену COI.

Species	*C. kamchatica*	*C. laevis*	*C. paninae*	*Cadlina* sp. 1	*Cadlina* sp. 2	*Cadlina* sp. 3	*Cadlina* sp. 4	*Cadlina* sp. 5	*Cadlina* sp. 6	*Cadlina* sp. 7	*C. umiushi*
C. kamchatica	0.16-0.32										
C. laevis	3.69-4.97	0-2.40									
C. paninae	4.65-4.97	4.17-4.65	0								
Cadlina sp. 1	4.33-4.65	4.65-5.61	4.17	n/a							
Cadlina sp. 2	4.97-5.13	3.85-4.97	4.33	3.85	n/a						
Cadlina sp. 3	2.08-2.72	3.37-4.65	4.17-4.49	3.69-3.85	4.17-4.49	0-0.32					
Cadlina sp. 4	6.25-6.57	5.61-6.57	6.25	5.93	7.21	5.77-6.09	n/a				
Cadlina sp. 5	5.77-5.93	4.49-4.97	5.13	5.29	5.61	4.65-4.81	6.73	n/a			
Cadlina sp. 6	6.09-6.41	4.97-5.61	5.29	4.81	5.13	5.13-5.29	6.57	2.08	n/a		
Cadlina sp. 7	4.81-5.29	4.81-5.13	4.97-5.13	5.93-6.09	6.41-6.57	4.33-4.65	5.93-6.09	3.37-3.53	4.17-4.33	0-0.16	
C. umiushi	4.33-5.13	4.17-4.97	5.13-5.45	5.61-6.09	5.29-5.77	4.33-4.97	5.93-6.09	3.69-3.85	4.01-4.33	2.88-3.53	0-0.64

herein, Fig. 2) and *Cadlina* sp. MIMB47980 (named *Cadlina* sp. 6 herein, Fig. 2) clustered together (PP = 1, BS = 97), but their relationships with other representatives of the *C. laevis* species complex received very low support. Finally, three specimens from the Sea of Japan (MIMB47981–MIMB47983 named *Cadlina* sp. 7 herein, Fig. 2) form a single monophyletic group (PP = 1, BS = 100), whose relationships to other species from the *C. laevis* species complex were unresolved.

Species delimitation

Species delimitation analyses based on different approaches resulted in different number of recovered operational taxonomical units (OTUs). In ASAP the lowest ASAP score (1.0) was received for partition with 13 OTUs, with all studied specimens of *C. laevis* species complex constituting a single OTU (Fig. 2). A scenario with 21 OTUs, which corresponded to the initial species hypothesis in most cases, received much higher ASAP score (4.5) (Fig. S2). Furthermore, mPTP also suggested a 'lumping' scenario with only 2 OTUs recovered within the *C. laevis* species complex (Figs 2, S4) and 14 OTUs in total (*Cadlina* sp. 4 was recovered as separate group, while the rest diversity of the *C. laevis* species complex was united in a single group). bPTP produced very different result with 25 OTUs in total (Figs 2, S4), and 13 OTUs recovered within the *C. laevis* species complex, however two groups were observed within both *C. laevis s.str.* and *C. paninae.* Finally, GMYC resulted in a 'splitting' hypothesis, with 14 OTUs within the *C. laevis* species complex (27 in total), with three separate OTUs within *C. laevis s.str.* and two within *C. umiushi* (Figs 2, S3).

Calculated *p*-distances of the COI marker among lineages of the *C. laevis* species complex are presented in Table 1.

To sum up, the results of the species delimitation analyses gave inconclusive results suggesting either the presence of a complex of closely related incipient species or a single species with genetically distinct populations and rather restricted gene-flow between them.

Haplotype network

The COI-based TCS haplotype network (Fig. 3) was well-structured and corroborated the results of the molecular phylogenetic and species delimitation analyses (Fig. 2). Overall, putative species formed separate haplotypes/haplogroups differed in 13–27 substitutions. Haplotypes of two species, *C. laevis* and *C. umiushi* formed two heterogeneous groups with high intraspecific haplotype diversity. Within *C. umiushi* most specimens were recovered in a single common haplotype, and eight specimens were represented by five haplotypes, which differed from the common haplotype by 1–2 mutation steps. Also, two specimens from Korea had two unique haplotypes, differed by six substitutions from haplotypes

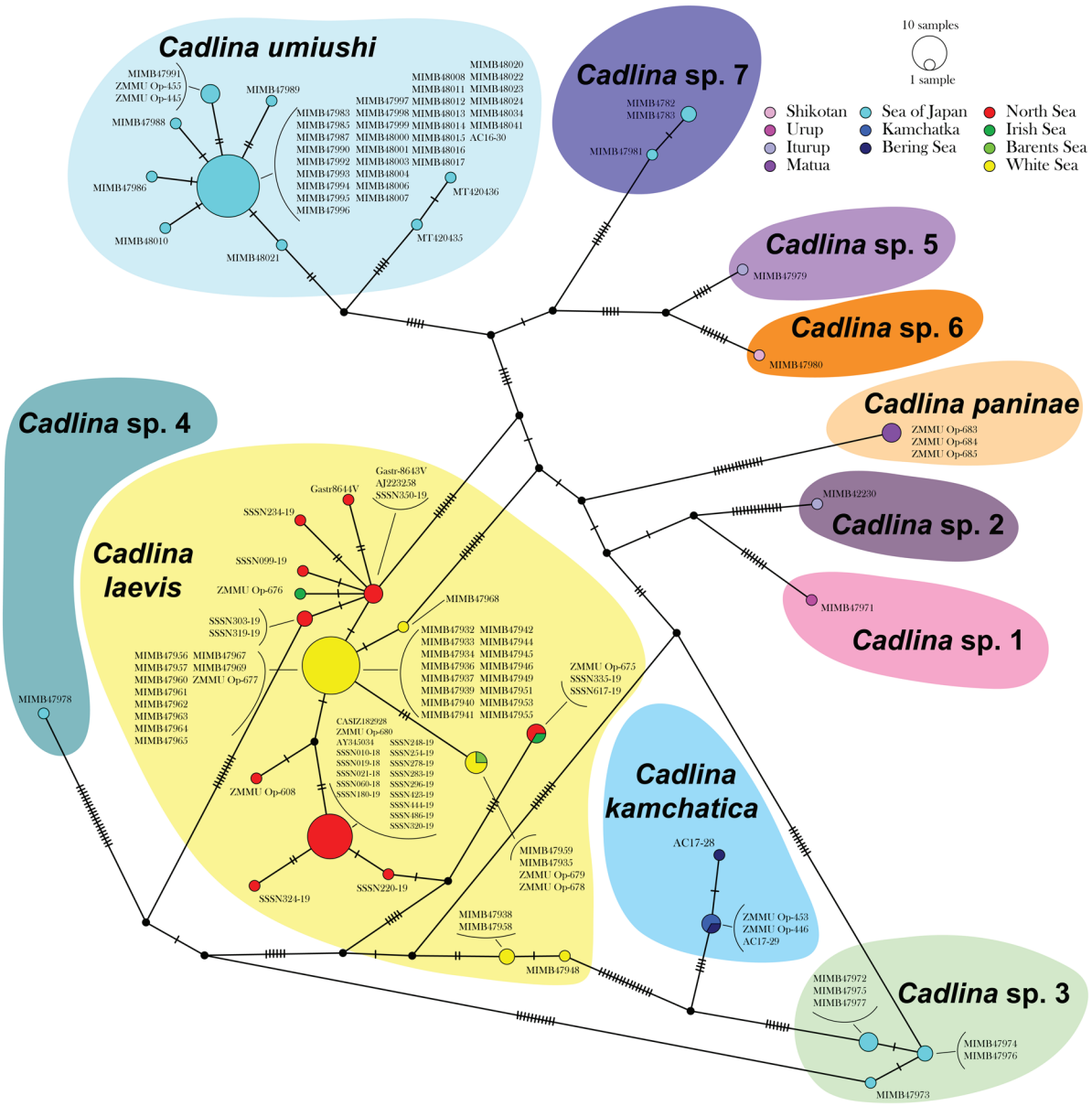

FIG. 3. COI haplotype network of *Cadlina laevis* species complex produced with TCS method in PopART. Colours of circles refer to the geographic origin of each haplotype. The relative size of circles is proportional to the number of sequences of that same haplotype.

РИС. 3. Сеть гаплотипов COI комплекса видов *Cadlina laevis*, полученная методом TCS в PopART. Цвета кружков обозначают географическое происхождение каждого гаплотипа. Относительный размер кружков пропорционален количеству последовательностей одного и того же гаплотипа.

represented by specimens from the Sea of Japan Russian coast. The haplotype network of the North-East Atlantic and subarctic *C. laevis* displayed high geographic structure. Specimens were represented by 18 haplotypes, among which two were most common and contained specimens exclusively from the White Sea (1) and from the North Sea (2), there were three mutation steps between these haplotypes, the rest haplotypes were represented by 1–2 specimens and differed from the most common ones by 1–4 substitutions. Specimens MIMB47948, MIMB47958 and MIMB47938 formed a diverged haplogroup, with nine substitutions separating them from the North Sea haplotypes.

Morphological analysis

Morphological traits of studied specimens are summarized in Table S3. Overall, the main differences in external morphology were related to coloration, especially to the presence/absence of pigmented notal spots, the presence and color of subepidermal glands, the presence/absence of a pigmented lines along notal margin (Figs 2, 4). *Cadlina* sp. 1 (MIMB47971) and *Cadlina* sp. (MIMB42230) did not possess any

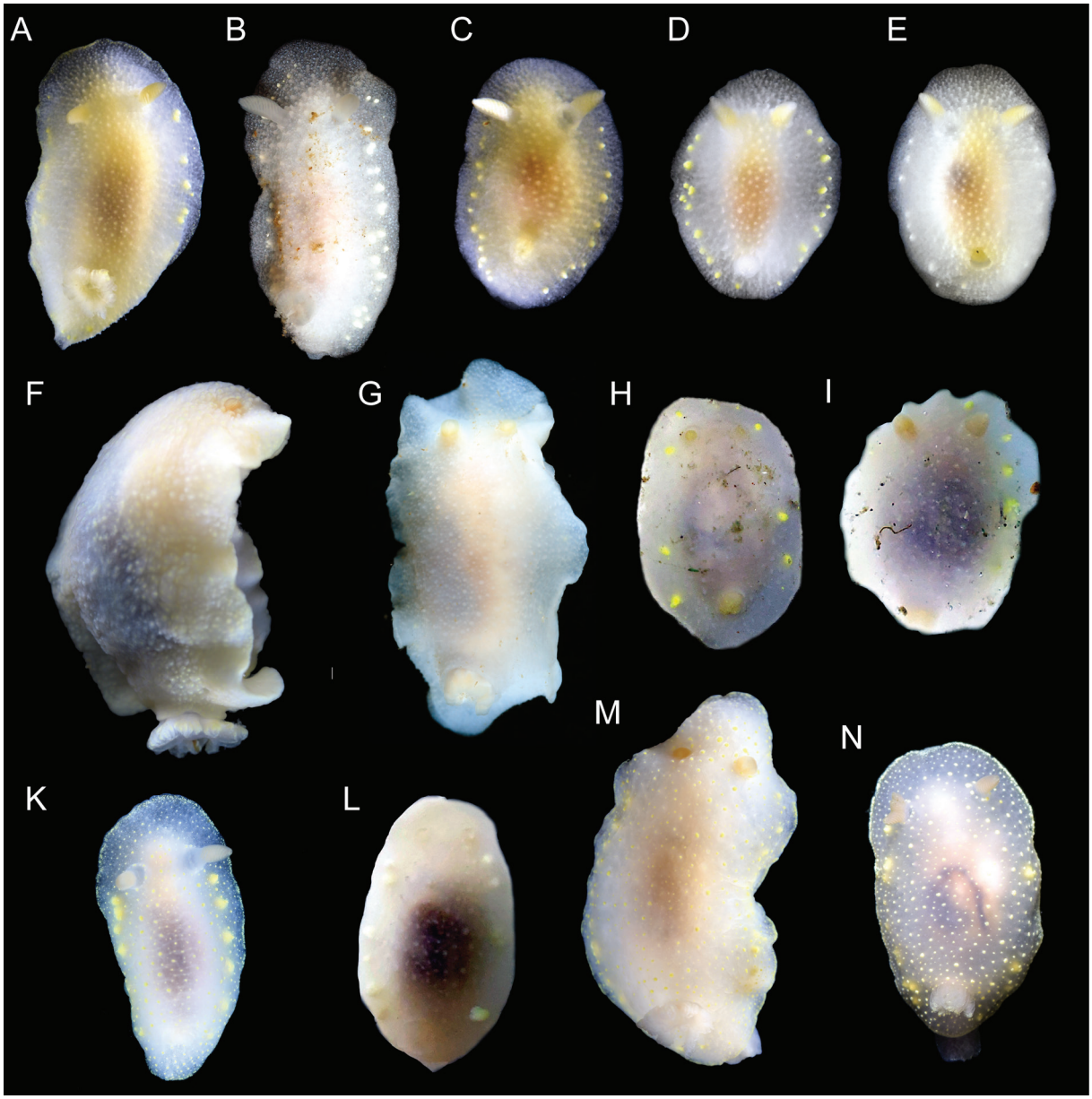

FIG. 4. Photos of studied specimens from different localities, all measurements are indicated in preserved state. **A.** *Cadlina laevis,* MIMB47953, White Sea, 16 mm in length. **B.** *Cadlina laevis,* MIMB47970, Barents Sea, 13 mm in length. **C.** *Cadlina laevis,* MIMB47948, White Sea, 10 mm in length. **D.** *Cadlina laevis,* MIMB47965, White Sea, 9 mm in length. **E.** *Cadlina laevis,* MIMB47958, White Sea, 10 mm in length. **F.** *Cadlina* sp. 1, MIMB47971, Urup Is., Sea of Okhotsk, 22 mm in length. **G.** *Cadlina* sp. 2, MIMB42230, Iturup Is., Sea of Okhotsk, 18 mm in length. **H.** *Cadlina* sp. 3, MIMB47974, Sea of Japan, 10 mm in length. **I.** *Cadlina* sp. 3, MIMB47972, Sea of Japan, 15 mm in length. **K.** *Cadlina* sp. 5, MIMB47979, Iturup Is., Sea of Okhotsk, 11 mm in length. **L.** *Cadlina* sp. 7, MIMB47981, Sea of Japan, 25 mm in length. **M.** *Cadlina* sp. 6, MIMB47980, Shikotan Is., Sea of Okhotsk, 29 mm in length. **N.** *Cadlina umiushi,* MIMB48000, Sea of Japan, 17 mm in length.

РИС. 4. Фотографии изученных экземпляров из разных регионов, размер тела указан для фиксированного состояния. **A.** *Cadlina laevis*, MIMB47953, Белое море, длина 16 мм. **B.** *Cadlina laevis*, MIMB47970, Баренцево море, длина 13 мм. **C.** *Cadlina laevis*, MIMB47948, Белое море, длина 10 мм. **D.** *Cadlina laevis*, MIMB47965, Белое море, длина 9 мм. **E.** *Cadlina laevis*, MIMB47958, Белое море, длина 10 мм. **F.** *Cadlina* sp. 1, MIMB47971, о. Уруп, Охотское море, длина 22 мм. **G.** *Cadlina* sp. 2, MIMB42230, о. Итуруп, Охотское море, длина 18 мм. **H.** *Cadlina* sp. 3, MIMB47974, Японское море, длина 10 мм. **I.** *Cadlina* sp. 3, MIMB47972, Японское море, длина 15 мм. **K.** *Cadlina* sp. 5, MIMB47979, о. Итуруп, Охотское море, длина 11 мм. **L.** *Cadlina* sp. 7, MIMB47981, Японское море, длина 25 мм. **M.** *Cadlina* sp. 6, MIMB47980, о. Шикотан, Охотское море, длина 29 мм. **N.** *Cadlina umiushi*, MIMB48000, Японское море, длина 17 мм.

pigmented spots and colored dots on notum or notal margin and no clear signs of yellow notal glands (Figs 2; 4F, G), while both notal yellow spots, yellow subepidermal glands and marginal yellow line were characteristic for *Cadlina* sp. 5 (MIMB47979), *Cadlina* sp. 6 (MIMB47980) and *C. umiushi* (Figs 2; 4K, M, N). Unfortunately, only several photos were taken for all specimens representing *Cadlina*

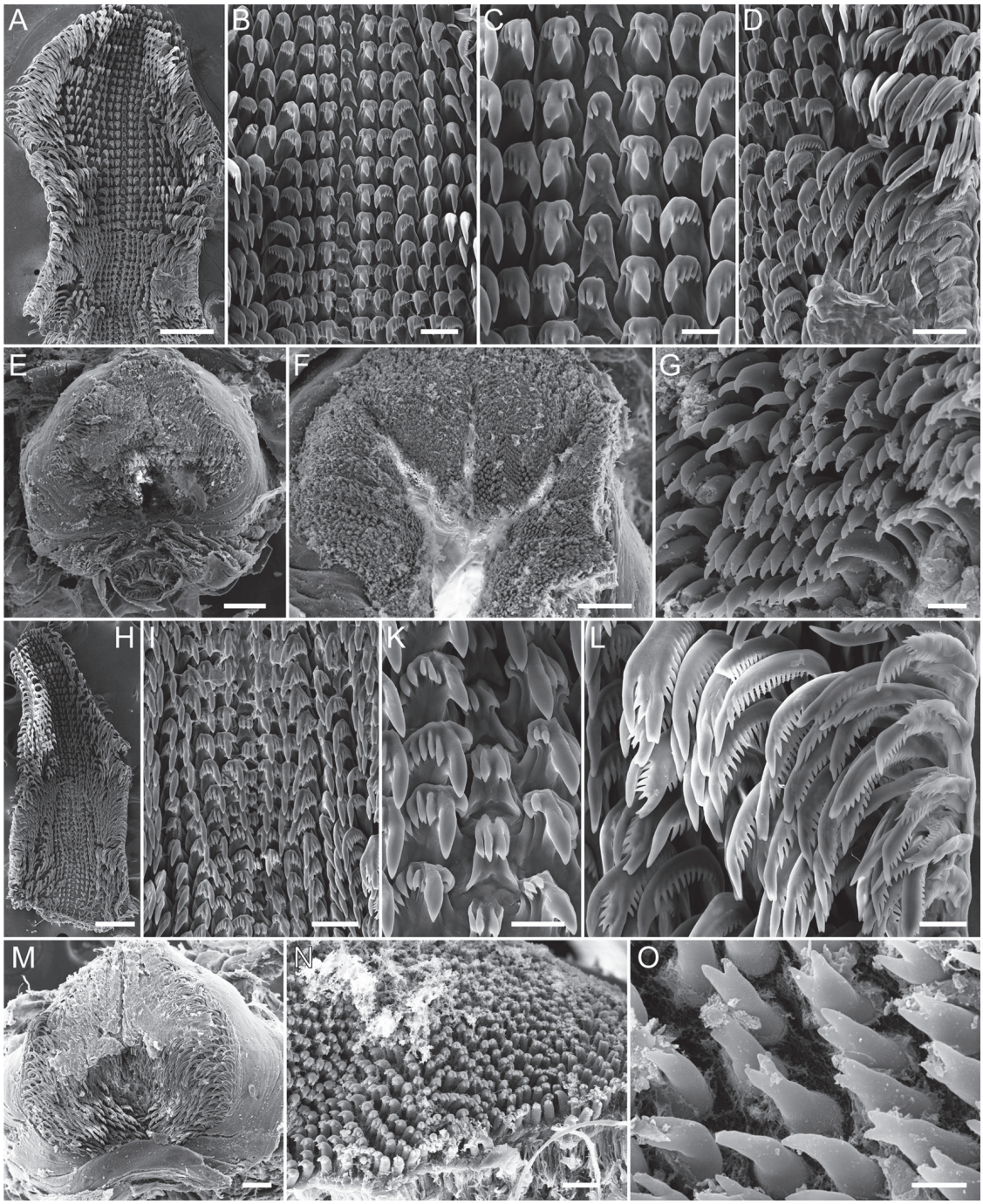

FIG. 5. Buccal armature of *Cadlina laevis s.str.,* specimens from the White Sea (SEM). **A.** MIMB47939, radula. **B.** MIMB47939, anterior radular portion, rachidian and inner lateral teeth. **C.** MIMB47939, same as **B**, enlarged. **D.** MIMB47939, anterior radular portion, outer lateral teeth. **E.** MIMB47939, odontophore with radula. **F.** MIMB47939, labial cuticle. **G.** MIMB47939, labial cuticle rodlets. **H.** MIMB47963, radula. **I.** MIMB47963, middle radular portion, rachidian and inner lateral teeth. **K.** MIMB47963, middle radular portion, rachidian and innermost laterals. **L.** MIMB47963, outer lateral teeth. **M.** MIMB47963, odontophore with radula. **N.** MIMB47963, labial cuticle. **O**. MIMB47963, labial cuticle rodlets. Scale bars: A, E, H = 200 μm; B, D, I = 50 μm; C, K, L, N = 20 μm; F, M = 100 μm; G = 10 μm; O = 5 μm.

РИС. 5. Буккальное вооружение *Cadlina laevis s.str*., особи из Белого моря (СЭМ). **A.** MIMB47939, радула. **B.** MIMB47939, передняя часть радулы, центральные и внутренние латеральные зубы. **C.** MIMB47939, то же, что B, увеличенное. **D.** MIMB47939, передняя часть радулы, внешние латеральные зубы. **E.** MIMB47939, одонтофор с радулой. **F.** MIMB47939, лабиальная кутикула. **G.** MIMB47939, родлеты лабиальной кутикулы. **H.** MIMB47963, радула. **I.** MIMB47963, средняя часть радулы, центральные и внутренние латеральные зубы. **K.** MIMB47963, средняя часть радулы, центральные и внутренние латеральные зубы. **L.** MIMB47963, наружные латеральные зубы. **M.** MIMB47963, одонтофор с радулой. **N.** MIMB47963, лабиальная кутикула. **O.** MIMB47963, родлеты лабиальной кутикулы. Масштабные линейки: A, E, H = 200 мкм; B, D, I = 50 мкм; C, K, L, N = 20 мкм; F, M = 100 мкм; G = 10 мкм; O = 5 мкм.

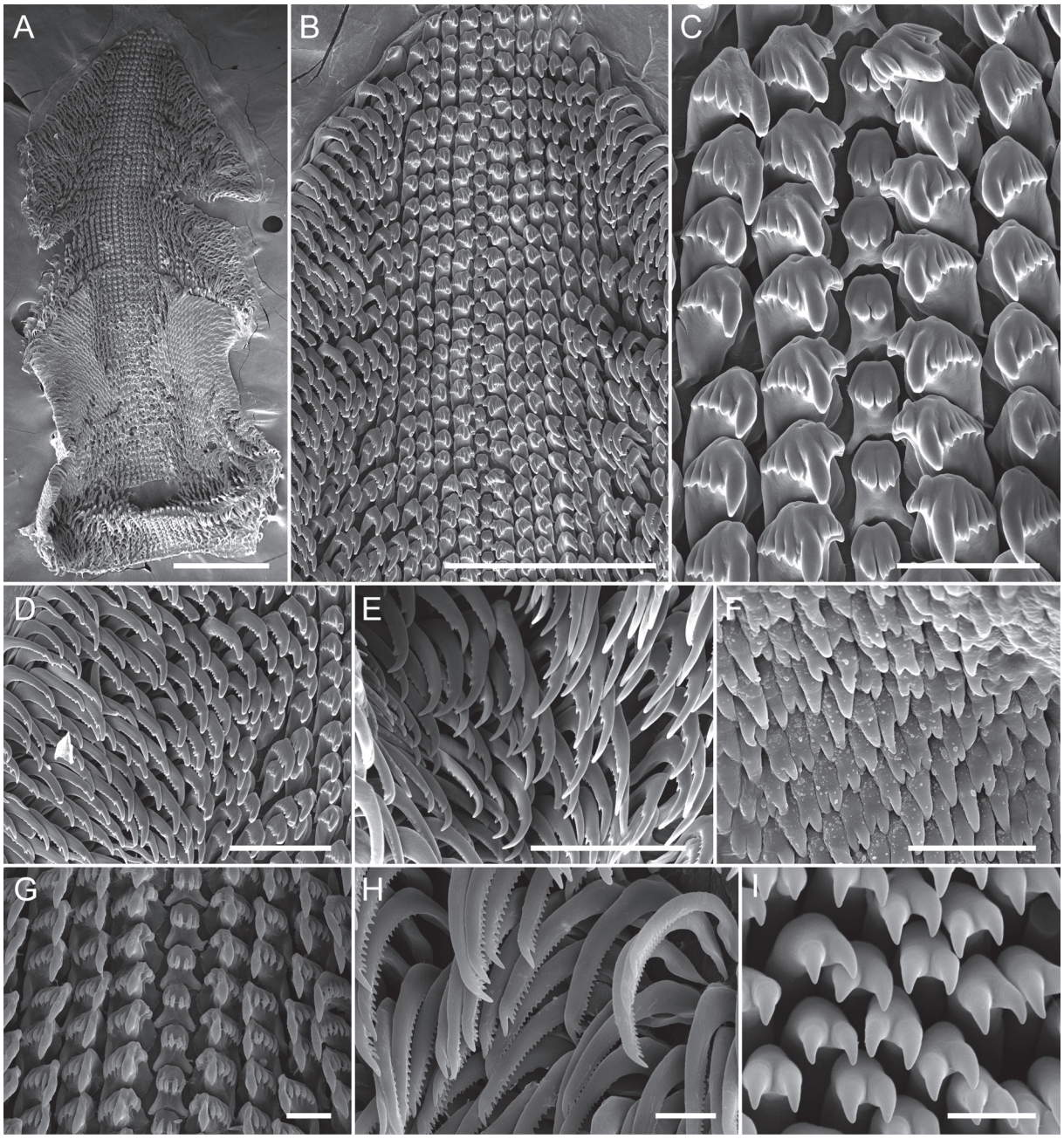

FIG. 6. Buccal armature of *Cadlina* sp. 1 (MIMB47971, Urup Is., Sea of Okhotsk) and *Cadlina* sp. 2 (MIMB42230, Iturup Is., Sea of Okhotsk) (SEM). **A.** *Cadlina* sp. 1, radula. **B.** *Cadlina* sp. 1, anterior radular portion, rachidian and lateral teeth. **C.** *Cadlina* sp. 1, rachidian and innermost lateral teeth. **D.** *Cadlina* sp. 1, outer lateral teeth. **E.** *Cadlina* sp. 1, outer lateral teeth. **F.** *Cadlina* sp. 1, labial cuticle rodlets. **G.** *Cadlina* sp. 2, anterior radular portion, rachidian and inner lateral teeth. **H.** *Cadlina* sp. 2, outer lateral teeth. **I.** *Cadlina* sp. 2, labial cuticle rodlets. Scale bars: A = 500 μm; B = 300 μm; C = 50 μm; D, E = 100 μm; F = 20 μm; G, H = 30 μm; I = 10 μm.

РИС. 6. Буккальное вооружение *Cadlina* sp. 1 (MIMB47971, о. Уруп, Охотское море) и *Cadlina* sp. 2 (MIMB42230, о. Итуруп, Охотское море) (СЭМ). **A.** *Cadlina* sp. 1, радула. **B.** *Cadlina* sp. 1, передняя часть радулы, центральные и внутренние латеральные зубы. **C.** *Cadlina* sp. 1, центральные и внутренние латеральные зубы. **D.** *Cadlina* sp. 1, внешние латеральные зубы. **E.** *Cadlina* sp. 1, внешние латеральные зубы. **F.** *Cadlina* sp. 1, элементы лабиальной кутикулы. **G.** *Cadlina* sp. 2, передняя часть радулы, центральные и внутренние латеральные зубы. **H.** *Cadlina* sp. 2, внешние латеральные зубы. **I.** *Cadlina* sp. 2, элементы лабиальной кутикулы. Масштабные линейки: A = 500 мкм; B = 300 мкм; C = 50 мкм; D, E = 100 мкм; F = 20 мкм; G, H = 30 мкм; I = 10 мкм.

sp. 3 and *Cadlina* sp. 4, so the precise identification of their specific traits in coloration was not possible (see explanation in the Material and Methods section, Fig. 2). However, we suggest that at least *Cadlina* sp. 3 contained both pigment-less and pigmented forms (Fig. 4H, I). In the case of *Cadlina* sp. 7 most specimens lacked pigmented spots on notum, possessed subepidermal yellow glands and in one specimen

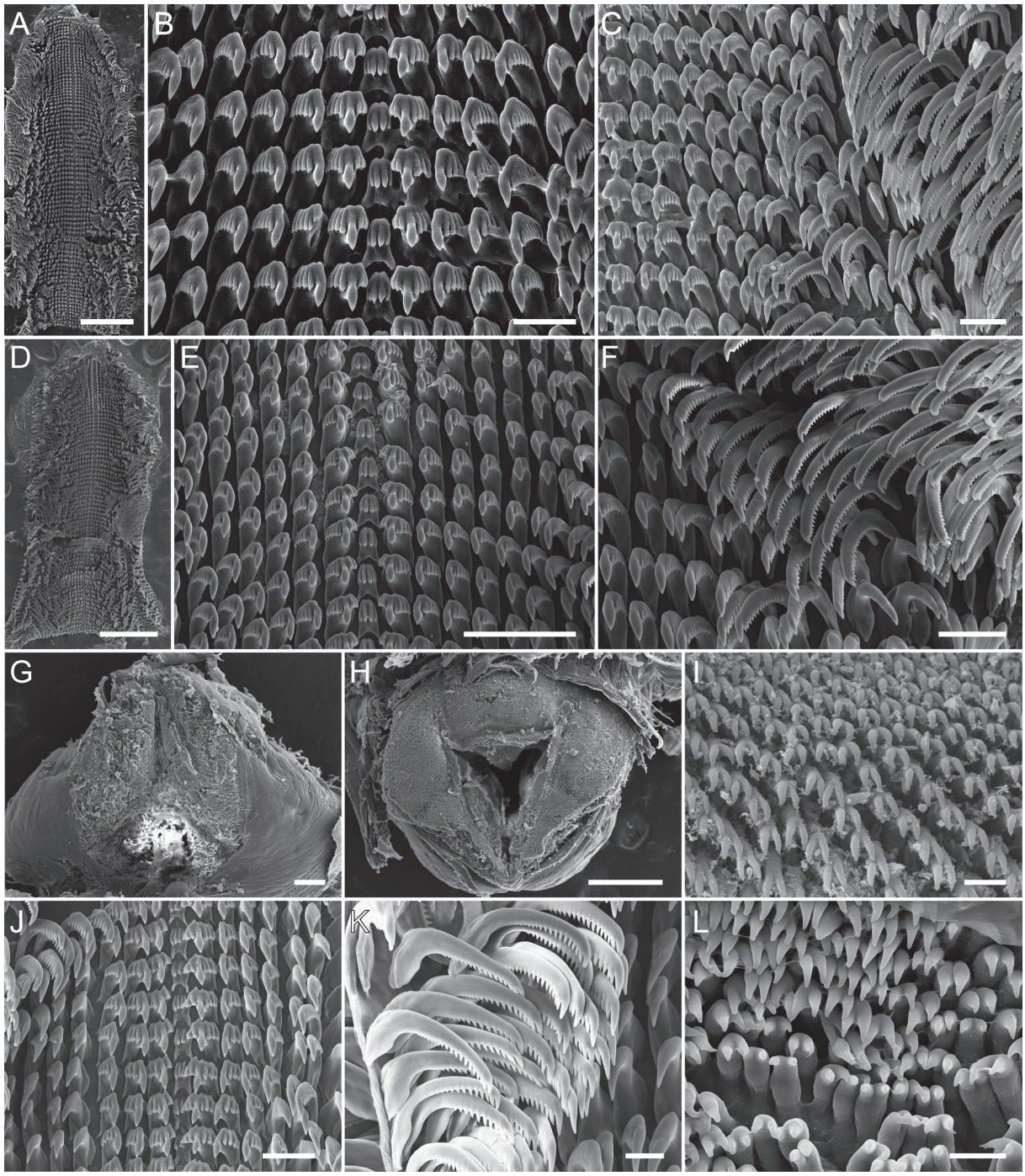

FIG. 7. Buccal armature of *Cadlina* sp. 3 from the Sea of Japan (SEM). **A.** MIMB47975, radula. **B.** MIMB47975, anterior radular portion, rachidian and inner lateral teeth. **C.** MIMB47975, anterior radular portion, outer lateral teeth. **D.** MIMB47976, radula. **E.** MIMB47976, anterior radular portion, rachidian and inner lateral teeth. **F.** MIMB47976, anterior radular portion, outer lateral teeth. **G.** MIMB47976, odontophore with radula. **H.** MIMB47976, labial cuticle. **I.** MIMB47976, labial cuticle rodlets. **J.** MIMB47973, anterior radular portion, rachidian and inner lateral teeth. **K.** MIMB47973, anterior radular portion, outer lateral teeth. **L.** MIMB47973, labial cuticle rodlets. Scale bars: A, D, H = 500 μm; B, C, F, J = 50 μm; E = 100 μm; G = 200 μm; I, L = 10 μm; K = 20 μm.

РИС. 7. Буккальное вооружение *Cadlina* sp. 3 из Японского моря (SEM). **A.** MIMB47975, радула. **B.** MIMB47975, передняя часть радулы, центральные и внутренние латеральные зубы. **C.** MIMB47975, передняя часть радулы, внешние латеральные зубы. **D.** MIMB47976, радула. **E.** MIMB47976, передняя часть радулы, центральные и внутренние латеральные зубы. **F.** MIMB47976, передняя часть радулы, внешние латеральные зубы. **G.** MIMB47976, одонтофор с радулой. **H.** MIMB47976, лабиальная кутикула. **I.** MIMB47976, элементы лабиальной кутикулы. **J.** MIMB47973, передняя часть радулы, центральные и внутренние латеральные зубы. **K.** MIMB47973, передняя часть радулы, внешние латеральные зубы. **L.** MIMB47973, элементы лабиальной кутикулы. Масштабные линейки: A, D, H = 500 мкм; B, C, F, J = 50 мкм; E = 100 мкм; G = 200 мкм; I, L = 10 мкм; K = 20 мкм.

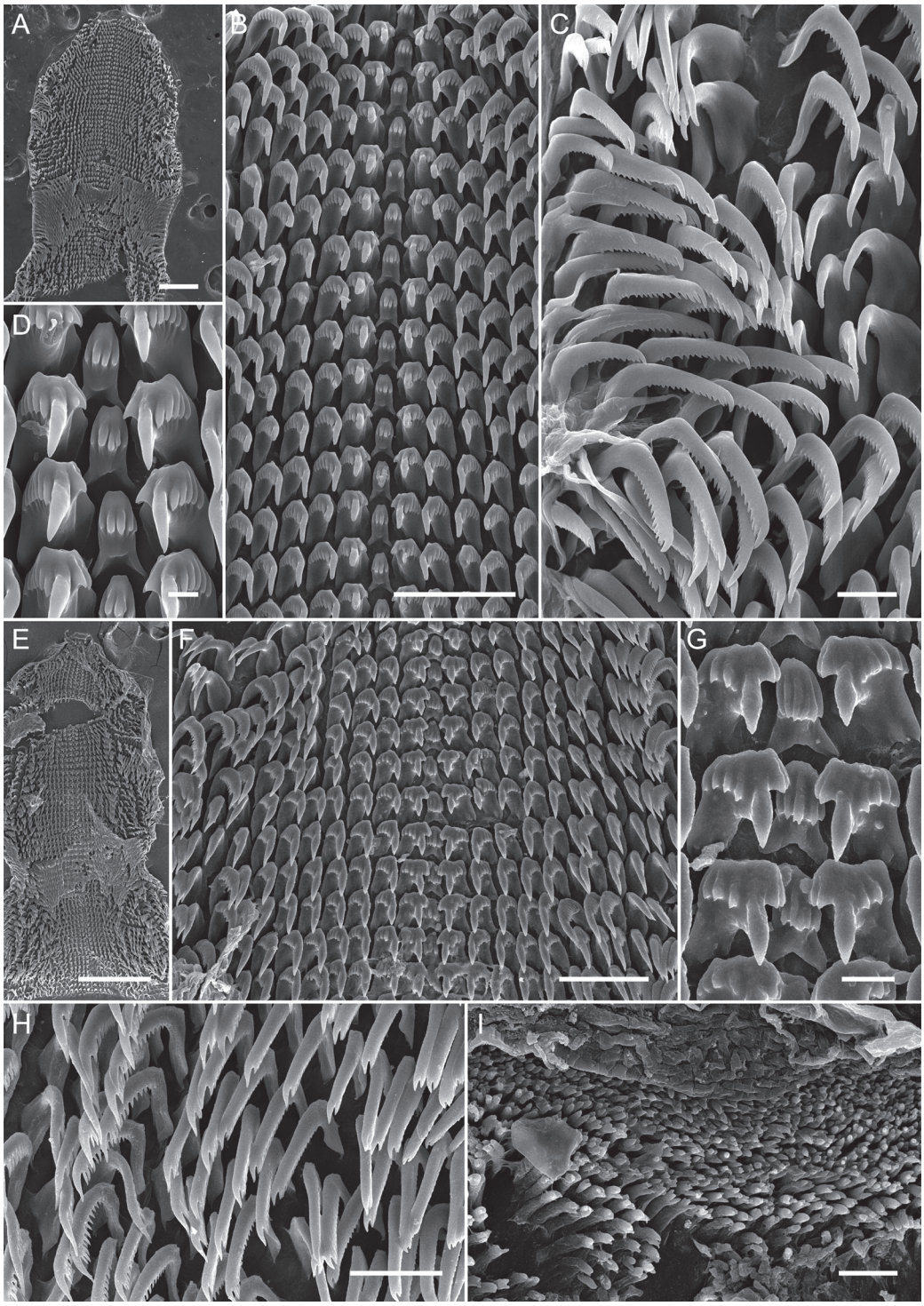

FIG. 8. Buccal armature of *Cadlina* sp. 7 (MIMB47981, Sea of Japan) and *Cadlina* sp. 4 (MIMB47978, Sea of Japan) (SEM). **A.** *Cadlina* sp. 7, radula. **B.** *Cadlina* sp. 7, anterior radular portion, rachidian and inner lateral teeth. **C.** *Cadlina* sp. 7, outer lateral teeth. **D.** *Cadlina* sp. 7, rachidian and innermost lateral teeth. **E.** *Cadlina* sp. 4, radula. **F.** *Cadlina* sp. 4, anterior radular portion, rachidian and inner lateral teeth. **G.** *Cadlina* sp. 4, rachidian and innoermost lateral teeth. **H.** *Cadlina* sp. 4, outer lateral teeth. **I.** *Cadlina* sp. 4, labial cuticle rodlets. Scale bars: A = 300 μm; B, F = 100 μm; C = 30 μm; D = 10 μm; E = 400 μm; G, I = 20 μm; H = 50 μm.

РИС. 8. Буккальное вооружение *Cadlina* sp. 7 (MIMB47981, Японское море) и *Cadlina* sp. 4 (MIMB47978, Японское море) (СЭМ). **A.** *Cadlina* sp. 7, радула. **B.** *Cadlina* sp. 7, передняя часть радулы, центральные и внутренние латеральные зубы. **C.** *Cadlina* sp. 7, наружные латеральные зубы. **D.** *Cadlina* sp. 7, центральные и внутренние латеральные зубы. **E.** *Cadlina* sp. 4, радула. **F.** *Cadlina* sp. 4, передняя часть радулы, центральные и внутренние латеральные зубы. **G.** *Cadlina* sp. 4, передняя часть радулы, центральные и внутренние латеральные зубы. **H.** *Cadlina* sp. 4, внешние латеральные зубы. **I.** *Cadlina* sp. 4, элементы лабиальной кутикулы. Масштабные линейки: A = 300 мкм; B, F = 100 мкм; C = 30 мкм; D = 10 мкм; E = 400 мкм; G, I = 20 мкм; H = 50 мкм.

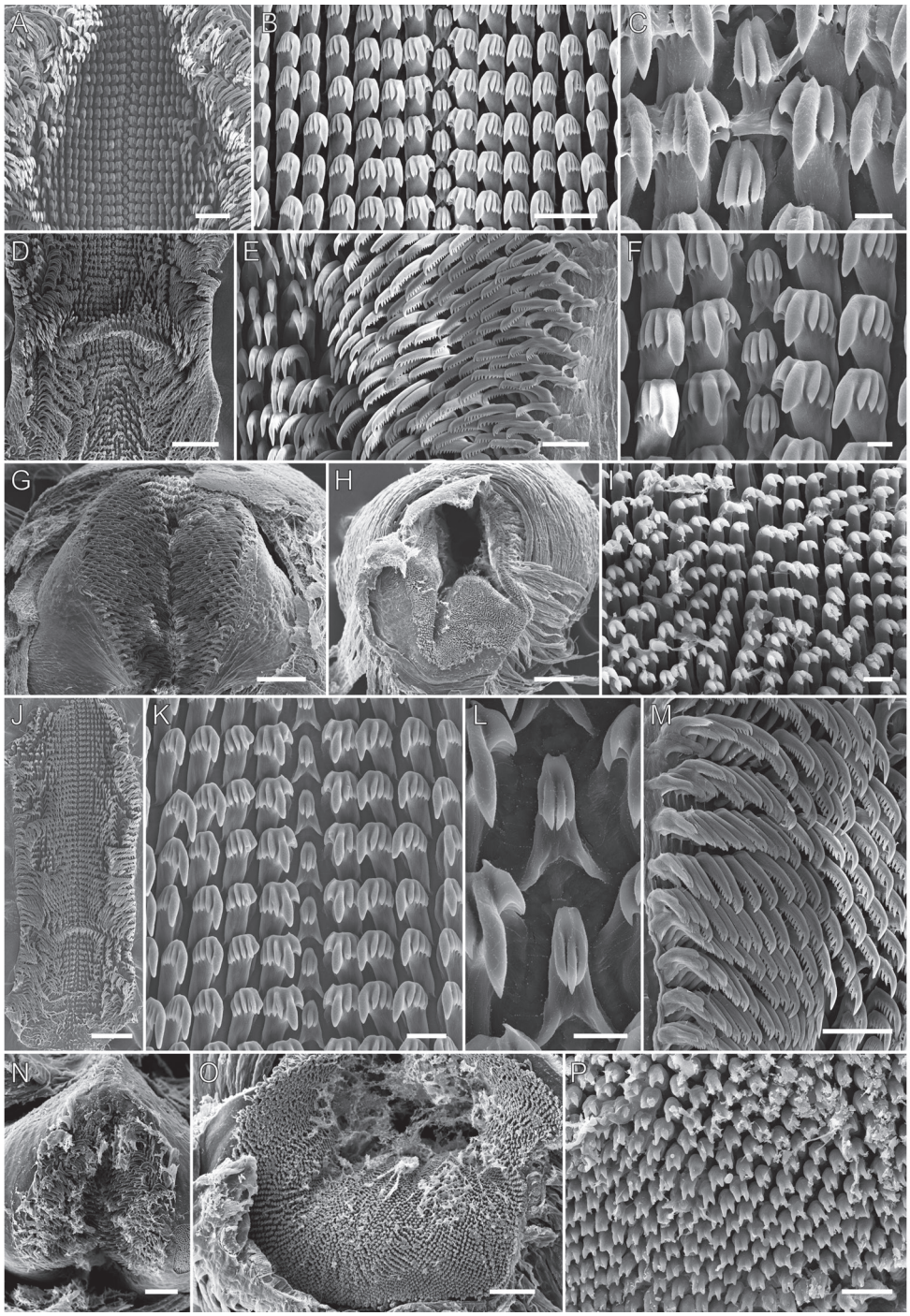

FIG. 9. Buccal armature of *Cadlina* sp. 6 (MIMB47980, Shikotan Is., Sea of Okhotsk) and *Cadlina* sp. 5 (MIMB47979, Iturup Is., Sea of Okhotsk) (SEM). **A.** *Cadlina* sp. 6, anterior radular portion. **B.** *Cadlina* sp. 6, rachidian and inner lateral teeth. **C.** *Cadlina* sp. 6, rachidian and innermost lateral teeth. **D.** *Cadlina* sp. 6, middle radular portion. **E.** *Cadlina* sp. 6, outer lateral teeth. **F.** *Cadlina* sp. 6, rachidian and innermost lateral teeth. **G.** *Cadlina* sp. 6, radula on odontophore. **H.** *Cadlina* sp. 6, labial cuticle. **I.** *Cadlina* sp. 6, labial cuticle elements. **J.** *Cadlina* sp. 5, radula. **K.** *Cadlina* sp. 5, rachidian and inner lateral teeth. **L.** *Cadlina* sp. 5, rachidian teeth. **M.** *Cadlina* sp. 5, outer lateral teeth. **N.** *Cadlina* sp. 5, radula on odontophore. **O.** *Cadlina* sp. 5, labial cuticle. **P.** *Cadlina* sp. 5, labial cuticle rodlets. Scale bars: A, N, O = 100 μm; B, E, M = 50 μm; C, F, I, L = 10 μm; D, G, H, J = 200 μm; K, P = 20 μm.

РИС. 9. Буккальное вооружение *Cadlina* sp. 6 (MIMB47980, о. Шикотан, Охотское море) и *Cadlina* sp. 5 (MIMB47979, о. Итуруп, Охотское море) (СЭМ). **A.** *Cadlina* sp. 6, передняя часть радулы. **B.** *Cadlina* sp. 6, центральные и внутренние латеральные зубы. **C.** *Cadlina* sp. 6, центральные и внутренние латеральные зубы. **D.** *Cadlina* sp. 6, средняя часть радулы. **E.** *Cadlina* sp. 6, внешние латеральные зубы. **F.** *Cadlina* sp. 6, центральные и внутренние латеральные зубы. **G.** *Cadlina* sp. 6, радула на одонтофоре. **H.** *Cadlina* sp. 6, лабиальная кутикула. **I.** *Cadlina* sp. 6, элементы лабиальной кутикулы. **J.** *Cadlina* sp. 5, радула. **K.** *Cadlina* sp. 5, центральные и внутренние латеральные зубы. **L.** *Cadlina* sp. 5, центральные зубы. **M.** *Cadlina* sp. 5, внешние латеральные зубы. **N.** *Cadlina* sp. 5, радула на одонтофоре. **O.** *Cadlina* sp. 5, лабиальная кутикула. **P.** *Cadlina* sp. 5, элементы лабиальной кутикулы. Масштабные линейки: A, N, O = 100 мкм; B, E, M = 50 мкм; C, F, I, L = 10 мкм; D, G, H, J = 200 мкм; K, P = 20 мкм.

there was a faint yellow line along notal margin (Figs 2, 4L). Finally, in studied *C. laevis s.str.* the coloration traits were more or less uniform with no yellow spots on the notum; in several specimens there was a discontinuous yellow marginal line along the notal edge and the color of the subepidermal glands varied from white to yellow (Figs 2, 4A-E).

We identified several differences in internal characters across the putative species within the *C. laevis* species complex, especially in the labial cuticle rodlet morphology, radular morphology and features of the reproductive system (Figs 5–12). Rodlets in the labial cuticle were bifid and unicuspid in *C. laevis s.str.* (Fig. 5G, O) and *Cadlina* sp. 1 (Fig. 6F); bifid and trifid in *Cadlina* sp. 4 (Fig. 8I), and exclusively bifid in other studied representatives of the *C. laevis* species complex (Figs 6I, 7I, L, 9I, P). The odontophore form was similar in all studied species, only outer lateral teeth were visible on working plane (Figs 5E, M, 7G, 9G, N, 10D). The radular formula was similar in most species, however in *C. umiushi* and *Cadlina* sp. 1 the radulae possessed more teeth rows than in other species of the complex (Figs 6A, 10A): 70–100 rows in *C. umiushi,* 86 rows in *Cadlina* sp. 1 and up to 80 rows in other species (Table S3). The rachidian tooth morphology varied greatly across the studied species, in was elongated and narrow with 2–4 denticles (*Cadlina* sp. 5, Fig. 9L), elongated, trapezoidal, with 3–6 denticles (*Cadlina* sp. 7, Fig. 8D), or trapezoidal with 4–6 denticles in other species (Figs 5C, K, 6C, G, 7B, E, J, 8G, 9C, F, 10H). In the latter case, denticles were almost equal in size (*C. laevis, Cadlina* sp. 2, *Cadlina* sp. 3, *Cadlina* sp. 4, *Cadlina* sp. 6), or the inner denticles were larger than outer ones (*Cadlina* sp. 1, Fig. 6C). In *Cadlina* sp. 1 and *Cadlina* sp. 4 denticles sometimes had bifurcations at tips.

Outer lateral teeth were hook-shaped, with numerous denticles, the number of denticles display a slight variation within each specimen in a single transversal row and in different rows (Figs 5D, L, 6D, E, H, 7C, F, K, 8C, H, 9E, M, 10C, I), and therefore this feature cannot be strictly compared across different individuals and species.

In the reproductive system morphology (Fig. 11) we identified several variations in the form of the ampulla, the length of the prostate and the deferent duct, and the relative proportion of the bursa copulatrix and the receptaculum seminis size (see Table S3 for details). Spines on penis were identified in two species, *Cadlina* sp. 3 and *C. umiushi* (Fig. 12A, B) in other species penis was either retracted (Fig. 12C) or hidden in penial sheath.

Discussion

Our analyses have shown that the taxonomy of the *C. laevis* species complex is challenging and the precise identification of morphological synapomorphies of putative species remains elusive. This is due to the low rates of divergence between putative species (Figs 2, 3) and high rate of intraspecific variability in morphological characters (Table S3). The complexity of *C. laevis* diversity has already been shown in a recent comparative study [Korshunova *et al.*, 2020], which concluded that the nominal species *C. laevis* inhabits exclusively boreal Atlantic and subarctic waters (the Barents and the White seas). In the North Pacific, the species *C. olgae* and *C. umiushi* were described from the Sea of Japan [Martynov *et al.*, 2015a, b; Chichvarkhin, 2016]. They were later considered conspecific with *C. umiushi* taking priority, see Korshunova *et al.* [2020]. Furthermore, two additional species were described: *C. kamchatica* from the Pacific coast of Kamchatka and *C. paninae* from the Kurile Islands (the Sea of Okhotsk). At the same time, researchers highlighted the importance of further research on this species complex, since only few and distant geographic areas of the North-West Pacific were sampled [Ekimova *et al.*, 2021]. Our results improve our understanding of the *C. laevis* species complex intra- and interspecific diversity; however it also introduces new uncertainties about the taxonomic status of the recently described species and newly discovered phylogenetic lineages.

Most of species studied herein as well as those described in previous studies – *C. kamchatica, C. umiushi, C. paninae* – have several diagnostic features in their external and internal morphology. For example, three putative species that form a single clade in the phylogenetic tree – *C. paninae, Cadlina* sp. 1, *Cadlina* sp. 2 have a white notum with pale white pigmented dots, more evident in *C. paninae* and *Cadlina* sp. 1 (Figs 2, 4). In all three species the subepidermal yellow glands are hardly visible or completely absent, they also lack pigmentation on the notal edge (Figs 2, 4). At the same time, the radular morphology seems to display several small differences: the rachidian tooth in *Cadlina* sp. 1 is large, trapezoidal, bearing 4–5 large denticles; the inner denticles are larger than the outer ones and sometimes have bifurcations at the tips (Fig. 6A–F). The rachidian teeth in *Cadlina* sp. 2 have a more typical morphology for the *C. laevis* species complex: they are trapezoidal with 5–6 small denticles approximately equal in size (Fig. 6G–I). *Cadlina paninae* has elongated rachidian teeth which are most similar to those of the phylogenetically distant *Cadlina* sp. 5 from the Kuril Islands [Korshunova *et al.*, 2020; Fig. 9G–P].

Cadlina sp. 3 from the Sea of Japan formed a single clade with *C. kamchatica* from the Pacific coast of Kamchatka. *Cadlina kamchatica* differs from all species of the *C. laevis* complex, having an opaque dark yellowish notum and lacking visible subepidermal glands; the marginal notal pigmentation is also

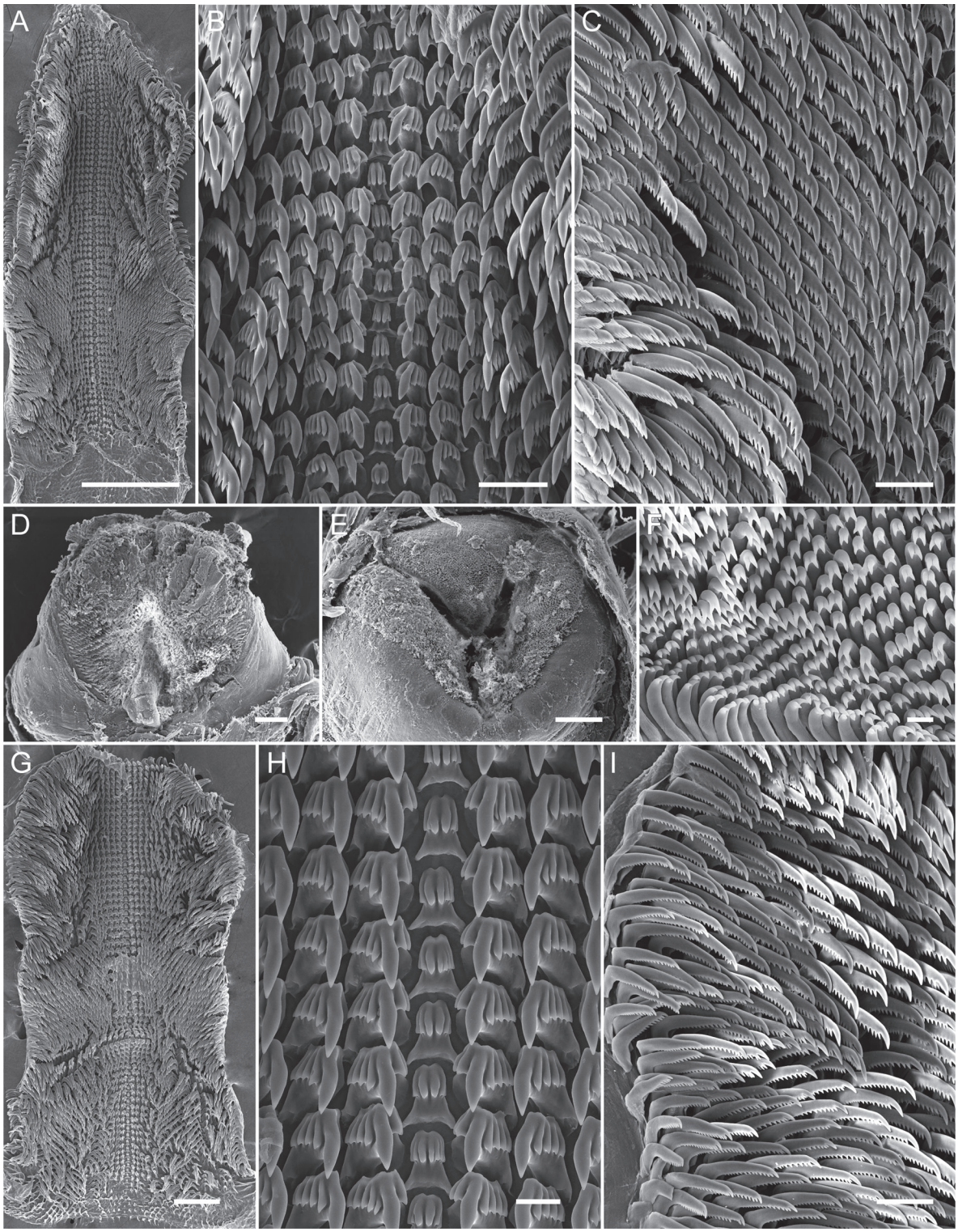

FIG. 10. Buccal armature of *Cadlina umiushi* from the Sea of Japan (SEM). **A.** MIMB47995, radula. **B.** MIMB47995, anterior radular portion, rachidian and inner lateral teeth. **C.** MIMB47995, outer lateral teeth. **D.** MIMB47995, radula on odontophore. **E.** MIMB47995, labial cuticle. **F.** MIMB47995, labial cuticle elements. **G.** MIMB47996, radula. **H.** MIMB47996, rachidian and innermost lateral teeth. **I.** MIMB47996, outer lateral teeth. Scale bars: A = 500 μm; B, C, I = 50 μm; D, E, G = 200 μm; F = 10 μm; H = 20 μm.

РИС. 10. Буккальное вооружение *Cadlina umiushi* из Японского моря (СЭМ). **A.** MIMB47995, радула. **B.** MIMB47995, передняя часть радулы, центральные и внутренние латеральные зубы. **C.** MIMB47995, внешние латеральные зубы. **D.** MIMB47995, радула на одонтофоре. **E.** MIMB47995, лабиальная кутикула. **F.** MIMB47995, элементы лабиальной кутикулы. **G.** MIMB47996, радула. **H.** MIMB47996, центральные и внутренние латеральные зубы. **I.** MIMB47996, внешние латеральные зубы. Масштабные линейки: A = 500 мкм; B, C, I = 50 мкм; D, E, G = 200 мкм; F = 10 мкм; H = 20 мкм.

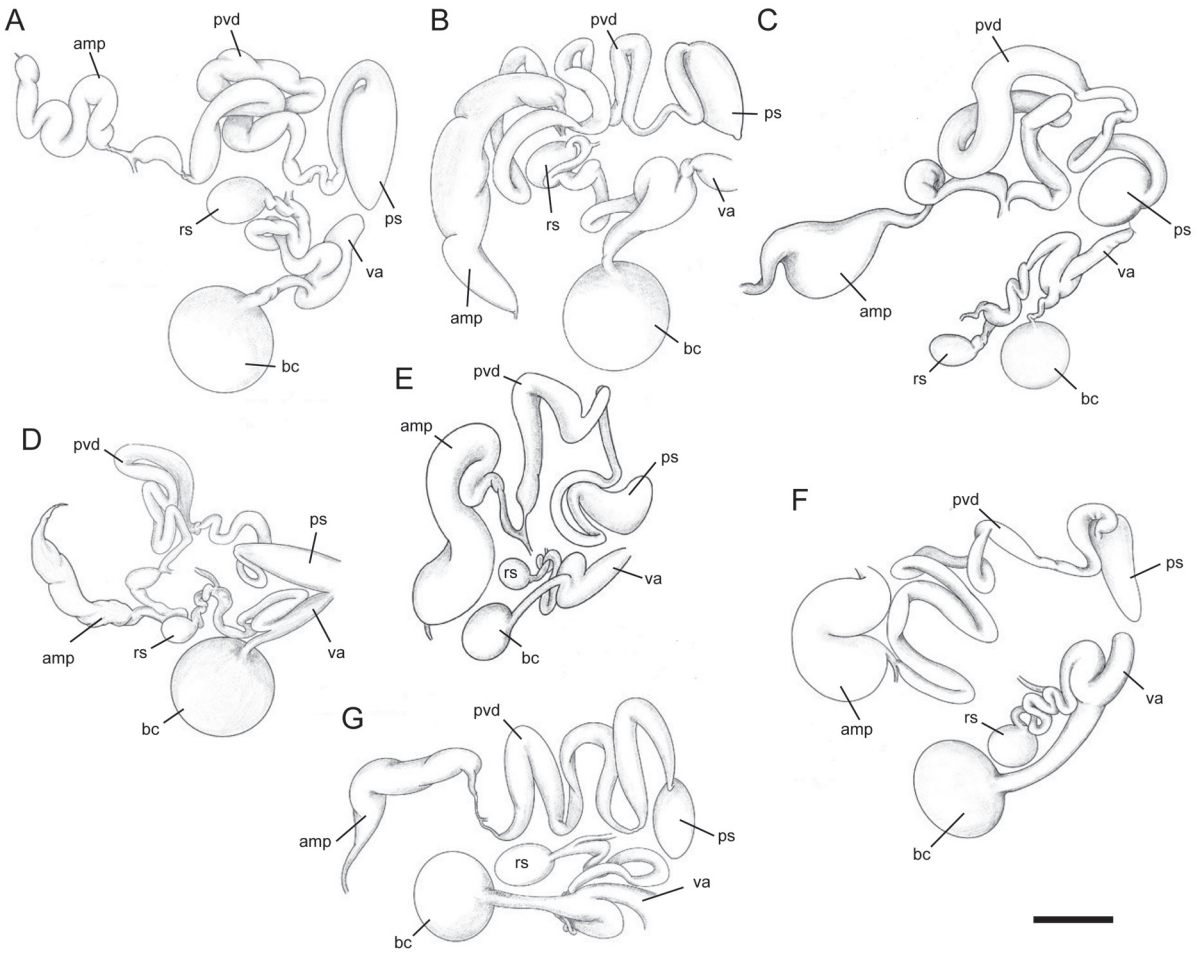

FIG. 11. Configuration of male and female reproductive organs in *Cadlina* spp., female gland mass removed. **A.** *Cadlina laevis,* MIMB47963. **B.** *Cadlina umiushi*, MIMB47996. **C.** *Cadlina* sp. 3, MIMB47975. **D.** *Cadlina* sp. 4, MIMB47978. **E.** *Cadlina* sp. 1, MIMB47971. **F.** *Cadlina* sp. 7, MIMB47981. **G.** *Cadlina* sp. 6, MIMB47980. Abbreviations: amp = ampulla; bc = bursa copulatrix; ps = penial sheath; pvd = prostatic vas deferens; rs = receptaculum seminis; va = vagina. Scale bar: 1 mm.

РИС. 11. Морфология мужских и женских репродуктивных органов *Cadlina* spp., комплекс женских желез удален. **A.** *Cadlina laevis*, MIMB47963. **B.** *Cadlina umiushi*, MIMB47996. **C.** *Cadlina* sp. 3, MIMB47975. **D.** *Cadlina* sp. 4, MIMB47978. **E.** *Cadlina* sp. 1, MIMB47971. **F.** *Cadlina* sp. 7, MIMB47981. **G.** *Cadlina* sp. 6, MIMB47980. Сокращения: amp = ампулла; bc = копулятивная сумка; ps = мешок пениса; pvd = простатический семяпровод; rs = семяприемник; va = вагина. Масштабная линейка: 1 мм.

absent. Unfortunately, the variation in coloration in *Cadlina* sp. 3 from the Sea of Japan cannot be precisely described, since during the collection only a photo of a single specimen per sample was taken. However, all specimens from the stations 63 and 75 (R/V *Akademik Oparin*, 2021) have distinct bright yellow subepidermal glands. Also, it seems that most specimens of *Cadlina* sp. 3 lack pigmentation on the notal edge (Figs 2, 4); this pigmentation is not evident in the preserved material either. Surprisingly, *Cadlina* sp. 4, collected from same stations as some of *Cadlina* sp. 3 forms a separate diverged branch in the tree (Fig. 2) and likely they have similar external appearance. In radular morphology, *Cadlina* sp. 3 and *Cadlina* sp. 4, and also *C. kamchatica*, have similar teeth shapes, but *C. kamchatica* and *Cadlina* sp. 4 sometimes have bifurcations at the tips of the denticles, while in *Cadlina* sp. 3 the rachidian teeth are most similar to the typical *C. laevis* morphology (Figs 7, 8E–I). *Cadlina umiushi* from the Sea of Japan, *Cadlina* sp. 5 and *Cadlina* sp. 6 from the Kuril Islands (the Sea of Okhotsk) have white to yellowish bodies and possess numerous bright yellow spots on the notum (Figs 2, 4). Therefore, although for each clade it is possible to identify several subtle diagnostic characters, the entire group displays a mosaic of morphological traits lacking apparent phylogenetic signal.

Such variability in external and internal morphology was previously shown and discussed for the Atlantic and subarctic species *C. laevis s.str.* [Korshunova *et al.*, 2020]. In addition, the boundaries of the morphological variability in *C. laevis s.str.* in both in external and internal features overlap

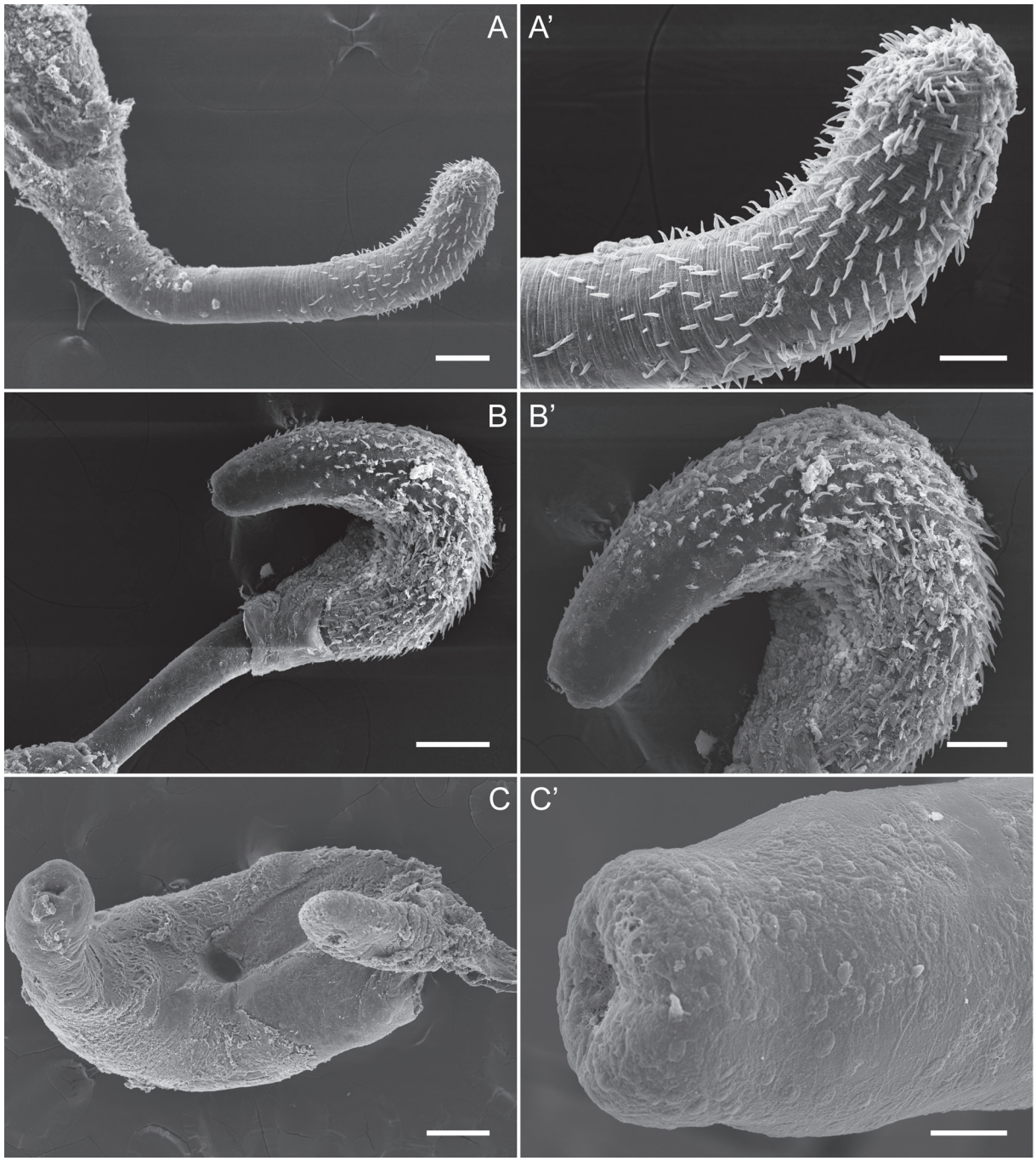

FIG. 12. Penial morphology in *Cadlina* spp. **A.** *Cadlina* sp. 3, MIMB47975. **B.** *Cadlina umiushi,* MIMB47996. **C.** *Cadlina laevis,* MIMB47963. Scale bars: A, C = 100 µm; A', B' = 50 µm; C = 200 µm; C' = 20 µm.

РИС. 12. Морфология пениса *Cadlina* spp. **A.** *Cadlina* sp. 3, MIMB47975. **B.** *Cadlina umiushi,* MIMB47996. **C.** *Cadlina laevis,* MIMB47963. Масштабные линейки: A, C = 100 мкм; A', B' = 50 мкм; C = 200 мкм; C' = 20 мкм.

with morphological traits in *Cadlina* spp. from the northwestern Pacific. Although our material did not contain specimens of *C. laevis s.str.* with yellow spots on the notum (also characteristic of *C. umiushi, Cadlina* sp. 5, *Cadlina* sp. 6), such specimens are known from Norway and the UK, and their species identity is confirmed by molecular data [Korshunova *et al.*, 2020; this study]. The same is true for radular morphological characters: seemingly species-specific characters (shape of teeth, number of denticles on the central tooth, etc.) vary greatly within *C. laevis s.str* (Fig. 5). Also, we did not find any differences in morphological characters between the two divergent subclades of *C. laevis s.str.* [comprised by (1) MIMB47958, MIMB47938, MIMB47948 and (2) other specimens]. Representatives of both subclades show a slight variation in the pigmentation of the notum, with subepidermal glands varying from

white to yellow in color and the presence/absence of a yellow band on the notal edge (Fig. 2). In radular characters they also show different morphology of the rachidian teeth, which may possess 2, 3, or 4–6 denticles (Fig. 5).

Overall, our results suggest that morphological differences found across divergent North Pacific phylogenetic lineages cannot be used by themselves to confirm their status as distinct species new to science (especially considering the fact that some "species" in our material are represented by a single specimen). At the same time, species delimitation analyses failed to provide unambiguous support for the status of these forms as distinct species: the results of the ASAP, bPTP, mPTP and GMYC analyses give different results (Figs 2, S2–S4). *P*-distances between Pacific lineages and Atlantic *C. laevis s.str.* for the COI gene are quite low, ranged from 2.08% (between *C. kamchatica* and *Cadlina* sp. 3) to 7.21% (between *Cadlina* sp. 2 and *Cadlina* sp. 4). These values are less than the p-distances between other species of the genus *Cadlina* (*ca.* 8–16%) (Table S4).

At the present time, it is difficult to evaluate the geographical range limits of each phylogenetic lineage received in this study because our material is limited in geographic scope and we lack samples from transitional areas such as Sakhalin and Hokkaido islands. Nevertheless at least four species – *Cadlina umiushi*, *Cadlina* sp. 3, *Cadlina* sp. 4, *Cadlina* sp. 7 inhabit the Sea of Japan and were found in close proximity or even at a single station (Fig. 1; Table S1). One possible explanation for the genetic divergence across *C. laevis s.l.* lineages may be adaptive radiation, including possible sexual selection or dietary specialization [Ekimova *et al*., 2019]. The first possibility seems to be dubious, as no major differences among studied specimens were found in the reproductive system (Fig. 11). At the same time, the active reproductive period and exact developmental mode are unknown for most species except the North Atlantic *C. laevis s.str.* Another explanation may be allopatric speciation due to historical climatic conditions during the Pleistocene or restricted contemporary gene flow due to habitat specialization or different bathymetry adaptations. The latter explanation is supported by the fact that the North Atlantic *C. laevis s.str.* lacks a free-swimming veliger stage [Thompson, 1967], which likely considerably reduces dispersal capabilities of this species and limits gene flow between populations, as shown for other gastropods lacking free-living larvae [Blakeslee *et al*., 2021]. Since the dietary preferences and developmental mode of most North-West Pacific *Cadlina* are unknown, further studies on this species complex would largely benefit from comparative ecological studies.

In conclusion, the observed genetic and morphological diversity of *C. laevis s.l.* may represent either a complex of at least 11 very closely related and cryptic species, or a single amphiboreal species with geographically restricted, partially isolated populations and blurry boundaries of morphological variability. This suggests an extremely complex evolutionary history of the *Cadlina laevis* species complex, making this group an interesting model system for studying speciation in boreal and Arctic communities. Further studies will require the inclusion of more genes into the dataset, preferably fast-evolving nuclear markers, to test species boundaries and possible geneflow between different mitochondrial lineages.

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Supplementary Material

Table S1. List of specimens used in this study. Voucher numbers, collection locality and collectors are given.

Table S2. List of specimens used for molecular analysis. Voucher numbers, collection locality and GenBank accession numbers are given.

Table S3. Variability of morphological characters within *Cadlina laevis* species complex

Table S4. Intra- and interspecific uncorrected p-distances (%) based on the COI gene

Fig. S1. Bayesian uncollapsed phylogenetic tree based on the COI alignment.

Fig. S2. ASAP results for COI alignments of the *Cadlina laevis* species complex applying Simple distances.

Fig. S3. GMYC results for the *Cadlina laevis* species complex.

Fig. S4. bPTP and mPTP results of the *Cadlina laevis* species complex.