Arthropoda Selecta 33(2): 183–192 © ARTHROPODA SELECTA, 2024

Morphological and genetic identification of an invasive species of the genus *Melita* (Amphipoda: Melitidae) from the Azov–Black Sea basin

Морфологическая и генетическая идентификация инвазивного вида рода *Melita* (Amphipoda: Melitidae) из Азово-Черноморского бассейна

Vladimir A. Grintsov[1], Ludmila V. Bondarenko[1], Vitalii A. Timofeev[1], Ulyana V. Simakova[2]

В.А. Гринцов[1], Л.В. Бондаренко[1], В.А. Тимофеев[1], У.В. Симакова[2]

[1] A.O. Kovalevsky Institute of Biology of the Southern Seas, Russian Academy of Sciences.

[1] Институт биологии южных морей им. А.О. Ковалевского Российской академии наук.

[2] Shirshov Institute of Oceanology, Russian Academy of Sciences.

[2] Институт океанологии им. П.П. Ширшова Российской академии наук.

Vladimir Grintsov vgrintsov@gmail.com ORCID 0000-0002-9003-3054

Ludmila Bondarenko bondarenko.luda@gmail.com ORCID 0000-0003-4755-2593

Vitalii Timofeev Tamplier74@mail.ru ORCID 0000-0002-1112-7141

Ulyana Simakova yankazeisig@gmail.com ORCID 0000-0002-7250-2611

KEY WORDS: Amphipoda, *Melita*, biological invasions, genetic analysis, Azov–Black Sea basin, Kerch Strait.

КЛЮЧЕВЫЕ СЛОВА: амфиподы, *Melita*, биологические инвазии, генетический анализ, Азово-Черноморский бассейн, Керченский пролив.

ABSTRACT. The species of the genus *Melita* that was found in the Kerch Strait is morphologically similar to *M. setiflagella* and *M. nitida*. A comparative analysis of the morphological parameters of 14 body parts with those in the known melitid amphipods *M. setiflagella* and *M. nitida*, and also those in a species identified as *M.* cf. *setiflagella* recorded from the Kerch Strait waters in March 2019, has shown that such character as setation of antennae 2 pair cannot be diagnostic at the species level and is probably a response of individuals to variations in their habitat conditions. According to the results of a genetic analysis, the species *M.* cf. *setiflagella* is actually *M. nitida*.

How to cite this paper: Grintsov V.A., Bondarenko L.V., Timofeev V.A., Simakova U.V. 2024. Morphological and genetic identification of an invasive species of the genus *Melita* (Amphipoda: Melitidae) from the Azov–Black Sea basin // Arthropoda Selecta. Vol.33. No.2. P.183–192. doi: 10.15298/arthsel.33.2.05

РЕЗЮМЕ. Обнаруженный в Керченском проливе вид из рода *Melita* морфологически сходен с *M. setiflagella* и *M. nitida*. Сравнительный анализ морфологических параметров 14 элементов тела у известных ранее мелитид *M. setiflagella* и *M. nitida*, а также обнаруженного в марте 2019 г. в акватории Керченского пролива вида, идентифицированного как *M.* cf. *setiflagella*, показал, что признак опушения второй пары антенн не может быть диагностическим на уровне видов и является, вероятно, реакцией особей на изменения условий среды их обитания. По результатам генетического анализа вид *M.* cf. *setiflagella* соответствует виду *M. nitida*.

Introduction

Species identification is one of the most important aspects in the study of living organisms, the lack of which makes it impossible to address a variety of biological, ecological, ethological and biodiversity issues for the species under study. To identify a species of the genus *Melita* new to the Black Sea [Grintsov *et al*., 2022], mainly morphological characteristics were used in the taxonomic research. According to various publications, from 55 to 80 species from the amphipod genus *Melita* have been recorded from the world's oceans to date [Lowry, 2010; Krapp-Schickel, Sket, 2015; World Register of Marine Species, 2022].

Barnard [Barnard, 1962] distinguished three groups of species of this genus based on the presence or lack of teeth on the pleonal and urosomal segments. Group A included species that lack teeth on the pleonal and urosomal segments. Group B included species with urosomal teeth. Group C included species with teeth on both pleonal segments and urosome. Also, he divided group A into two subgroups: individuals with spines on urosome and those without them. According to this classification, the species *Melita nitida* S.I. Smith in Verrill, 1873 (type locality: New Jersey, USA) and *Melita setiflagella* Yamato, 1988 belong to group A, i.e., with urosomal spines.

In 2015 [Krapp-Schickel, Sket, 2015], a group of *Melita* species was identified on the basis of the lack of teeth on urosome and pleon and the lack of second article on exopodite of uropod 3. The species *M. nitida* and *M. setiflagella* were assigned to this group, and the authors suggested that the resemblance of these species was so great that they could be considered extremely similar.

Another two species considered close to *M. setiflagella* and *M. nitida* are *M. mirzajani* Krapp-Schickel, Sket, 2015 and *M. elongata* Scheridan, 1979. *Melita elongata* is distinguished by a smaller number of dorsolateral spines (two spines) on urosomite 2 relative to those in the species *M. nitida* and *M. setiflagella* that have three or more spines in this part of the segment [Krapp-Schickel, Sket, 2015]. In *M. mirzajani*, the antenna 2 flagellum is significantly less setose than that in *M. nitida* and *M. setiflagella.*

According to Bousfield [1973], the species *M. nitida* is found in the northwestern Atlantic Ocean from the southwestern Gulf of St. Lawrence (Canada) to the Yucatán Peninsula (Mexico). Outside of its natural range [Chapman, 1988], the species *M. nitida* has been recorded from the northeastern Pacific Ocean waters from British Columbia to California. In 1998, the species was found in the Netherlands; in 2010, in Germany; and in 2013, in France [Gouillieux, 2016]. In 2014, the species was discovered in the waters off Gdansk. Potentially, *M. nitida* can occur off the Baltic coast of Kaliningrad Oblast [Burukovsky, Sudnik, 2018]. To date, two species belonging to the genus *Melita* are known from the Black Sea: *M. palmata* [Greze, 1985] and *M. nitida* that was recorded in 2019 from off the Georgia coast [Copilaş-Ciocianu *et al*., 2020].

In September 2019, *Melita* cf. *setiflagella* was found in the waters of the Kerch Strait [Grintsov *et al*., 2022]. It is very similar in morphology to *M. setiflagella* inhabiting river estuaries in Japan [Yamato, 1988]. The issue is even more complicated by the fact that *M. setiflagella* and *M. nitida* are so close morphologically that some authors [Krapp-Schickel, Sket, 2015] consider these species extremely similar, while others treat them as synonyms [Jarrett, Bousfield, 1996, Faasse, van Moorsel, 2003; Reichert, Beermann, 2011]. Clarification of the taxonomic position of the presented species requires further genetic research.

The molecular barcoding method, based on analysis of gene sequences of certain organisms and comparison of the sequences with those available in databases, is currently widely used to address the issue of identification of alien species [Trebitz *et al*., 2017; Darling, Frederick, 2018]. Cytochrome c oxidase subunit I (*COI*), a fragment of the mitochondrial DNA (mtDNA) genome referred to as DNA barcode, is most frequently selected to identify marine invertebrate species [Hebert *et al*., 2003; Ratnasingham, Hebert, 2013]. The ratio of intra- and interspecific variations and the rate of evolution for this gene allow differentiation of even very closely related species in many cases. Since this gene is protein-coding, its variability is still limited, deletions and insertions are rare, and, therefore, this DNA region can relatively easily be amplified [Hebert *et al*., 2003] and aligned. However, there are some limitations as regards the use of this gene for phylogenetic reconstructions or identification purposes, e.g., the presence of nuclear copies of mtDNA [Funk, Omland, 2003]. Of equivalent importance is the information about the group under study available in the reference genetic databases such as GenBank [Sayers *et al*., 2023] and BOLD [Ratnasingham, Hebert, 2007]. Not all entries in the databases and publications are attributed to certain species names (which may be misidentified) and to morphological descriptions. However, analysis of even incomplete information can give a clue to the geographical distribution and diversity of the species under consideration.

In view of the above facts, this study aimed to revise the species status of *M.* cf. *setiflagella* found in the Black Sea by reanalyzing its morphological parameters and by molecular barcoding. The objectives of the study were as follows: obtain sequences of the *COI* gene region, analyze haplotype diversity, and compare them to the previously published sequences. Comparison of the morphological features of the closely related species *M. setiflagella* and *M. nitida* to those of *M.* cf. *setiflagella* from the Black Sea was also among the objectives.

Material and Methods

A comparative analysis of the morphological and genetic parameters of the two closest species, *M. setiflagella* and *M. nitida*, and also those of *M.* cf. *setiflagella* was carried out on the basis of the characters considered in relevant publications [Scheridan, 1979; Yamato, 1988; Kim *et al*., 1992; Jarrett, Bousfield, 1996; Gouillieux *et al*., 2015, 2016; Burukovsky, Sudnik, 2018; Tomikawa *et al*., 2018, 2022; Grintsov *et al*., 2022].

Samples were collected with a hand-held bottom grab sampler from a depth of 0.1–0.2 m in the waters of the Kerch Strait in September 2019 and March 2020. The samples were fragments of "reefs" consisting of tubes built by the polychaete *Ficopomatus enigmaticus* (Fauvel, 1923). Representatives of the species *M.* cf. *setiflagella* were fixed in a 96% ethanol solution. The individuals were identified under an MBS 9 biological light microscope and a Mikmed 5 microscope. Measurements were carried out using an eyepiece micrometer for the MBS 9 microscope. Photographs of the found *M.* cf. *setiflagella* individuals were taken through a Hitachi SU 3500 microscope. A total of more than 100 individuals were analyzed.

DNA was extracted from seven ethanol-fixed specimens by the direct lysis method with the WLB buffer [Williams *et al*., 1992]. The specimens were incubated, first, for 2 h at 56 °C and then for 10 min at 98 °C. Afterwards, the lysate was centrifuged, and the supernatant was transferred to new test tubes to be used for polymerase chain reaction (PCR). For amplification (and subsequent sequencing) of the 5' part of the mitochondrial *COI* gene, a pair of primers commonly applied for crustaceans was selected [Costa *et al*., 2009]: UCOIF (TAWACTTCDGGRTGRCCRAAAAAYCA) and UCOIr (ACWAAYCAYAAAGAYATYGG). The fragments were amplified using the HS-ScreenMix kit (Evrogen) in a volume of 20 μL according to the manufacturer's protocol: 15 s at 95 °C, 30 s at 50 °C, and 45 s at 72 °C, in 35 cycles. Sanger sequencing was performed with the same primers as for the PCR, using the ABI PRISM® BigDye™ Terminator v. 3.1 kit, followed by an analysis of reaction products on an ABI PRISM 3500 automated sequencer.

The resulting chromatograms were processed in the Codon Code Aligner software (Codon Code Corporation, Dedham,

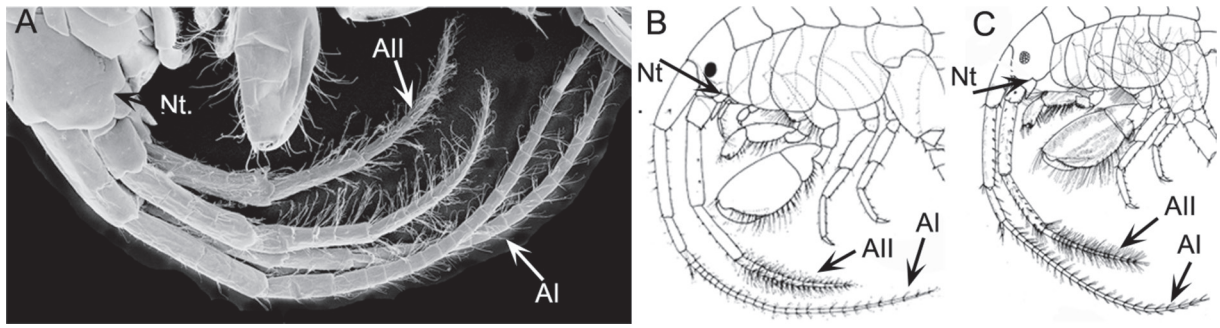

Fig. 1. Head and antennae. A — *M.* cf. *setiflagella*; B — *M. setiflagella* (after Yamato [1988]); C — *M. nitida* (after Jarrett & Bousfield [1996]). Designations: Nt — notch; AI — first pair of antennae; AII — second pair of antennae.

Рис. 1. Внешний вид головы и антенн. A — *M.* cf. *setiflagella*; B — *M. setiflagella* (по Yamato [1988]); C — *M. nitida* (по Jarrett & Bousfield [1996]). Обозначения: Nt — выемки; AI — антенны 1-й пары; AII — антенны 2-й пары.

Massachusetts). All sequences were aligned by the MAFFT algorithm [Katoh, Standley, 2013] with subsequent manual verification. For initial comparison, the GenBank database was used [Sayers *et al*., 2023] with the BLAST algorithm [Camacho *et al*., 2009]. Then the BOLD database was used for a more detailed analysis [Ratnasingham, Hebert, 2007], as it contains larger amounts of data than GenBank. Using the built-in algorithm (identification engine), we identified the clusters (BIN) to which the obtained sequences belonged [Ratnasingham, Hebert, 2013]. All sequences from the same BIN and also all identified as *M. setiflagella* and *M. nitida* sequences were used in the analysis. The resulting alignment included 293 sequences of length 571 bp, while shorter sequences were discarded. The TCS haplotype network [Clement *et al*., 2002] built in the POPART program [Leigh *et al*., 2015] was used for genealogy reconstruction using statistical parsimony since the divergence is low. Based on the obtained alignment and additional data for other *Melita* species in the MEGA X software [Kumar *et al*., 2018], the genetic distances were calculated using the Tajima-Nei model for nucleotide substitutions [Tajima, Nei, 1984] with the gamma parameter = 4. The position in the codon was taken into account. For this, representatives of *M. nitida* were divided into two groups: inhabitants of the Atlantic (AO) and Pacific (PO) coasts of the United States.

Results and discussion

A comparative analysis of morphological characters of three closely related species of the genus *Melita*: *M.* cf. *setiflagella*, *M. setiflagella*, and *M. nitida.*

HEAD

M. cf. *setiflagella*: head lobes evenly convex, rounded, with notches (Fig. 1A, Nt), forming accessory lobes ventrally [Grintsov *et al*., 2022].

M. setiflagella: lateral cephalic lobes subround, with notch (Fig. 1B, Nt), forming accessory lobes ventrally [Yamato, 1988].

M. nitida: head lobe broadly rounded, with squared inferior notch (Fig. 1C, Nt) [Jarrett, Bousfield, 1996].

ANTENNAE 1

M. cf. *setiflagella* (Fig. 1A, AI): depending on individual's size, the number of segments in the accessory flagellum of antenna 1 can range from two complete segments and one rudimentary to three complete and one rudimentary. As individuals grow in size, the number of segments in the accessory flagellum of antenna 1 increases.

M. setiflagella (Fig. 1B, AI): accessory flagellum (with 25–27 articles of main flagellum) consists of three articles [Yamato, 1988].

M. nitida (Fig. 1C, AI): accessory flagellum (with 22 articles of main flagellum) consists of four articles with the terminal article rudimentary [Gouillieux *et al*., 2016; Burukovsky, Sudnik, 2018]. Accessory flagellum (with 16–21 articles of main flagellum) consists of two articles with the terminal article rudimentary [Scheridan, 1979]. This species, recorded from the Bay of Biscay (northeastern Atlantic Ocean), showed a positive correlation between the size of animal and the number of articles in the accessory flagellum of antenna 1 (three to five) [Gouillieux *et al*., 2016].

As the comparison showed, the number of articles in the accessory flagellum varies between the three species within a narrow range. Taking into account the possible increase in the number of articles with the growth of individuals, this difference cannot be considered significant.

ANTENNAE 2

M. cf. *setiflagella* (Fig. 1A, AII): peduncle, peduncular article 5 and flagellar articles with numerous long setae.

M. setiflagella (Fig. 1B, AII): flagellum densely setose [Yamato, 1988; Kim *et al*., 1992].

M. nitida (Fig. 1C, AII): flagellum with many setae [Gouillieux *et al*., 2016; Jarrett, Bousfield, 1996].

Setation of this pair of antennae is indicated as one of the main characters by which Yamato [1988] distinguished the species *M. setiflagella* from *M. nitida.* In *M. nitida*, not only the flagellum but also peduncular article 5 of antenna is setose [Mills, 1964]. However, only the flagellum is setose in *M. setiflagella*. The discovered species *M.* cf. *setiflagella* is closer in setation to *M. nitida.*

Due to the discrepancies in the descriptions of the species *M. nitida* [Kim *et al*., 1992; Jarrett, Bousfield, 1996; Gouillieux *et al*., 2016], setation of flagellum and peduncular articles of antennae 2 cannot be a reliable distinguishing character, especially because the authors above do not mention setation of peduncular article 5.

MANDIBLES

M. cf. *setiflagella*: palp three-articulated (Fig. 2A, Ap); article 3 with long setae at end; articles 2 and 3 with setae ventrally [Grintsov *et al*., 2022].

M. setiflagella: 3rd article of mandible palp (Fig. 2B, Ap) setose only along medial margin, lacking setae along lateral margin [Yamato, 1988].

M. nitida: palp segments (Fig. 2C, Ap) weakly setose [Jarrett, Bousfield, 1996].

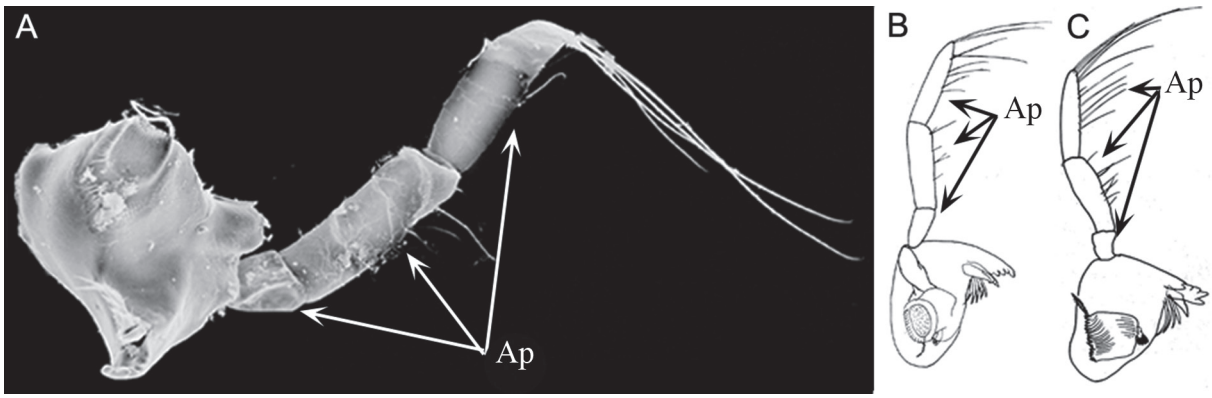

Fig. 2. Mandible and palp. A — *M.* cf. *setiflagella*; B — *M. setiflagella* (after Yamato [1988]); C — *M. nitida* (after Jarrett & Bousfield, [1996]). Designations: Ap — articles of palp.

Рис. 2. Внешний вид мандибул и щупика. A — *M.* cf. *setiflagella*; B — *M. setiflagella* (по Yamato [1988]); C — *M. nitida* (по Jarrett & Bousfield [1996]). Обозначения: Ap — членики щупика.

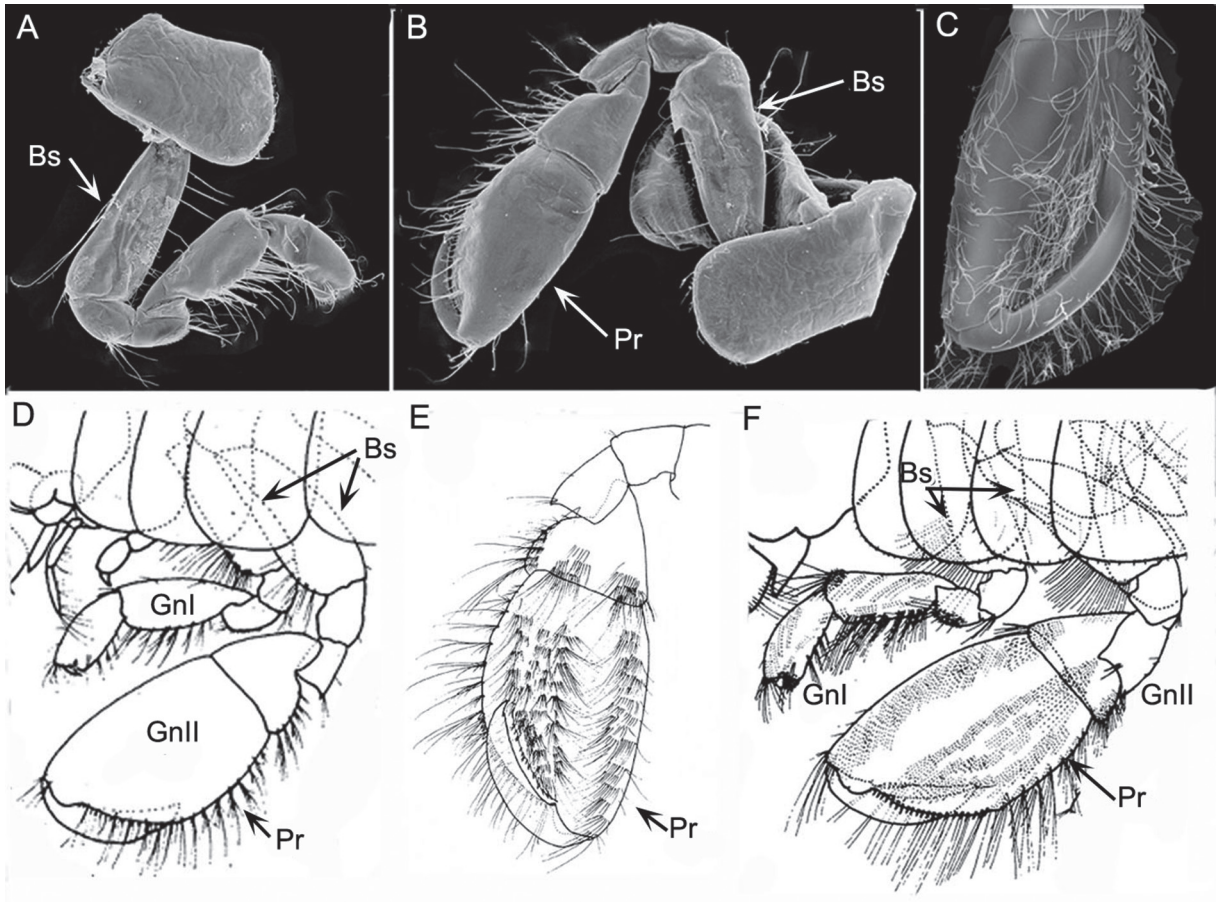

Fig. 3. Gnathopods. A — *M.* cf. *setiflagella*, gnathopod 1; B — *M.* cf. *setiflagella*, gnathopod 2, external view; C — *M.* cf. *setiflagella*, gnathopod 2, propodus, internal view; D — *M. setiflagella* (after Yamato [1988]), gnathopods 1 and 2; E — *M. setiflagella* (after Yamato [1988]), gnathopod 2, propodus, internal view; F — *M. nitida* (after Jarrett & Bousfield [1996]), gnathopods 1 and 2. Designations: Bs — basis; Pr — propodus; GnI — first pair of gnathopods; GnII — second pair of gnathopods.

Рис. 3. Внешний вид гнатопод. A — *M.* cf. *setiflagella*, гнатопод I; B — *M.* cf. *setiflagella*, гнатопод II, вид снаружи; C — *M.* cf. *setiflagella*, гнатопод II, проподус, вид с внутренней стороны; D — *M. setiflagella* (по Yamato [1988]), гнатоподы I и II; E — *M. setiflagella* (по Yamato [1988]), гнатопод II, проподус, вид с внутренней стороны; F — *M. nitida* (по Jarrett & Bousfield [1996]), гнатоподы I и II. Обозначения: Bs — базиподит; Pr — проподус; GnI — 1-я пара гнатопод; GnII — 2-я пара гнатопод.

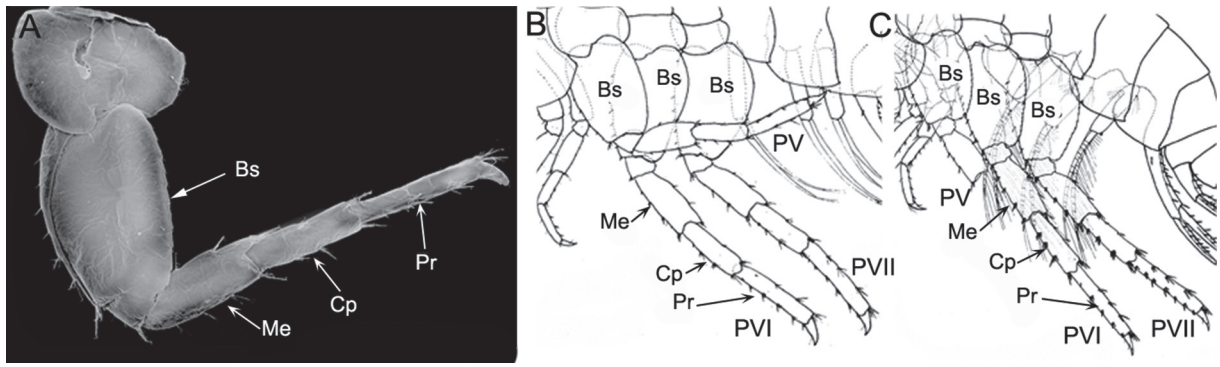

Fig. 4. Pereopods 5–7. A — *M.* cf. *setiflagella*; B — *M. setiflagella* (after Yamato [1988]); C — *M. nitida* (after Jarrett & Bousfield [1996]). Designations: Bs — basis; Me — merus; Cp — carpus; Pr — propodus; PV to PVII — pereopods 5–7.

Рис. 4. Внешний вид переопод V–VII пар. A — *M.* cf. *setiflagella*; B — *M. setiflagella* (по Yamato [1988]); C — *M. nitida* (по Jarrett & Bousfield [1996]). Обозначения: Bs — базиподит; Me — мерус; Cp — карпус; Pr — проподус; PV–PVII — переоподы 5–7-й пар.

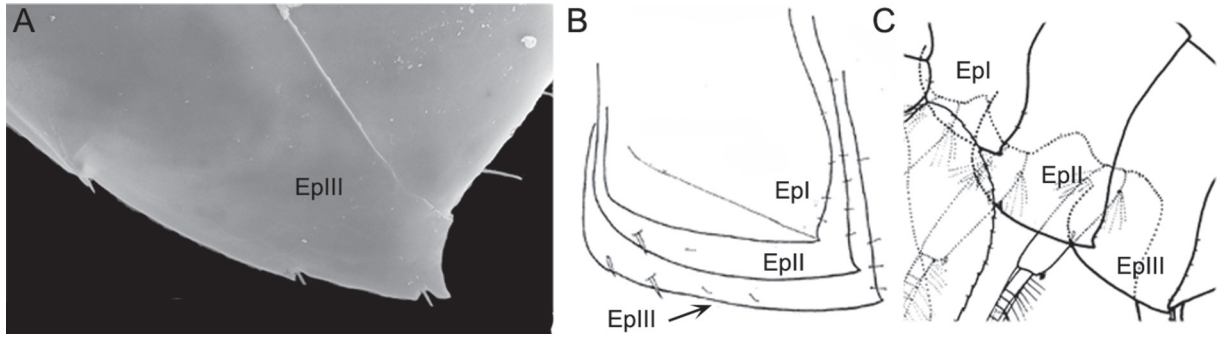

Fig. 5. Epimeral plate 3. A — *M.* cf. *setiflagella*; B — *M. setiflagella* (after Yamato [1988]); C — *M. nitida* (after Jarrett & Bousfield [1996]). Designations: EpI–EpIII — epimeral plates 1–3.

Рис. 5. Внешний вид эпимеральной пластинки III. A — *M.* cf. *setiflagella*; B — *M. setiflagella* (по Yamato [1988]); C — *M. nitida* (по Jarrett & Bousfield [1996]). Обозначения: EpI–EpIII — эпимеральные пластинки 1–3-й пар.

GNATHOPODS 1

Basis. *M.* cf. *setiflagella* (Fig. 3A, Bs): long setae located along posterior margin; setae variable in length anterodistally.

M. setiflagella (Fig. 3D, gn1 Bs): numerous long setae on distal half of anterior margin and some long setae on posterior margin [Yamato, 1988].

M. nitida (Fig. 3F, gn1 Bs): anterodistal margin densely setose [Jarrett, Bousfield, 1996].

GNATHOPODS 2

Basis. *M.* cf. *setiflagella* (Fig. 3B, Bs): long setae located anterodistally along posterior margin; short setae posterodistally.

M. setiflagella (Fig. 3D, E, Bs): many long setae along anterodistal margin and some long setae along posterior margin [Yamato, 1988].

M. nitida (Fig. 3F, Bs): less densely setose anterodistally [Jarrett, Bousfield, 1996].

This description of the cuticular formations on basis does not indicate any clear difference between the species.

Propodus. *M.* cf. *setiflagella* (Fig. 3C, Pr; 3 B): numerous long setae located along inner margin.

M. setiflagella (Fig. 3D Pr; 3E Pr): inner surface covered with numerous long setae [Yamato, 1988].

M. nitida (Fig. 3 F Pr): medial face with strong superior and inferior submarginal setal groups [Jarrett, Bousfield, 1996].

The arrangements and states of cuticular formations on this article are almost identical in all three cases, which does not allow considering these species as distinct.

PEREOPODS 5–7

Basis. *M.* cf. *setiflagella* (Fig. 4A, Bs): in all three pairs of pereopods, bases narrowing distally.

M. setiflagella (Fig. 4B, Bs): basis of pereopods 5–7 narrowing distally [Yamato, 1988].

M. nitida (Fig. 4C, Bs): bases of 6 and 7 narrowing distally [Jarrett, Bousfield, 1996].

The shapes of bases in all three cases are almost identical, which does not allow distinguishing any of the species.

Merus–propodus. *M.* cf. *setiflagella* (Fig. 4 A, Me, Cp, Pr): merus–propodus linear.

M. setiflagella (Fig. 4 B, Me, Cp, Pr): articles 4–6 linear [Yamato, 1988].

M. nitida (Fig. 4 C, Me, Cp, Pr): segment 4 little broadened; segment 6 distinctly longer and more slender than 5 [Jarrett, Bousfield, 1996].

EPIMERAL PLATE 3

M. cf. *setiflagella* (Fig. 5A, EpIII): ventroposteriorly, with tooth.

M. setiflagella (Fig. 5B, EpIII): slightly produced ventroposteriorly, with minute setae [Yamato, 1988].

M. nitida (Fig. 5C, EpIII): slightly acuminate [Jarrett, Bousfield, 1996]. Despite the incomplete information [Jarrett, Bousfield, 1996], the illustrations provided in this publication fully match those in the two previous cases.

Fig. 6. Uropods 1 and 2. A — *M.* cf. *setiflagella*; B — *M. setiflagella* (after Yamato [1988]); C — *M. nitida* (after Jarrett & Bousfield [1996]). Designations: UI and UII — uropods 1 and 2.

Рис. 6. Внешний вид уропод I–II. A — *M.* cf. *setiflagella*; B — *M. setiflagella* (по Yamato, [1988]); C — *M. nitida* (по Jarrett & Bousfield, [1996]). Обозначения: UI–UII — уроподы 1–2-й пар.

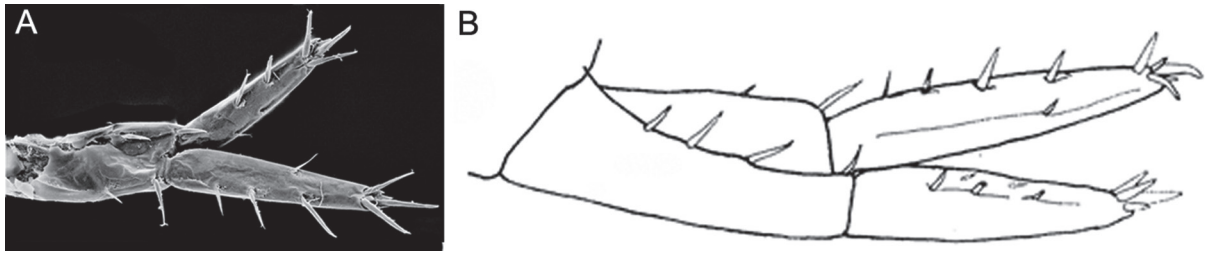

Fig. 7. Uropods 2. A — *M.* cf. *setiflagella*; B — *M. setiflagella* (after Yamato [1988]).

Рис. 7. Внешний вид уропод II. A — *M.* cf. *setiflagella*; B — *M. setiflagella* (по Yamato [1988]).

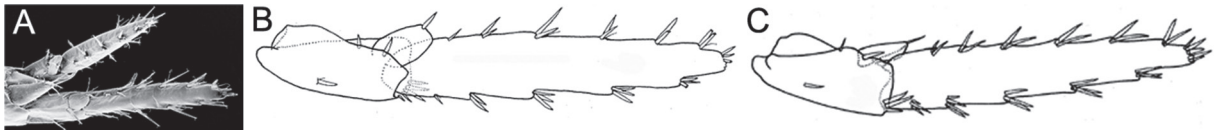

Fig. 8. Uropods 3. A — *M.* cf. *setiflagella*; B — *M. setiflagella* (after Yamato [1988]); C — *M. nitida* (after Jarrett & Bousfield [1996]).

Рис. 8. Внешний вид уроподов III. A — *M.* cf. *setiflagella* (два уропода); B — *M. setiflagella* (по Yamato [1988]); C — *M. nitida* (по Jarrett & Bousfield [1996]).

UROSOME SEGMENT 2

M. cf. *setiflagella*: with two clusters of spines and setae on each side of posterodorsal margin [Grintsov *et al*., 2022].

M. setiflagella: with dorsolateral spines [Yamato, 1988].

M. nitida: with clusters of 3–5 short spines on either side of postero-dorsal margin [Jarrett, Bousfield, 1996]. This species, recorded from the Bay of Biscay (northeastern Atlantic Ocean), showed a positive correlation between the size of animal and the number of spines on urosome 2 (one to five) [Gouillieux *et al*., 2016].

No differences in this character were found that would be sufficient for distinguishing at the species level.

UROPOD 1

M. cf. *setiflagella* (Fig. 6A, UI): spines on peduncle located exterodorsally and interodorsally; largest spines, distally; peduncle equal to inner ramus length, both rami with spines.

M. setiflagella (Fig. 6B, UI): spinose along dorsal margin of peduncle, as well as both rami; peduncle slightly shorter than inner ramus [Yamato, 1988].

M. nitida (Fig. 6C, UI): distal peduncular spine relatively short; rami subequal; peduncle slightly longer than inner ramus [Jarrett, Bousfield, 1996].

UROPOD 2

M. cf. *setiflagella* (Fig. 7A): outer ramus slightly shorter than inner ramus.

M. setiflagella (Fig. 7B): outer ramus slightly shorter than inner ramus [Yamato, 1988].

M. nitida (Fig. 6C, UII): outer ramus distinctly the shorter [Jarrett, Bousfield, 1996].

UROPOD 3

M. cf. *setiflagella* (Fig. 8A): peduncle much shorter than outer ramus; outer ramus with many spines on lateral margins and terminally, uni-articulate; inner ramus small, scale-like, with apical spines.

M. setiflagella (Fig. 8B): peduncle much shorter than outer ramus; inner ramus scale-like, with one apical spine; outer ramus uni-articulate, with groups of spines along lateral margins [Yamato, 1988].

M. nitida (Fig. 8C): outer ramus 2.5 X peduncle, with clusters of medium spines on lateral margins; inner ramus short, scale-like, with one apical spine [Jarrett, Bousfield, 1996].

TELSON.

M. cf. *setiflagella* (Fig. 9A): lobes of telson separated to base, with spines distally and on inner margin.

M. setiflagella (Fig. 9B): incised to base, with groups of subapical spines and spines on inner margin [Yamato, 1988].

M. nitida (Fig. 9C): lobes separated to base, apical spines short, inner margins with short spines [Jarrett, Bousfield, 1996].

As regards the sex-related characters of females, the following data are provided (COXA 6):

M. cf. *setiflagella* (Fig. 10A, CVI): anterior lobe much larger than in male, forming a posteriorly directed curl with notch in the middle of the end; a row of scale-like denticles present.

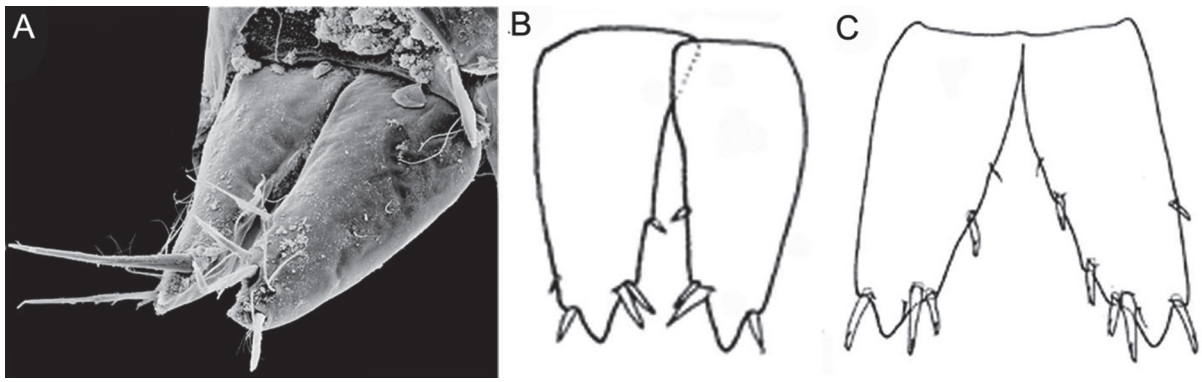

Fig. 9. Telson. A — *M.* cf. *setiflagella*; B — *M. setiflagella* (after Yamato [1988]); C — *M. nitida* (after Jarrett & Bousfield [1996]).
Рис. 9. Внешний вид тельсона. A — *M.* cf. *setiflagella*; B — *M. setiflagella* (по Yamato [1988]); C — *M. nitida* (по Jarrett & Bousfield [1996]).

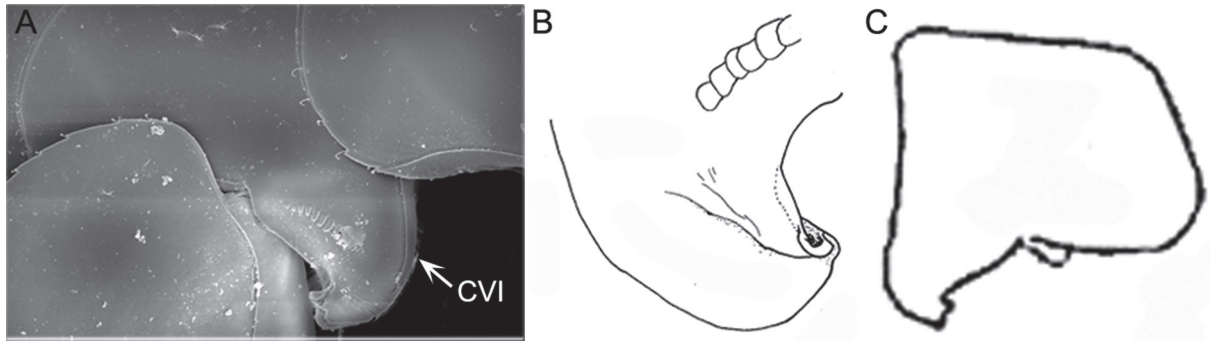

Fig. 10. Coxa 6. A — *M.* cf. *setiflagella*; B — *M. setiflagella* (after Yamato [1988]); C — *M. nitida* (after Jarrett & Bousfield [1996]). Designation: CVI — coxa 6.
Рис. 10. Внешний вид коксальной пластинки VI. A — *M.* cf. *setiflagella*; B — *M. setiflagella* (по Yamato [1988]); C — *M. nitida* (по Jarrett & Bousfield [1996]). Обозначение: CVI — коксальная пластинка VI.

M. setiflagella (Fig. 10B): anterior lobe hooked, round apically, with a row of scale-like denticles [Yamato, 1988].

M. nitida (Fig. 10C): weakly hooked process present, with lower submarginal row of pits (not shown in the figure) [Jarrett, Bousfield, 1996].

The differences between the descriptions of the row of denticles in the case of *M.* cf. *setiflagella* or the row of pits in *M. nitida* are not fundamental.

Thus, after analyzing 14 body parts and their certain details in the above-listed representatives of the genus *Melita*, we did not find any differences at the species level. Only the setation of mandible palp turned out to vary. The main results that we obtained are fully consistent with the statement about the extreme similarity between two species, *M. nitida* and *M. setiflagella* [Krapp-Schickel, Sket, 2015]. No reliable characters were identified to distinguish the above species from each other.

Sequences of the partial *COI* gene with lengths of 639–661 bp were obtained for seven specimens (GenBank accession nos. OR491059–OR491065). Two haplotypes were revealed with a 99.4% identity (four substitutions). One haplotype was found in six out of seven specimens. After a comparison with previously published data, the greatest similarity of the sequences under study was found with representatives of the species *M. nitida* from the Atlantic coast of the United States (98.6–100%). The similarity with *M. nitida* from the U.S. Pacific coast amounted to 79.8–80.0%. The Pacific species *M. choshigawaensis* turned out to be even slightly closer (81.1–81.6% similarity). The similarity with the rest of the representatives of the genus was also lower than 80%.

The estimated intraspecific distances (Table 1) between representatives of the genus *Melita* were mainly within 0.001–0.045. The representatives of *M. dentata*, however, differed to a significantly greater extent (0.26), which indicates a possible hidden diversity. The distances between the groups were by an order of magnitude greater and, in most cases, higher than 0.16. The distances between *M. nitida* AO (Atlantic Ocean) and *M.* cf. *setiflagella* BS (Black Sea) (0.01) and also between *M. nitida* PO (Pacific Ocean) and *M. setiflagella* (0.01) fit within the range of intraspecific variation. Nevertheless, the distances between these groups match interspecific values.

The TCS haplotype network inferred from the alignment of sequences in this study, represented in the BOLD database (designated as *Melita nitida* and *Melita setiflagella*), is shown in Figure 11. All specimens appeared to be divided into two groups: (1) *M. nitida* PO with *M. setiflagella* and also specimens of *Melita* sp. from the Indian Ocean; (2) *M. nitida* AO with *M. nitida* BS. These two groups were separated by 110 nucleotide substitutions. In group 1, the sequences were represented by one, the most common haplotype and several ones that differed from it

Table 1. Estimates of evolutionary distances between all pairs of sequences between the groups (correspond to the species present in the database; of them, only *M. nitida* is divided into two groups: Atlantic (AO) and Pacific Oceans (PO); the species under study, *M*. cf. *setiflagella* Black Sea (BS) and within these groups.

Таблица 1**.** Оценки эволюционных дистанций между всеми парами последовательностей между группами (соответствуют видам, обозначенным в базе данных, только *M. nitida* подразделены на две группы: Атлантический АО и Тихий океаны РО, отдельно представлен объект исследования *M*.cf.*setiflagella* BS) и внутри этих групп.

		1	2	3	4	5	6	7	8	9	10	11	12	13
1	*M*. cf. *setiflagella* BS	**0.003**												
2	*M. nitida* AO	0.01	**0.003**											
3	*M. hergensis*	0.34	0.33	**0.002**										
4	*M. dentata*	0.38	0.38	0.44	**0.260**									
5	*M. shimizui*	0.26	0.26	0.30	0.34	**0.044**								
6	*M. choshigawaensis*	0.22	0.22	0.27	0.37	0.16	**0.001**							
7	*M. palmata*	0.33	0.32	0.30	0.42	0.36	0.29	**0.045**						
8	*M. formosa*	0.43	0.44	0.47	0.34	0.44	0.43	0.47	**0.001**					
9	*M. nitida* PO	0.24	0.25	0.32	0.38	0.23	0.22	0.33	0.42	**0.002**				
10	*M. plumulosa*	0.33	0.32	0.32	0.41	0.28	0.27	0.34	0.39	0.30	**0.006**			
11	*M. matilda*	0.31	0.30	0.34	0.39	0.32	0.31	0.37	0.45	0.32	0.25	**0.004**		
12	*Melita* sp.	0.00	0.01	0.35	0.38	0.26	0.23	0.33	0.44	0.25	0.33	0.31	**n/c**	
13	*M. setiflagella*	0.24	0.25	0.31	0.38	0.22	0.22	0.33	0.41	0.01	0.30	0.32	0.25	**n/c**

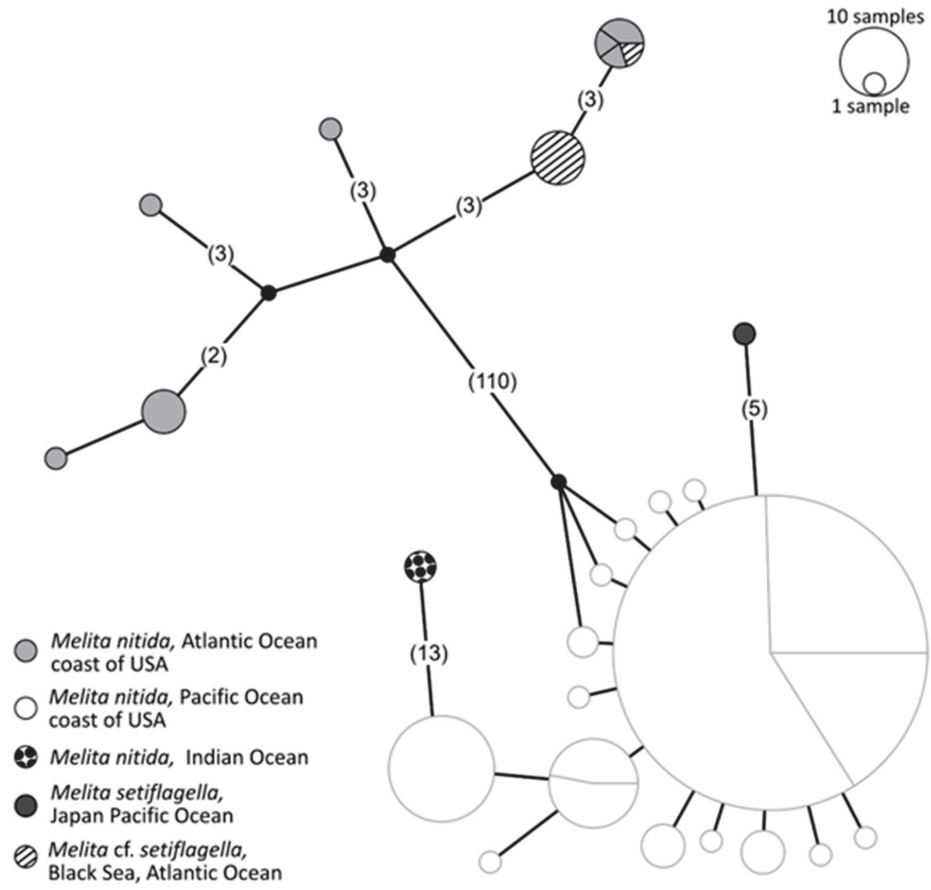

Fig. 11. The TCS network of haplotypes of *M. nitida*, *M. setiflagella*, and *M*. cf. *setiflagella* BS.

Рис. 11. TCS сеть гаплотипов *M. nitida*, *M. setiflagella*, *M*. cf. *setiflagella* ЧМ.

by 1–3 nucleotides. There were a total of 17 haplotypes in this group, of which most were found only once. The specimen belonging to *M. setiflagella* from the western Pacific Ocean differed from the rest of the specimens in this group by five substitutions. The specimens from the Indian Ocean belonging to a single haplotype and differing from the nearest haplotype by 13 nucleotide substitutions also got into this group. Group 2 comprised all specimens collected from the Atlantic coasts of North America and Europe that differed from each other by 1–3 nucleotide substitutions. These were represented by six haplotypes, of which one was found only in the Black Sea (six specimens). The second Black Sea haplotype was also found off the Virginia coast, U.S.A.

Thus, an assumption can be made that at least two species under the name *M. nitida* are represented in the databases of genetic sequences. One of them occurs in the Atlantic Ocean and is invasive off the eastern Atlantic coast and in the Black Sea. The status of the species from the eastern Pacific coast, which has been found to be a close relative to *M. setiflagella*, is unclear since there are no morphological descriptions of the sequenced specimen available. Also, sequences of nuclear genes are needed as secondary genetic markers to clarify the phylogeny and species diversity. The morphological descriptions of *M. nitida*, *M. setiflagella*, and *M.* cf. *setiflagella* has not shown significant differences [Yamato, 1988; Jarrett, Bousfield, 1996; Faasse, van Moorsel, 2003; Reichert, Beermann, 2011; Grintsov *et al*., 2022; Tomikawa *et al*., 2022], which necessitates further research.

Ecological remarks

M. nitida is a polyhaline species. It is found mainly in shellfish farms (cultivating the oyster *Crassostrea gigas* (Thunberg, 1793)), among oyster shells, under small stones, on the underside of boulders, on a silty seafloor, and also among intertidal rocks and algae [Paulmier, 1905; Kunkel, 1918; Watling, Maurer, 1972; Fasse, Van Moorse, 2003; Gouillieux, 2016]. This species occurs in wide ranges of water temperatures (from 0 to 32 °C) and salinities (from 0 to 35‰) [Bousfield, 1973; Sheridan, 1979; Chapman, 1988; Faasse, van Moorsel, 2003; Reichert, Beermann, 2011]. It forms also high abundances in seagrass beds in more saline waters (20–33‰) [Gouillieux, 2016].

In the waters of the Kerch Strait, the salinity at the time of finding of the amphipod *M.* cf. *setiflagella* was 16‰. Sediments were represented by a clay substrate with an ochre-colored silt deposit and fine-grained gravel. The water temperature in the study area varied from 9.8 to 24°C between seasons. The environment-forming component at the site with the recorded high abundance of representatives of *M.* cf. *setiflagella* was colonies of the polychaetes *F. enigmaticus* composed of their tubes. In the Kerch Strait, *M.* cf. *setiflagella* has a limited distribution range, since this species was not found in neighboring waters where the substrate formed by the polychaete *F. enigmaticus* was absent.

The tolerance to a wide range of temperatures, salinities, and the level of anthropogenic pollution makes *M.* cf. *setiflagella* a potential invader in various regions of the world's oceans [Faasse, van Moorse, 2003].

Conclusion

The species from the genus *Melita* that was previously found in the Kerch Strait has been identified as *M.* cf. *setiflagella* [Grintsov *et al*., 2022] on the basis of setation of antenna 2 flagellum, which is more similar to that in *M. setiflagella* than in *M. nitida*. The setation of antennae 2 was the character that Yamato used for distinguishing between these two species [Yamato, 1988]. On the basis of presence of a notch in the lower part of head lobes, as Yamato pointed out, our comparative analysis has not revealed any differences between all three species under study, and this character, therefore, cannot not be considered sufficiently reliable. We did not use other characters for choosing one of these two species (*M. setiflagella* and *M. nitida*) to which the discovered individuals belong due to the very close similarity of their morphologies, as reported earlier [Krapp-Schickel, Sket, 2015]. To further clarify the status of *M.* cf. *setiflagella*, we carried out a genetic analysis. As the results have shown, the partial *COI* gene sequences of *M.* cf. *setiflagella* from the Black Sea are genetically close to the published sequences of *M. nitida* from the Atlantic Ocean. In view of the data obtained through the genetic analysis, the setation of the mandible palp, observed in our morphological analysis, cannot be diagnostic at the species level and is probably a response of individuals to changes in their habitat conditions.

Compliance with ethical standards

CONFLICT OF INTEREST: The authors declare that they have no conflict of interest.

Ethical approval: No ethical issues were raised during our research.

Funding. This work was carried out within the framework of state assignments of the Federal Research Center of the Kovalevsky Institute of Biology of the Southern Seas, Russian Academy of Sciences, nos. No. 1023032700554-2-1.6.16 and No. 1023032000049-6-1.6.21, state assignment to the Institute of Oceanology of the Russian Academy of Sciences, nos. FMWE-2024-0021.

References

Barnard J.L.1962. Benthic marine Amphipoda of Southern California: families Tironidae to Gammaridae // Pacific Naturalist. Vol.3. P.74–115.

Bousfield E.L. 1973. Shallow-water Gammaridean Amphipoda of New England. Ithaca & London: Cornell Univ. Press. 312 p.

Burukovsky R.N., Sudnik S.A. 2018. [Atlas-manual of amphipods (Crustacea, Amphipoda) of the south-eastern Baltic estuaries of the Kaliningrad region: a textbook]. Kaliningrad. 105 p. [In Russian]

Bulycheva A.I. 1957. [Sea fleas of the seas of the USSR and adjacent waters (Amphipoda-Talitroidea)]. Moscow, Leningrad: AN SSSR Publ. 185 p. [In Russian]

Camacho C. *et al*. 2009. BLAST+: architecture and applications // BMC bioinformatics. Vol.10. P.1–9.

Chapman J.W. 1988. Invasions of the northeast Pacific by Asian and Atlantic Gammaridean Amphipod Crustaceans, including a new species of *Corophium* // J. Crust. Biol. Vol.8. P.364–382.

Clement M., Snell Q., Walker P., *et al*. 2002. TCS: estimating gene genealogies // Parallel and Distributed Processing Symposium, International. IEEE Computer Society. Vol.3. P.184.

Copilaş-Ciocianu D., Berchi G., Mumladze L. 2020. First survey of shallow-water Amphipoda along the Georgian Black Sea coast reveals new faunistic records and the unexpected Atlantic invader *Melita nitida* // Mediterranean Marine Science. Vol.21. No.2. P.460–463. https:// doi.org/10.12681/mms.22844.

Costa F.O., Henzler C.M., Lunt D.H., Whiteley N.M., Rock J. 2009. Probing marine *Gammarus* (Amphipoda) taxonomy with DNA barcodes // Systematics and Biodiversity. Vol.7. No.4. P.365–379.

Darling J.A., Frederick R.M. 2018. Nucleic acids-based tools for ballast water surveillance, monitoring, and research // Journal of sea research. Vol.133. P.43–52.

Faasse M., Moorse G., van. 2003. The North-American amphipods, *Melita nitida* Smith, 1873 and *Incisocalliope aestuarius* (Watling and Maurer, 1973) (Crustacea: Amphipoda: Gammaridea), introduced to the Western Scheldt estuary (The Netherlands) // Aquatic Ecology. Vol.37. P.13–22.

Funk D.J., Omland K.E. 2003. Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA // Annual Review of Ecology, Evolution, and Systematics. Vol.34. No.1. P.397–423.

Gouillieux B., Lavesque L., Leclerc J.C., Le Garrec V., Viard F., Bachelet G. 2015. Three non-indigenous species of *Aoroides* (Crustacea: Amphipoda: Aoridae) from the French Atlantic coast // Journal of the Marine Biological Association of the United Kingdom. Vol.96. Is.8. P.1651–1659 http://dx.doi.org/10.1017/S0025315415002027

Gouillieux B., Lavesque N., Blanchet H., Bachelet G. 2016. First record of the non-indigenous *Melita nitida* Smith, 1873 (Crustacea: Amphipoda: Melitidae) in the Bay of Biscay (NE Atlantic) // Bioinvasions Records. Vol.5. Is.2. P.85–92. http://dx.doi.org/10.3391/bir.2016.5.2.05

Greze I.I. 1985. [Higher crustaceans. Amphipods] // Fauna Ukrayiny. Kyiv: Naukova dumka. Vol.26. Vip.5. 172 p. [In Russian]

Grintsov V.A., Bondarenko L.V., Timofeev V.A. 2022. [A new species of the Amphipoda *Melita* leach, 1814 (Crustacea: Amphipoda: Melitidae) for the Azov-Black sea basin] // Rossiiskii zhurnal biologicheskikh invazii. Vol.5. No.1. P.41–54. [in Russian]. https:// doi.org/10.35885/1996-1499-15-1-41-54

Hebert P.D.N., Ratnasingham S., De Waard J.R. 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species // Proceedings of the Royal Society of London. Series B: Biological Sciences. Vol.270. No.suppl.1. P.S96–S99.

Jarrett N.E., Bousfield E.L. 1996. The amphipod superfamily Hazdioidea on the Pacific Coast of North America: Family Melitidae. The *Melita* group: systematics and distributional ecology // Amphipacifica. Vol.2. Part 1. P.3–74.

Katoh K., Standley D.M. 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability // Molecular biology and evolution. Vol.30. No.4. P.772–780.

Kim Chang Bae, Kim Won, Kim Hoon Soo. 1992. Three Species of the Genus *Melita* from Korea (Crustacea, Amphipoda, Melitidae) // The Korean Journal of Systematic Zoology. Special Issue. No.3. P.113–120.

Krapp-Schickel T., Sket B. 2015. *Melita mirzajanii* n. sp. (Crustacea: Amphipoda: Melitidae), a puzzling new member of the Caspian fauna // Zootaxa. Vol.3948. P.248–262. https://doi.org/10.11646/zootaxa.3948.2.6

Kunkel BW. 1918. The Arthrostraca of Connecticut // State of Connecticut, State Geological and Natural History Survey Bull. No.26. P.1–261

Leigh J.W., Bryant D. 2015. Popart: full-feature software for haplotype network construction // Methods in Ecology and Evolution. Vol.6. No.9. P.1110–1116.

Lowry J. 2010. *Melita* // Horton T., Lowry J., De Broyer C., Bellan-Santini D., Coleman C.O., Daneliya M., Dauvin J.-C., Fišer C., Gasca R., Grabowski M., Guerra-García J.M., Hendrycks E., Holsinger J., Hughes L., Jazdzewski K., Just J., Kamaltynov R.M., Kim Y.-H., King R., Krapp-Schickel T., Le Croy S., Lörz A.-N., Senna A.R., Serejo C., Sket B., Tandberg A.H., Thomas J., Thurston M., Vader W., Väinölä R., Vonk R., White K., Zeidler W. World Amphipoda Database. Accessed through: World Register of Marine Species at http://www.marinespecies.org/aphia.php?p=taxdetails&id=101679 on 2016-03-08

Mills E.L. 1964. Noteworthy Amphipoda (Crustacea) in the collection of the Yale Peabody Museum // Postilla. No.79. 41 p.

Paulmier F.C. 1905. Higher Crustacea of New York City // Bulletin of the New York State Museum. Vol.91. Zoology 12. P.117–189. http://dx.doi.org/10.5962/bhl.title.54915

Ratnasingham S., Hebert P.D.N. 2013. A DNA-based registry for all animal species: the Barcode Index Number (BIN) system // PloS one. Vol.8. No.7. Art.e66213.

Reichert K., Beermann J. 2011. First record of the Atlantic gammaridean amphipod *Melita nitida* Smith, 1873 (Crustacea) from German waters (Kiel Canal) // Aquatic Invasions. Vol.6. Is.1. P.103–108. doi: 10.3391/ai.2011.6.1.13

Sayers E.W., Kavanaugh M., Clark K., Pruitt K.D., Sherry S.T., Vankie L., Karsch-Mizrachi I. 2023. GenBank 2023 update // Nucleic acids research. Vol.51. No.D1. P.D141–D144.

Sheridan P.F. 1979. Three new special [sic] of Melita (Crustacea: Amphipoda), with notes on the amphipod fauna of the Apalachicola Estuary of northwest Florida. // Northeast Gulf Science. Vol.3. No.2. P.60–73.

Tomikawa K., Hirashima K., Hirai A., Uchiyama R. 2018. A new species of *Melita* from Japan (Crustacea, Amphipoda, Melitidae) // ZooKeys. Vol.760. P.73–88. doi: 10.3897/zookeys.760.24778 http://zookeys.pensoft.net

Tomikawa K., Sasaki T., Aoyagi M., Nakano T. 2022. Taxonomy and phylogeny of the genus *Melita* (Crustacea: Amphipoda: Melitidae) from the West Pacific Islands, with descriptions of four new species // Zoologischer Anzeiger. Vol.296. P.141–160.

Trebitz A.S., Hoffman J.C., Darling J.A., Pilgrim E.M., Kelly J.R., Brown E.A., Chadderton W.L., Egan S.P., Grey E.K., Hashsham S.A., Klymus K.E., Mahon A.R., Ram J.L., Schultz M.T., Stepien C.A., Schardt J.C. 2017. Early detection monitoring for aquatic non-indigenous species: Optimizing surveillance, incorporating advanced technologies, and identifying research needs // Journal of Environmental Management. Vol.202. P.299–310.

Watling L., Maurer D. 1972. Marine shallow water amphipods of the Delaware Bay area, U.S.A. // Crustacea. Suppl. No.3. P.251–266.

Williams B.D., Schrank B., Huynh C., Shownkeen R., Waterston R.H. 1992. A genetic mapping system in *Caenorhabditis elegans* based on polymorphic sequence-tagged sites // Genetics. Vol.131. No.3. P.609–624.

World Register of Marine Species at: https://www.marinespecies.org/aphia.php?p=taxdetails&id=101679 on 2022-03-18

Yamato S. 1988. Two species of the genus *Melita* (Crustacea: Amphipoda) from Brackish Waters in Japan // Publ. Seto Mar. Biol. Lab. Vol.33. No.1/3. P.79–95. http://hdl.handle.net/2433/176148

Responsible editor K.G. Mikhailov