



Morphological description and DNA barcoding of *Thalassomya paraskevae* sp. nov. (Diptera: Chironomidae: Telmatogetoninae) from coast of the Black Sea

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Abstract

Illustrated description of adult male, as well as DNA barcoding, of *Thalassomya paraskevae* sp. nov. in comparison with close related species *T. frauenfeldi* Schiner from coast of the Black Sea are provided. Interspecific p-distances using gene *COI* 5P between *T. paraskevae* sp. nov. and *T. frauenfeldi* from Madeira (Portugal) were 8.94%. Divergence between described species and *T. frauenfeldi* collected near the type locality (Friuli Venezia-Giulia, Italy) using *COI* 3P were 7.72%. Obtained values corresponds to species level.

Key words: Diptera, Chironomidae, Telmatogetoninae, *Thalassomya*, new species, DNA barcoding, coast of the Black Sea

Introduction

The genus *Thalassomya* was erected by Schiner (1856) for the marine species *T. frauenfeldi* Schiner from Trieste (Adriatic coast, Italy). Currently, the genus is represented by 12 species living in different areas of the world's oceans (Ashe & O'Conner 2009; Neubern de Oliveira *et al.* 2013). The most widespread species is the *T. frauenfeldi*, which is recorded from many islands of the Atlantic Ocean and from the coasts of the Baltic, Adriatic, Mediterranean and Black Seas (Ashe & O'Conner 2009). However, molecular studies of this species from different areas have shown that it is not one species, but a group of species that are poorly distinguished or not at all distinguished by morphology, but have different DNA barcoding data. This is confirmed by our study of the *T. paraskevae* sp. nov. from the Black Sea coast of Abkhazia and Russia, which is well distinguished by molecular data from the *T. frauenfeldi* collected near the type locality (Adriatic coast, Italy) (Cranston *et al.* 2012) and in Madeira, but hardly differs in morphology. Below we provide a morphological description with a study of DNA barcoding of adult male of this species and comments on taxonomy of *Thalassomya*.

Material and methods

The material was preserved in 96% ethanol and slide-mounted in polyvinyl lactophenol. The terminology follows Sæther (1980). The photographs were taken using an Axio Lab.A1 (Carl Zeiss) microscope with an AxioCam ERc5s digital camera and an Olympus SZX16 stereomicroscope with an Olympus DP74 digital camera, and then stacked using Helicon Focus software. The final illustrations were post-processed for contrast and brightness using Adobe®.

Holotype and paratypes as well as all other material are deposited in the Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far East Branch of the Russian Academy of Sciences, Vladivostok, Russia (FSCEATB FEB RAS).

Genomic DNA was extracted from whole body by the ExtractDNA Blood & Cells (Evrogen, Moscow, Russia) followed the manufacturer's instructions. A two partial mitochondrion fragments, 5P and 3P ends of the cytochrome *c* oxidase I (COI) gene were amplified using primers LCO1490 and HCO2198 (Folmer *et al.* 1994) and s2183 and a3014 (Simon *et al.* 1994) respectively. The preparation of samples for sequencing was carried out as described by Makarchenko *et al.* (2022a, 2022b, 2023). The PCR products were bidirectionally sequenced on an ABI 3500 sequencer (Applied Biosystems). Intra and interspecific genetic divergence values were calculated using p-distances implemented in MEGA7 (Kumar *et al.* 2016) software. We also used this program to reconstruct phylogenetic trees using the neighbor joining algorithm (Saitou & Nei 1987). Molecular species delineation was achieved by Assemble Species by Automatic Partitioning (ASAP) (Puillandre *et al.* 2021, web site <https://bioinfo.mnhn.fr/abi/public/asap>) using simple p-distances as the substitution model. The obtained sequence have been deposited in GenBank under number PP554679–PP554681 (contains both COI-5P and COI-3P sequences) and PQ120403–PQ120404 (contains only COI-5P sequences).

Taxonomy

Thalassomya paraskevae Makarchenko, Semenchenko *et* Palatov, sp. nov.

<http://zoobank.org/NomenclaturalActs/ED222392-FC41-44C4-BCA1-0E74C1AB7640>

(Figs. 1–11)

Type material. Holotype, adult male, ABKHAZIA: Gudautra District, New Athos Town, breakwater near the pier on the beach of the village, 01.01.2024, 43.083339 N, 40.816681 E, leg. D. Palatov. Paratypes: 6 adult males, the same data as holotype; 3 adult males, RUSSIA: Krasnodar Territory, Tuapse District, Cape Kadosh cliffs on the Black Sea coast, near the village of Agoy, south of the mouth of the river of the same name, 25.04.2024, 44.132806 N, 39.026056 E, leg. D. Palatov.

Derivatio nominis. The species is named in honour of Bulgarian chironomidologist Dr. Paraskeva Mikhailova (Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, Sofia) who studied *Thalassomya frauenfeldi* from Bulgaria's Black Sea coast.

Description

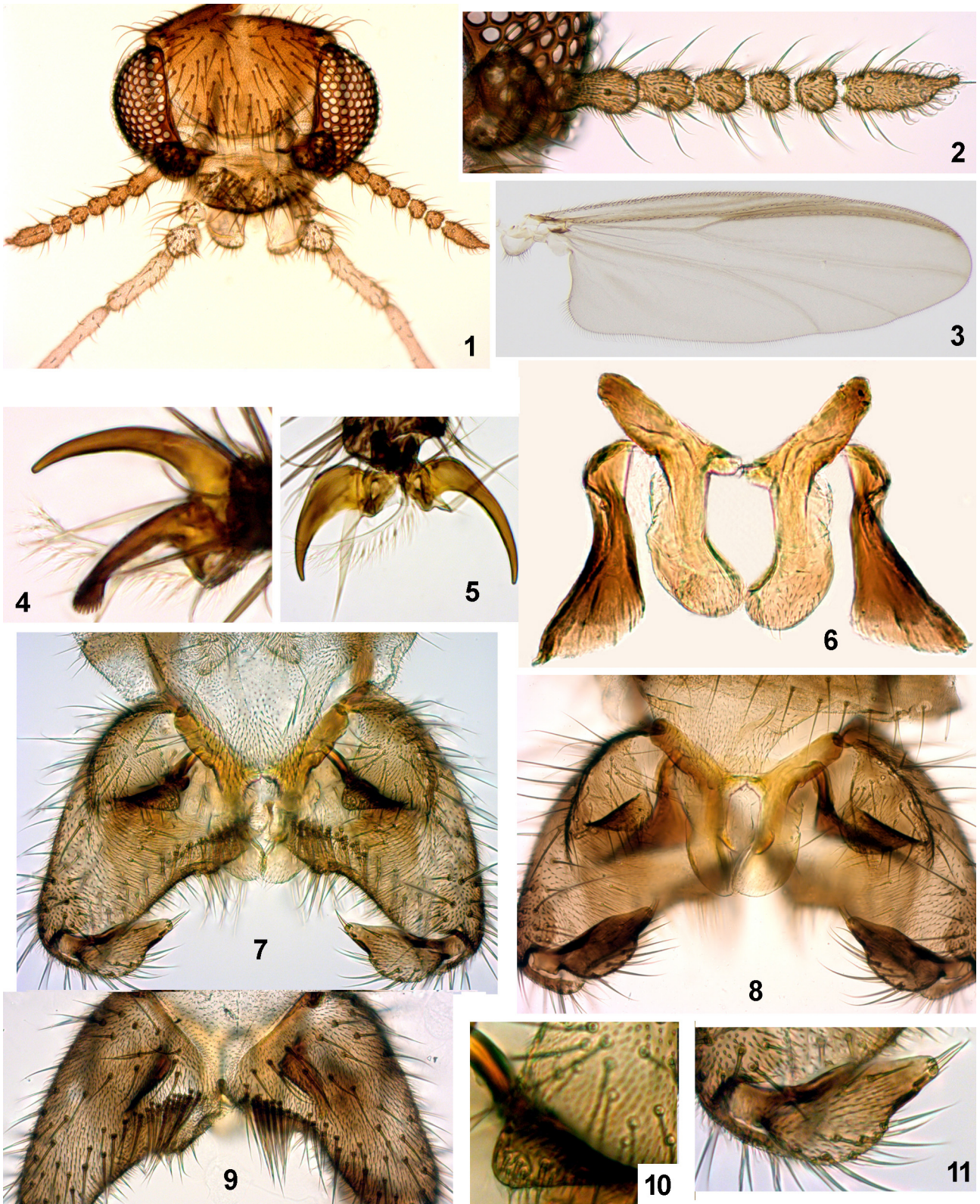
Adult male (n = 5, except when otherwise stated). Total length 3.4–4.2 mm. Total length/wing length 0.98–1.0.

Coloration. Dark brown. Wings grey, with brownish veins.

Head (Fig. 1). Eyes bare, rounded, without dorsomedial projections. Temporal setae strong and numerous—75–108. Clypeus with 49–58 strong setae. Antenna with scape covered *ca* 30 strong setae and 6 flagellomeres with reduced plume of setae (Fig. 2); number and length of these setae on 1–5 flagellomeres respectively: 5–6 (44–68 μ m), 4 (60–68 μ m), 4 (44–52 μ m), 4 (48–56 μ m), 4 (44–68 μ m); terminal flagellomere with 4 setae, 48–60 μ m long in basal part and with 1 subapical setae, 32–40 μ m long. Length of 1–6 flagellomeres (μ m): 96–112, 52–56, 44–52, 40–48, 40–44, 96–120; terminal flagellomere with a distinct nipple-like apex. AR 0.35–0.39. Palpomere length (μ m): 40–42, 32–44, 80–88, 136–188, 208–252. Palpomere 1 without setae, palpomeres 2–3 with setae 68–96 μ m long, palpomeres 4–5 with setae 24–32 μ m long; palpomere 3 in distal part with sensilla capitata with diameter 16 μ m. Head width/palpal length 1.12–1.26. Antennal length/palpal length 0.74–0.75.

Thorax. Anteprepronotum with 37–40 ventrolateral setae, 148–256 μ m long. Acrostichals 30–32, 104–128 μ m long, in anterior part in 1 row, in posterior part in 2 rows; dorsocentrals 23–39, 156 μ m long; humerals 5–6, 100–104 μ m long; prealars 16–21, 100–112 μ m long (in 2 groups). Scutellum with 30–34 setae. Preepisternum without setae.

Wing (Fig. 3). Length 3.44–4.08 mm, width 1.08–1.28 mm. R_{2+3} absent. R and R_1 with 62–68 setae, R_{4+5} with 35–38 setae. fCu well beyond base of r-m. Anal lobe rounded-angular. Squama with 31–45 setae, 100–120 μ m long.



FIGURES 1–8. Adult male of *Thalassomyia paraskevae* sp. nov. 1, head; 2, antenna; 3, wing; 4, claws of mid leg; 5, claws of hind leg; 6, endomeres and phallapodemes; 7–8, hypopygium, in dorsal view; 9, part of hypopygium, in ventral view; 10, dorsobasal lobe of gonocoxite; 11, gonostylus.

Legs. Spur of front tibia 80–100 μm long. Spurs of mid tibia 76–80 μm and 96–120 μm long. Spurs of hind tibia 92 μm and 92–100 μm long. Anterior tarsal claws on front and middle legs pectinate at tip, other claws sharp (Figs. 4–5). Length (μm) and proportions of leg segments are as in Table 1.

TABLE 1. Lengths (in μm) and proportions of leg segments of *Thalassomya paraskevae* sp. nov., male (n=4)

	fe	ti	ta ₁	ta ₂	ta ₃	ta ₄	ta ₅	LR	BV	SV	BR
P ₁	1960-2080	2240-2360	1240-1320	508-525	295-328	98-120	197-213	0.45-0.55	4.69-4.99	3.39-3.81	2.0-2.2
P ₂	2680-2920	2640-2720	1000-1040	360-410	262-280	98-120	160-213	0.37-0.39	6.48-6.96	5.15-5.64	2.0-2.1
P ₃	2800-3000	3080-3160	1360-1440	600-705	360-377	115-120	213-230	0.43-0.48	5.28-5.72	4.17-4.38	2.2-2.5

Hypopygium (Figs. 7–11). Tergite IX reduced, with 25–29 setae in two groups. Laterosternite IX with 10–11 setae. Gonocoxite 304–308 μm long, broad at base, rounded laterally, abruptly narrowed on distal half; dorsobasal lobe triangle rounded or rounded, with 4–5 setae 32–40 μm long and in subapical part with group of very short setae; on ventral side at base with a patch of strong and long (112–128 μm) setae. Gonostylus 144–156 μm long, broadest medially, tapering apically to 2 setae, 24–44 μm long, with some setae 72–88 μm along the outer edge. Endomere yellow and with setae, 6–8 μm long in distal part. Phallapodeme dark brown, 184–188 μm long. HR 1.93–2.17.

Pupa and larva unknown.

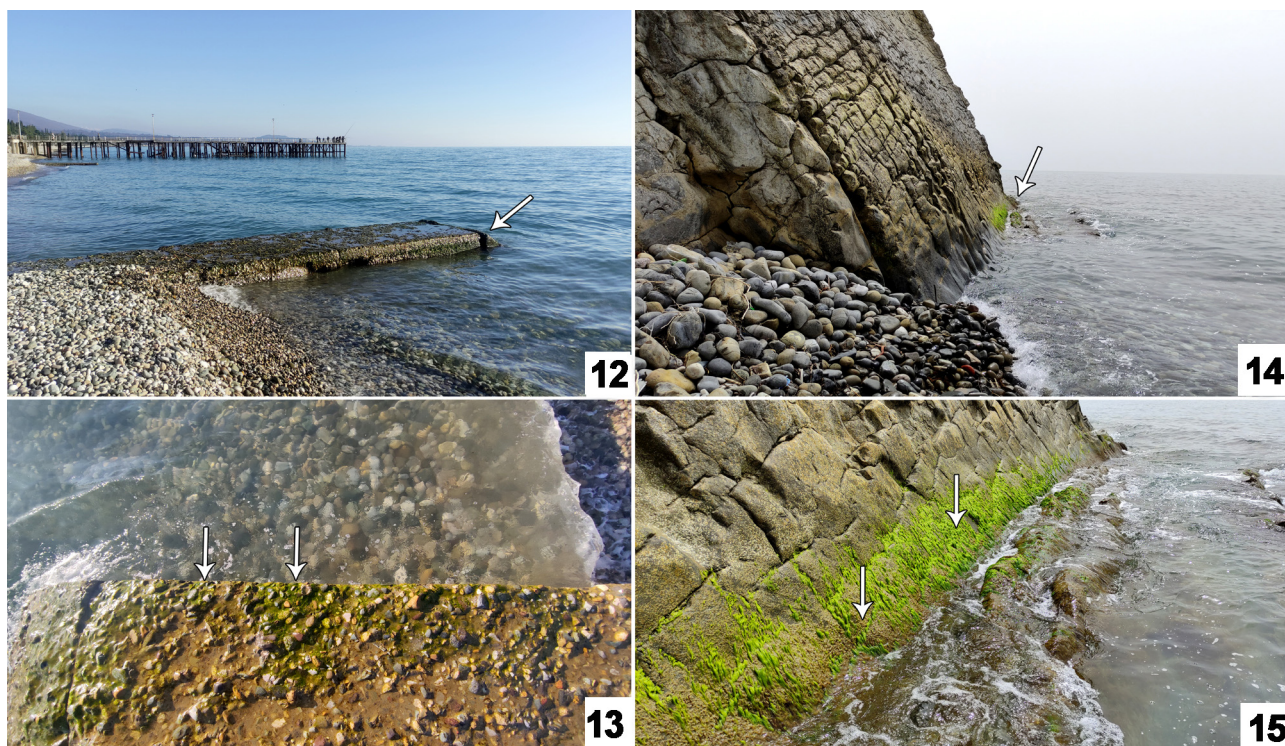
Diagnosis and comments. Adult male total length 3.4–4.2 mm. Eyes bare, without dorsomedial projections. Temporal setae strong and numerous. Clypeus with 49–58 strong setae. Antenna with 6 flagellomeres and reduced plume of setae; terminal flagellomere with a distinct nipple-like apex; AR 0.35–0.39. Palpomere 3 in distal part with sensilla capitata. Head width/palpal length 1.12–1.26. Antennal length/palpal length 0.74–0.75. Anteprepronotum with 37–40 ventrolateral setae. Acrostichals 30–32, in anterior part in 1 row, in posterior part in 2 rows; dorsocentrals 23–39; humerals 5–6; prealars 16–21 (in 2 groups). Scutellum with 30–34 setae. Preepisternum without setae. Wing length 3.44–4.08 mm, R₂₊₃ absent. Anal lobe rounded-angular. Squama with 31–45 setae. Anterior tarsal claws on front and middle legs pectinate at tip, other claws sharp. LR₁ 0.45–0.55, BV₁ 4.69–4.99, SV₁ 3.39–3.81. Tergite IX reduced, with 25–29 setae in two groups. Gonocoxite broad at base, rounded laterally, abruptly narrowed on distal half; dorsobasal lobe triangle rounded or rounded, with 4–5 setae and in subapical part with group of very short setae; on ventral side at base with a patch of strong and long setae. Gonostylus broadest medially, tapering apically to 2 setae, with some setae along outer edge. Endomere yellow and with setae in distal part. HR 1.93–2.17.

As noted above, the new species is most closely related to *T. frauenfeldi* and is poorly distinguished from the latter in morphological features. The matter is further complicated by the fact that *T. frauenfeldi* was described by Schiner (1856) from adult female and for comparison we can use only the description by Wirth (1947), which is based on an adult male, a pupa and a larva from San Remo in Italy. This description is not complete enough. However, it can be noted that structure of the male hypopygium of these two species do not differ as well as value of AR, wing venation, shape of terminal flagellomere and some other features. There are differences in the number of ventrolateral setae on the anteprepronotum and the values of LR, BV and SV. Thus, a male *T. paraskevae* sp. nov. with 37–40 ventrolateral setae on the anteprepronotum, LR₁ 0.45–0.55, BV₁ 4.69–4.99, SV₁ 3.39–3.81, while male *T. frauenfeldi* with 8–10 ventrolateral setae, LR₁ 0.57, BV₁ 4.53, SV₁ 3.25. It is also possible that there will be a different number of acrostichals, dorsocentrals and other setae of mesonotum but unfortunately there is no such data in the description of *T. frauenfeldi* (Wirth 1947). Most likely, we are dealing with cryptic species, which can be reliably distinguished only by molecular methods. The use of this method will also allow in the future to analyze the species of *Thalassomya* from the Bulgarian Black Sea coast, which was recorded as *T. frauenfeldi* (Valkanov 1949; Valkanov & Michailova 1974; Michailova 1976) and may be *T. paraskevae* sp. nov.

Ecology. Like other species of *Thalassomya*, the new species is associated with algal fouling on rocks or similar artificial structures such as breakwaters located along the coastal area. Adult individuals actively moved along the rocks at the waterline, in the splash zone, occasionally flying from one place to another. The highest locomotor activity of imago was observed during daytime hours, correlated with maximum air temperature. However, the

activity of some individuals had been also noted at night. The species cohabits with *Telmatogeton* sp. and *Clunio* sp.

Distribution. Known only from the type locality in coast of the Black Sea (Figs. 12–15).



FIGURES 12–15. Habitats of *Thalassomyia paraskevae* sp. nov. on the coast of the Black Sea near New Athos (Abkhazia) (12–13, type locality) and Cape Kadosh in the vicinity of Tuapse (Krasnodar Territory, Russia) (14–15). Arrows indicate collection points.

Results of DNA barcoding

In this study we obtained DNA barcodes for five specimens of *T. paraskevae* sp. nov. Of these, three specimens collected in Abkhazia, were sequenced by both COI-5P (658 bp in length) and COI-3P (802–826 bp in length) DNA barcoding markers. For two specimens from the Krasnodar Region of Russia, only COI-5P (658 bp in length) was obtained. The use of DNA barcodes for two markers is associated with the need for comparison with a closely related species *T. frauenfeldi*. To date (accessed 02 August 2024), only two sequences are known for this species, the first for the gene COI-5P for the specimen from Madeira, Portugal (BOLD Process ID: MIDGE429-07) and the second for the gene COI-3P for the specimen from Friuli Venezia-Giulia, Italy (HQ441010). The specimen from Italy was collected close to the type locality of the species, which is located in Trieste, Italy (Schiner 1856).

Intraspecific sequence divergence within *T. paraskevae* sp. nov. did not exceed 1.2% by both markers. The average interspecific distances between *T. paraskevae* sp. nov. and *T. frauenfeldi* were 8.94% and 7.72% for COI-5P and COI-3P respectively. Such values corresponds to species level for other chironomid subfamilies (e.g. Montagna *et al.* 2016, Makarchenko *et al.* 2022a, 2022b, 2023), but species boundary calculations for the Telmatogetoninae are not available.

The neighbor joining tree using *Telmatogeton* as an outgroup support monophyly of *T. paraskevae* sp. nov. using both markers (Fig. 16). Morphologically close species *T. frauenfeldi* was sister to *T. paraskevae* sp. nov. ASAP with the best score (1.5) which was achieved at a distance threshold of 5.34% and 4.14% for COI-5P and COI-3P respectively also support *T. paraskevae* sp. nov. as distinct species (Fig. 16).

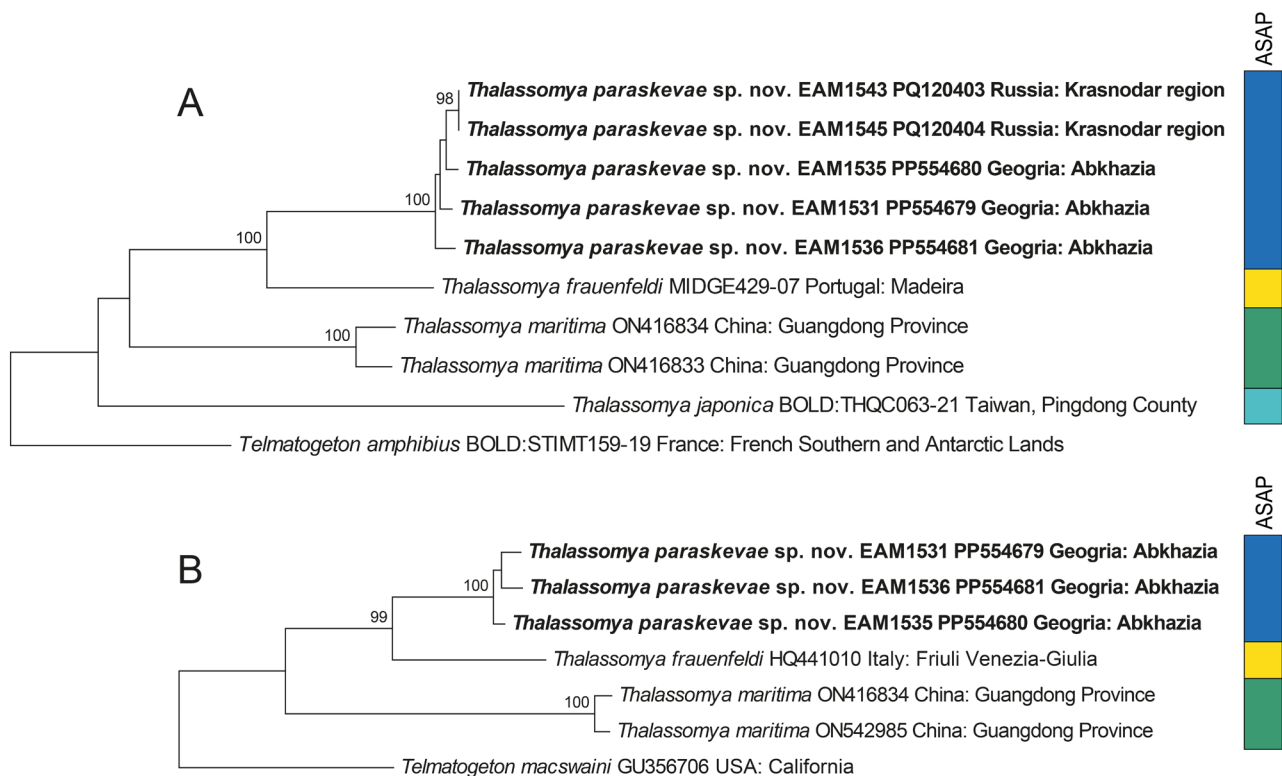


FIGURE 16. Neighbor joining trees based on the COI-5P (A) and COI-3P (B) nucleotide sequence data of the genus *Thalassomya* Schiner with *Telmatogeton* Schiner as an outgroup. Bootstrap support values (higher than 70%) are given above tree nodes. The sequences obtained in this study are in bold.

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