ZOOSYSTEMATICA ROSSICA

ISSN 2410-0226 [online] 0320-9180 [print]

Zoological Institute, Russian Academy of Sciences, St Petersburg ▪ https://www.zin.ru/journals/zsr/

Vol. 34(1): 18–27 ▪ Published online 22 January 2025 ▪ DOI 10.31610/zsr/2025.34.1.18

RESEARCH ARTICLE

The Black Sea *Pleurobrachia* (Ctenophora): *P. rhodopis* or *P. pileus*?

К какому виду относится черноморская плевробрахия (Ctenophora), *Pleurobrachia rhodopis* или *P. pileus*?

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Abstract. *Pleurobrachia* Fleming, 1822 is the native taxon of ctenophores in the Black Sea. Until now, its species affiliation has not been resolved: the Black Sea *Pleurobrachia* is referred to either *P. pileus* (O.F. Müller, 1776) or *P. rhodopis* (Chun, 1879). In the taxonomic reviews, both species are recognised as occurring in the Black Sea. The experts on zooplankton identified the Black Sea *Pleurobrachia* based solely on its definitive body size, but size cannot be a sufficient diagnostic feature. To clarify the species identity of the Black Sea *Pleurobrachia*, we carried out a phylogenetic analysis using the nucleotide sequences of the mitochondrial COI and the nuclear 18S rRNA gene fragments. As a result, we have established that the Black Sea *Pleurobrachia* is *P. pileus* and its identification as *P. rhodopis* in the global taxonomic databases is erroneous.

Резюме. Плевробрахия (*Pleurobrachia* Fleming, 1822) – аборигенный таксон гребневиков в Чёрном море. До сих пор не выяснена её точная видовая принадлежность: черноморскую плевробрахию относят либо к виду *P. pileus* (O.F. Müller, 1776), либо к *P. rhodopis* (Chun, 1879). В таксономических сводках оба вида указаны для Чёрного моря. Зоопланктонологи при определении вида черноморских плевробрахий ограничивались лишь дефинитивными размерами тела, которые нельзя считать достаточно информативными диагностическими признаками. Для уточнения видовой принадлежности черноморских плевробрахий мы провели филогенетический анализ с использованием фрагментов нуклеотидных последовательностей митохондриального гена COI и ядерного гена 18S рРНК. Результаты исследования подтвердили, что черноморская плевробрахия относится к виду *P. pileus*. Указание черноморской плевробрахии в мировых таксономических базах как *P. rhodopis* является ошибочным.

Key words: phylogeny, taxonomy, Black Sea, distribution, Ctenophora, Tentaculata, Cydippida, Cydippidae, *Pleurobrachia pileus*, *Pleurobrachia rhodopis*

Ключевые слова: филогения, систематика, Черное море, распространение, Ctenophora, Tentaculata, Cydippida, Cydippidae, *Pleurobrachia pileus*, *Pleurobrachia rhodopis*

ZooBank Article LSID: 6BA91123-A960-453B-9936-26D28EB9BC69

Introduction

The phylum Ctenophora comprises nearly 200 species of gelatinous marine organisms found throughout the World's oceans (Mills, 1998). The branching patterns of the gastrovascular system are traditionally used in the morphology-based taxonomy of ctenophores to distinguish orders and families. Lower-level systematics of Ctenophora is heavily based on organ size and position, includ-

ing the relative size of comb rows, the location of tentacle roots, body shape, and the presence or absence of tentilla on the tentacle (Harbison, 1985; Licandro & Lindsay, 2017). However, morphological identification of ctenophores is challenging due to damaged specimens, poor preservation under fixation, and confusing morphology (Haddock, 2004; Mills & Dubois, 2023).

Pleurobrachia Fleming, 1822 is a ctenophore taxon that is native to the Black Sea. Initially, experts on the Black Sea zooplankton attributed it to *Pleurobrachia pileus* (O.F. Müller, 1776), a small ctenophore species found in various ocean regions (Klyucharev, 1952; Dimov, 1960; Lazareva, 1961). However, after the appearance of two publications by Naumov (1968a*, 1968b), there was a confusion in species identification of the Black Sea *Pleurobrachia*. Naumov (1968a, 1968b) classified the Black Sea *Pleurobrachia* as *P. rhodopis* (Chun, 1879) and mentioned *P. pileus* as its junior synonym (Naumov, 1968a). According to Zaika (2012), an error in estimating the size of *Pleurobrachia* from the Black Sea led to the fact that Naumov classified it as *P. rhodopis*. As a result, some authors mentioned the Black Sea *Pleurobrachia* as *P. rhodopis* (Kovalev et al., 2001; Kideys & De Maddalena, 2004), while other researchers continued to mention the Black Sea species as *P. pileus* (Kideys et al., 2000; Anninsky et al., 2022; Shiganova, 2023). Zaika (2012) concluded that the Black Sea *Pleurobrachia* is *P. pileus* based on a comparison of the maximum definitive length of *P. pileus* (30 mm) and *P. rhodopis* (10 mm). However, the size of an animal can vary between the different parts of its distribution range, making it an unreliable characteristic for taxonomic identification. Therefore, molecular genetic tests would be useful for resolving this issue (Zaika, 2012).

Genomic approaches are increasingly used in studying the diversity of ctenophores, for their identification and reconstruction of phylogenetic relationships. The data on the nuclear 18S ribosomal gene have provided a phylogenetic framework for reconstructing the relationships among ctenophores in general (Podar et al., 2001; Simion et al., 2015). The mitochondrial cytochrome-c oxidase subunit-I (COI) gene fragment (so-called DNA barcode) is a commonly used molecular marker for species identification. Recently, Christianson et al. (2022) designed new primers and amplified the COI fragment from members of all major groups of ctenophores, providing species-level resolution for taxonomic assignments of ctenophores.

Nowadays, the authoritative classification and catalogue of marine animals World Register of Marine Species (WoRMS) indicates *P. rhodopis* for the Black Sea, while *P. pileus* is not listed from the Black Sea (WoRMS..., 2025). In a global open-access data and information clearing-house on marine biodiversity for science (Ocean Biodiversity Information System, OBIS), both species (*P. rhodopis* and *P. pileus*) are recognised as occurring in the Black Sea (OBIS..., 2024).

In this study, we used morphological analysis and phylogenetic reconstruction based on fragments of the mitochondrial COI and the nuclear 18S rRNA genes to clarify the species of *Pleurobrachia* inhabiting the Black Sea.

Material and methods

Sampling. The specimens of the Black Sea *Pleurobrachia* were caught using the Bogorov–Rass plankton net in the coastal waters of Sevastopol during the 122nd scientific cruise of the R/V "Professor Vodyanitsky" (7 June – 2 July, 2022) in two localities, 44°13′01″N 33°41′27″E and 44°24′69″N 34°24′45″E.

Morphological methods. Alive animals were placed in aquariums with seawater located in the laboratory on the R/V board. The morphological analysis of live Black Sea *Pleurobrachia* specimens was carried out in a Petri dish using a microscope at a minimum magnification (8×)

* *Note by the editor (A. Przhiboro).* The chapter "Phylum Ctenophora" in volume 1 of "A key to the fauna of the Black Sea and the Sea of Azov" (1968) is cited here as "Naumov, 1968a", although the authorship of the chapter was not indicated in the book (apparently, by a mistake). D.V. Naumov included this chapter in the unpublished list of his publications (typescript) kept in the personal record of D.V. Naumov at the Scientific Archive of the Zoological Institute of the Russian Academy of Sciences, St Petersburg (personal record No. 95, fund 1, inventory 3, storage unit 217: p. 132). The copy of volume 1 of "A key..." from the Library of the Zoological Institute of the Russian Academy of Sciences bears a glued-on typescript "D.V. Naumov" indicating his authorship of the chapter, which looks like a hand-made correction of the omitted authorship.

with an attached video camera (Zeiss Stemi 305 LAB). Measurements of the body sizes (in oral-aboral axis), relative length of ctene rows, and coloration assessments were conducted on 25 live specimens.

DNA extraction, amplification and sequencing. For phylogenetic analysis, whole specimens were frozen in 95% ethanol and stored at -20 °C.

DNA was extracted from two specimens of *Pleurobrachia* (body size 10 and 20 mm) using the QIAamp DNA Mini Kit (QIAGEN, Germany). DNA concentration was measured with an Invitrogen Qubit fluorometer.

Initial ctenophore COI primers were designed based on the published COI sequences of *P. pileus* (GenBank accession number JF760211): (Pl-COI-F) TGTTACCTTACACGCAGTTT (forward) / (Pl-COI-R) ATCGAATAGTAAGTAATGGC (reverse) (amplicon length: 1165 bp), and internal primers Pl-COI-D_FW CTTACTGATCTCCTTGCCTGT (forward) / Pl-COI-D_RW ACAGGCAAGGAGATCAGTAAG (reverse).

The cytochrome oxidase subunit I (COI) gene fragment was amplified using C1000 Touch thermal cycler (Bio-Rad). The PCR amplifications were performed in a 25 μL reaction volume per sample, and contained 2 μl of template DNA (0.3–1.4 ng/μl), 1 μl of forward and reverse primers (aliquoted to a standard concentration of -0.5 μM), 5 μl 5× ScreenMix (Evrogen, Russia), and 16 μl DNA-free H_2O. The PCR program consisted of an initial denaturing step at 94 °C for 3 min, 30 amplification cycles (denaturation at 94 °C for 30 s, annealing at 45 °C for 30 s, extension at 72 °C for 60 s), and a final extension at 72 °C for 5 min.

PCR products were verified on 1% agarose/TBE electrophoretic gel. Amplified fragments were purified using GeneJET Gel Extraction Kit and DNA Cleanup Micro Kit (Thermo Scientific, USA), according to the manufacturer's protocols, except for the last step: the final DNA elution was carried out with RNA-free water.

Sequencing of the double-stranded PCR products was conducted by the dideoxy termination method (Sanger et al., 1977) using BrilliantDye™ Terminator (v3.1) Cycle Sequencing Kit (Nimagen, the Netherlands). Dye-labelled cycle-sequence products were cleaned by ethanol-precipitation. PCR products were sequenced with the PCR forward and reverse primers using Genetic Analyser Nanophore 05 (Institute for Analytical Instrumentation of the Russian Academy of Sciences, St Petersburg).

Raw reads for each sequence were base-called using the software for analysing and editing sequencing results (PAR2SEC, Institute of Analytical Instrumentation), assembled and checked for improper base-calling manually. Newly obtained sequences were deposited in GenBank under accession numbers OR917931 and OR917932.

Obtaining genomic data from transcriptome. We searched for the 18S rRNA gene using a whole transcriptome sequence (GenBank SRR26700624) obtained from the Black Sea *Pleurobrachia* as a part of our previous project (GenBank PRJNA1036602). We conducted preliminary data processing, de novo transcriptome assembly, and an 18S sequence search, following the methodology outlined in our previous work (Krivenko et al., 2024) The identified sequence was deposited in GenBank under accession number OR918328.

Data processing and phylogenetic reconstructions. We have reconstructed the phylogeny of ctenophores based on the COI and the 18S rRNA sequences obtained by us, along with a set of sequences mined for the phylum Ctenophora from the NCBI (National Center for Biotechnology Information) database (txid10197). The dataset includes complete or partial sequences of the mitochondrial COI gene (225 sequences) and the 18S rRNA (104 sequences) loci of specimens that have been identified by the authors to species (see Addenda: Electronic supplementary material 1). The sequences were first aligned with MAFFT v.7.48 (Katoh & Toh, 2010) with option L-INS-I, ambiguously aligned 5'- and 3'-terminal regions were trimmed. The length of the resulting matrix was 1753 bp and 1167 bp for the 18S rRNA and the COI genes, respectively. Maximum likelihood phylogenetic analysis was performed using IQ-TREE 1.6.12 (Trifinopoulos et al., 2016) with a model K3Pu+F+I+G4 for the COI gene and TNe+I+G4 one for the 18S rRNA gene chosen by ModelFinder (Kalyaanamoorthy et al., 2017). We have launched the IQ-TREE with the following parameters:

number of ultrafast bootstrap alignments – 10000; SH-aLRT branch test (replicates) – 10000; approximate Bayes test – choosen, perturbation strength – 0,01; stopping rule – 1000. We calculated the Kimura two-parameter (K2P) distances using the MEGA 11 software (Kimura, 1980; Tamura et al., 2021).

Results

Phylum **Ctenophora** Eschscholtz, 1829

Class **Tentaculata** Eschscholtz, 1825

Order **Cydippida** Gegenbaur, 1856

Family **Cydippidae** Gegenbaur, 1856

Genus ***Pleurobrachia*** Fleming, 1822

Pleurobrachia pileus (O.F. Müller, 1776) (Fig. 1)

Material. **Black Sea**, *env. of Sevastopol*: 44°13′01″N 33°41′27″E, 7 June 2022, 11 specimens; 44°24′69″N 34°24′45″E, 10 June 2022, 14 specimens.

Morphological characters. We analysed the diagnostic characters of the Black Sea *Pleurobrachia* (Fig. 1) based on Chun (1879) and Licandro & Lindsay (2017). We observed the animals with a body length (from statocyst to mouth) ranging from 9.6 to 22.4 mm (average size 16.7 mm) (Table 1). The length of ctene rows varied among specimens: some were less than three-quarters of the body length (n = 9), while the others were longer (Table 1). The length of the stomodeum was about half of the body length (Table 1). The tentacular bulbs were far from the stomodeum. The adradial canal opened onto the meridional canal above the infundibulum (relative to the axis of the statocyst-mouth). The tentacles were very long, supplied with numerous tentilla. The body shape was nearly spherical. The body was transparent, without pigmentation or coloration.

A living specimen of the Black Sea *Pleurobrachia* is presented in our video (Addenda: Electronic supplementary material 2).

Molecular phylogeny. The lengths of sequenced DNA fragments for the Black Sea *Pleurobrachia* were 681 bp and 932 bp for the COI gene and 2511 bp for the 18S rRNA gene. The length of the resulting alignment was 1167 bp for the COI and 1753 bp for the 18S rRNA genes, respectively. The BLAST algorithm (McGinnis & Madden, 2004) implemented in NCBI revealed the highest percentage identity match with *P. pileus* for both genes, with 98.68–100% identity for the COI and 99.89–99.44% identity for the 18S rRNA. The second-best matches for the COI gene are *P. globosa* (Moser, 1903) with 86.39–87.62% and *P. bachei* (Agassiz, 1860) with 82.86–83.69% identity; for the gene 18S rRNA are *P. bachei* with 99.83% identity and *P. globosa* with 99.19%. The molecular data on *P. rhodopis* are not available in the NCBI. We calculated the Kimura two-parameter (K2P) distances within and between *P. pileus*, *P. globosa* and *P. bachei*. K2P distances among all studied *P. pileus* specimens (including our own material) ranged from 0 to 1.30%, while within the Black Sea *P. pileus*, the level of genetic divergence ranged from 0 to 0.44%. K2P distances between *P. pileus* and *P. globosa* ranged from 13.02 to 15.41%; between *P. pileus* and *P. bachei* ranged from 18.45 to 20.64%.

A phylogenetic reconstruction based on the COI sequences showed that the *Pleurobrachia* samples from the Black Sea clustered within the *P. pileus* clade in the ML tree with high supports (ultrafast bootstrap and SH-aLRT values equal to 100, aBayes equal to 1) (Fig. 2A). The Black Sea samples (including the specimen from Turkey, MW735824) formed a separate subgroup (the distance between the Black Sea clade and the other *P. pileus* samples is 0.008 nucleotide substitutions per site) with 100% ML support, 92.9% SH-aLRT support, and aBayes support equal to 1. Phylogenetic reconstruction for the 18S rRNA gene showed the same clustering pattern of the Black Sea specimens, though with a ultrafast bootstrap support of 90% (Fig. 2B). The supporting values obtained using SH-aLRT and aBayes methods were low (0 for SH-aLRT and less than 0.4 for aBayes), indicating that the optimal reconstruction conditions were not met. At the same time, the clade of *Pleurobrachia* has high support in the ML tree (ultrafast bootstrap value equal to 96%, SH-aLRT equal to 93.9%, aBayes equal to 1). Therefore, despite the fact that phylogenetic reconstructions based on the COI and the 18S rRNA gene fragments have a similar topology, in our conclusions we rely on the phylogeny obtained for the COI gene fragment.

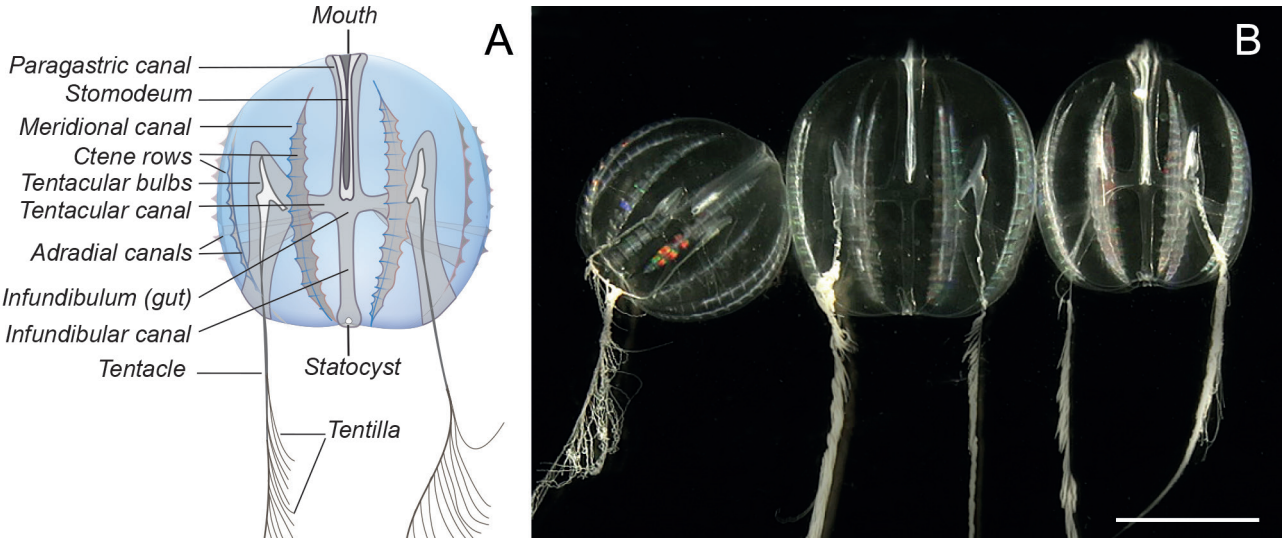


Fig. 1. *Pleurobrachia pileus* (O.F. Müller, 1776). Drawing of morphology **(A)** based on photos **(B)** of three specimens from our catches in the Black Sea. Scale bar: 1 cm.

Discussion

Pleurobrachia pileus is a cosmopolitan pelagic ctenophore recorded around the World Ocean, including the North Pacific, the North Atlantic, the Mediterranean, the North Sea, and the Southern Ocean (WoRMS..., 2025). Until now, researchers traditionally relied solely on dimensional characteristics to differentiate the species of *Pleurobrachia*, as well as to distinguish between *P. rhodopis* and *P. pileus*.

The Black Sea *Pleurobrachia* was first identified as "*P. rhodopis* Chun, 1879 (syn.: *P. pileus* O. Müller)" in Naumov (1968a). The latter source briefly describes it as having an ovoid body, the rows of ctenes starting at some distance from the aboral pole, very long tentacles with numerous filaments, and a transparent body measuring 5–7 mm in length (Naumov, 1968a). According to Chun's (1879) original description of *P. rhodopis*, "The largest specimen measured three-quarters of a centimeter. It is elliptical and has eight ribs (combs) of equal size arising not far from the sensory pole and extending over little more than half of the body. The strongly developed base of the tentacle is closer to the periphery and is oblique, so that its extended axis forms an acute angle with the stomach, the apex of which would be located at the mouth opening. The bases of the tentacle, as well as the tentacle filaments with its side branches are vividly pigmented pink, whereas the rest of the body is free of pigment spots. The tentacle emerges from the poorly developed sheath at the same level as the base of the funnel (infundibulum)". Thus, Chun (1879) has noted the body size and shape, the morphology of comb rows, gastrovascular system branching patterns, and pink coloration of tentacles and tentacle bulbs as *P. rhodopis* diagnostic characters. However, Naumov (1968a, 1968b) re-identified the Black Sea *Pleurobrachia* from *P. pileus* to *P. rhodopis* based solely on the differences in size, specifically, the smaller maximum size of *P. rhodopis* (Zaika, 2012).

Zaika (2012) noted that *Pleurobrachia* of different sizes were found in the Black Sea in samples from different depths; they are characterised by the body sizes up to 22 mm. Based on the dimensional characteristics, Zaika concluded that the native species of *Pleurobrachia* in the Black Sea is *P. pileus*. Probably, the wrong size estimation of *Pleurobrachia* from the Black Sea in the earlier publications (Naumov, 1968a, 1968b) led to an incorrect conclusion that the Black Sea *Pleurobrachia* is *P. rhodopis*.

The morphology of the Black Sea specimens of *Pleurobrachia* that we analysed is consistent with the original and subsequent descriptions of *P. pileus* from the different oceanic regions (Licandro & Lindsay, 2017), including the first description of the native Black Sea ctenophore (Naumov, 1968a).

The specimens of the Black Sea *Pleurobrachia* that we studied had an average body size of 16.7 mm and maximum size of 22.4 mm, same as indicated by Zaika (2012), which is corresponding to *P. pileus* (up to 25 mm) but not to *P. rhodopis* (up to 10 mm) (Licandro & Lindsay, 2017). The relative size of the ctene rows varied between individuals; apparently, animals with smaller body sizes and smaller ctene row lengths were most likely young individuals of *P. pileus*.

Furthermore, even in the Mediterranean Sea, the existence of *P. rhodopis* as a distinct species may be questionable. According to Licandro & Lindsay (2017), reliable diagnostic characters for *P. rhodopis* and *P. pileus* are size, length of ctene rows and pigmentation. We have found in the scientific literature only one image of *P. rhodopis* and it is not coloured, making it impossible to determine the colour of the animal (Licandro & Lindsay, 2017: 260, Fig. D). Due to the transparency of the *Pleurobrachia* body, the length of the ctene rows cannot be accurately depicted in greyscale images under different lighting conditions. We have found the only colour photo of *P. rhodopis* on the internet; the author gives a description "A sea gooseberry (*Pleurobrachia rhodopis*) photographed while snorkeling near the city of Pula, Adriatic Sea, Croatia" (Babic, 2023). It is possible to identify the specimen in this photo as *P. pileus*.

The researchers who identified *P. rhodopis* (Molinero et al., 2008; Pestorić et al., 2021) referred to Buecher & Gasser (1998) who mentioned "Although similar to the more common *P. pileus* of the Atlantic waters, the species present in the Mediterranean was identified as *P. rhodopis* (Fedele, 1940; Trégouboff & Rose, 1957; Riedl, 1983), due to its small size". Thus, Buecher and Gasser selectively quoted Fedele (1940) on the size of *P. rhodopis*. However, in the conclusion of his paper, Fedele (1940) summarised that "*P. rhodopis* was erroneously isolated from *P. pileus*, based on body size: *Pleurobrachia* with small body sizes, assigned to the species *P. rhodopis*, most likely were juveniles of *P. pileus*". Thus, all the researchers who mentioned *P. rhodopis* cited the authors who incorrectly quoted an earlier publication.

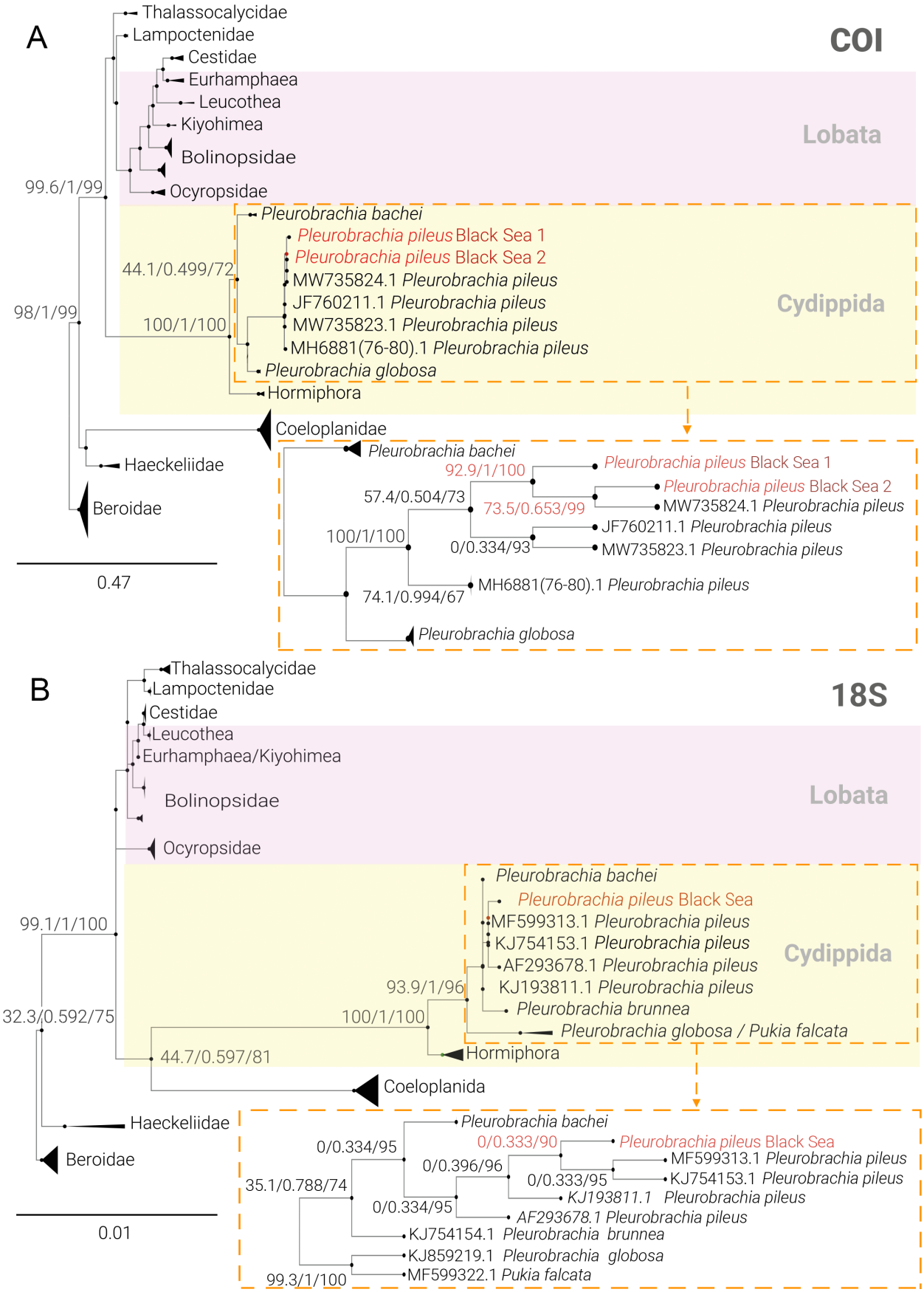
Red coloration of *Pleurobrachia* individuals mentioned in the description of *P. rhodopis* (Chun, 1879) may have been caused by the diet of the individuals. The main food source of *Pleurobrachia* is copepods (Buecher & Gasser, 1998), which feed on diatoms containing carotenoids

Table 1. Morphological measurements of Black Sea *Pleurobrachia*.

Specimen numbers	Body size (in oral–aboral axis), mm	Length of ctene rows, mm	Length of stomodeum, mm
1*	21.2	17.0	14.0
2	13.4	10.2	8.3
3	12.8	9.2	7.5
4	9.6	8.1	5.8
5	12.0	7.2	6.3
6	22.3	15.9	13.3
7	15.4	11.5	7.7
8	16.7	13.9	9.0
9	18.2	14.0	12.0
10	22.4	15.4	16.8
11	14.2	10.9	8.9
12*	13.6	9.8	8.0
13	11.0	8.6	6.1
14	10.7	7.7	5.6
15	21.9	14.2	13.3
16	20.2	16.0	10.0
17	20.9	17.0	11.2
18	17.4	15.0	8.5
19	18.9	13.0	10.1
20	13.7	9.2	12.6
21	14.2	8.2	14.2
22	22.3	19.1	11.1
23	18.4	13.8	10.5
24	16.0	9.8	8.4
25	20.2	17.0	11.0
Mean ± standard deviation	16.7±4.1	12.5±3.5	10.0±2.9
Min–Max	9.6–22.4	7.2–19.1	5.6–16.8

* Specimens used in the molecular analysis.

Sampling station coordinates: 44°13′01″N 33°41′27″E for specimens 1–11, 44°24′69″N 34°24′45″E for specimens 12–25.


A
COI
Thalassocalycidae
Lampoctenidae
Cestidae
Eurhamphaea
Leucothea
Kiyohimea
Bolinopsidae
Ocyropsidae
Lobata
99.6/1/99
Pleurobrachia bachei
Pleurobrachia pileus Black Sea 1
Pleurobrachia pileus Black Sea 2
44.1/0.499/72
MW735824.1 Pleurobrachia pileus
JF760211.1 Pleurobrachia pileus
98/1/99
100/1/100
MW735823.1 Pleurobrachia pileus
MH6881(76-80).1 Pleurobrachia pileus
Pleurobrachia globosa
Cydippida
Hormiphora
Coeloplanidae
Haeckeliidae
Beroidae
0.47
Pleurobrachia bachei
92.9/1/100
Pleurobrachia pileus Black Sea 1
Pleurobrachia pileus Black Sea 2
57.4/0.504/73
73.5/0.653/99
MW735824.1 Pleurobrachia pileus
JF760211.1 Pleurobrachia pileus
100/1/100
0/0.334/93
MW735823.1 Pleurobrachia pileus
MH6881(76-80).1 Pleurobrachia pileus
74.1/0.994/67
Pleurobrachia globosa
B
18S
Thalassocalycidae
Lampoctenidae
Cestidae
Leucothea
Eurhamphaea/Kiyohimea
Bolinopsidae
Ocyropsidae
Lobata
Pleurobrachia bachei
Pleurobrachia pileus Black Sea
99.1/1/100
MF599313.1 Pleurobrachia pileus
KJ754153.1 Pleurobrachia pileus
AF293678.1 Pleurobrachia pileus
93.9/1/96
KJ193811.1 Pleurobrachia pileus
Pleurobrachia brunnea
32.3/0.592/75
100/1/100
Pleurobrachia globosa / Pukia falcata
Cydippida
Hormiphora
44.7/0.597/81
Coeloplanida
Haeckeliidae
Beroidae
0.01
Pleurobrachia bachei
0/0.334/95
0/0.333/90
Pleurobrachia pileus Black Sea
0/0.396/96
MF599313.1 Pleurobrachia pileus
0/0.333/95
KJ754153.1 Pleurobrachia pileus
35.1/0.788/74
KJ193811.1 Pleurobrachia pileus
0/0.334/95
AF293678.1 Pleurobrachia pileus
KJ754154.1 Pleurobrachia brunnea
KJ859219.1 Pleurobrachia globosa
99.3/1/100
MF599322.1 Pukia falcata

that can affect red colour of animals when consumed (Vilgrain et al., 2023). The content of carotenoids can vary between seasons and localities, leading to a significant variation in the coloration of animals. Thus, the coloration of *Pleurobrachia* individuals cannot be considered a reliable species character.

Molecular genetic tests can serve as reliable indicators of the species, but there are no data on *P. rhodopis* in the NCBI. We used genetic tests to compare *Pleurobrachia* specimens from the Black Sea with the data from the NCBI for the genus *Pleurobrachia*. A more reliable differentiation between *P. rhodopis* and other species was revealed from the COI gene. This result aligns with previous conclusions that the 18S rRNA is more useful for distinguishing ctenophore genera, but not species (Podar et al., 2001; Haddock et al., 2017).

Phylogenetic reconstruction using the COI gene demonstrated that the sequences of *Pleurobrachia* from the Black Sea clustered with *P. pileus*. Interspecific pairwise sequence distances for the COI gene within the *Pleurobrachia* species we analysed were almost an order of magnitude higher than intraspecific ones. The distances we obtained for intra- and interspecific variability in *Pleurobrachia* coincided with those calculated for other taxa of Ctenophora (Christianson et al., 2022). The specimens of *Pleurobrachia* from the Black Sea form a separate group within the *P. pileus* cluster on the COI phylogenetic tree. However, the distances between the sequences from the Black Sea and other regions do not exceed intraspecific variability levels. Thus, the Black Sea population of *Pleurobrachia* undoubtedly belongs to *P. pileus*.

As of today, consequently, there are no reliable morphological characters that can distinguish *P. rhodopis* as a separate species. There is also a lack of molecular data available for this species. It may be necessary to consider the issue of synonymy between *P. pileus* and *P. rhodopis*.

In conclusion, based on our results, the *Pleurobrachia* inhabiting the Black Sea is *P. pileus*. We believe that there are no convincing reasons to classify it as *P. rhodopis*.

Addenda

Electronic supplementary material 1.

GenBank accession numbers of Ctenophora nucleotide sequences used in phylogenetic analysis. File format: PDF.

Electronic supplementary material 2.

Video recording of free movement of the Black Sea *Pleurobrachia* in a laboratory aquarium. File format: MPG.

All these materials are available from: https://doi.org/10.31610/zsr/2025.34.1.18

Acknowledgements

We are grateful to P.I. Donchik for capturing ctenophores using a plankton net during the 122th scientific cruise of the R/V “Professor Vodyanitsky”. This work was carried out within the framework of A.O. Kovalevsky Institute of Biology of the Southern Seas (IBSS) state research assignment. This work was funded by the IBSS government assignment No. 124022400148-4. The research was carried out at the core facility R/V “Professor Vodyanitsky” (IBSS) and scientific and educational research equipment sharing center “Phylogenomics and Transcriptomics” (IBSS).

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Fig. 2. Reconstructions of the phylogeny of Ctenophora based on the nucleotide sequences of the mitochondrial COI **(A)** and the nuclear 18S rRNA **(B)** gene fragments, obtained for the Black Sea *Pleurobrachia* (coloured in red) and the sequences of these genes presented in the NCBI database for *Pleurobrachia* and other ctenophores. SH-aLRT support (%), aBayes support and ultrafast bootstrap (Maximum Likelihood) support (%) values are given at the nodes. Scale bar for COI represents 0.47 nucleotide substitutions per site. Scale bar for 18S represents 0.01 nucleotide substitutions per site.

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Editorial responsibility: N.A. Shapoval & A.A. Przhiboro