


FIRST RECORD OF A PEA MUSSEL *EUGLESA MILIUM* (MOLLUSCA: BIVALVIA: SPHAERIIDAE) FROM JAPAN, WITH ITS MORPHOLOGICAL AND GENETIC CHARACTERISTICS

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ABSTRACT: *Euglesa milium* is a Holarctic freshwater mussel species characterised by its unique subtrapezoidal shell and elongated perisiphonal suture. In this study, *E. milium* is recorded for the first time at two sites in Hokkaido, Japan, based on the investigation of the shell, anatomical, and genetic characteristics. The shell and anatomical features of the Japanese specimens were consistent with those of *E. milium* previously reported, while variations in shell outline shapes were observed between localities. Identical or very similar 16S rRNA haplotypes among Japanese and Russian specimens supported the morphological identification. The species richness of Japanese Sphaeriidae may have been underestimated because of the lack of surveys; therefore, further faunal investigations and taxonomic revisions are required.

KEYWORDS: anatomy; distribution; freshwater mollusk; Hoppe-mameshijimi; mitochondrial haplotype

INTRODUCTION

The family Sphaeriidae Deshayes, 1855 is a group of tiny freshwater mussels, with 244 species (LEE 2019, GRAF & CUMMINGS 2026). Members of this family are common in various freshwater environments and are distributed worldwide, except in Antarctica (GRAF 2013, LEE 2019). Many sphaeriid species have extensive distribution ranges, which is thought to be due to their passive dispersal abilities and flexibility of life-history traits (KILEEN et al. 2004, CLEWING et al. 2013, BESPALAYA et al. 2020, 2024).

Japan is one of the regions with limited knowledge of Sphaeriidae as indicated by SAITO et al. (2022). The taxonomy and distribution of Japanese sphaeriids were studied by a sequential nationwide study of Syuiti MORI (1933, 1935a, 1935b, 1936, 1937, 1938a, 1938b) after descriptions of several species (WESTERLUND 1883, PILSBRY & HIRASE 1908,

KURODA 1930), reporting 24 sphaeriid taxa, including 18 new species and subspecies. However, subsequent studies have been limited to the redescrptions of several Japanese species (IEYAMA & TAKAHASHI 2004, IEYAMA 2008), and investigations into the domestic distributions of *Euglesa* Jenyns, 1832 and *Odhneripisidium* Kuiper, 1962 (e.g. STAROBOGATOV et al. 2004, INABA et al. 2011, AKIYAMA & USUI 2017, OYAGI & IEYAMA 2017). Owing to insufficient investigation, several species with extensive distribution ranges in the Northern Hemisphere may have been unrecorded in Japan (WELTER-SCHULTES 2012). One such species is *Euglesa milium* (Held, 1836).

Euglesa milium has a Holarctic distribution, and extant specimens have been recorded from wide areas of North America, Northwest Africa, Europe, and North Asia (KERNEY 1999, KEBAPCI 2023, GRAF &

CUMMINGS 2026). Fossils from after the last interglacial period have also been obtained from Europe and Africa (KUIPER 1968, 2009, STELFOX et al. 1972, HOLOPAINEN & KUIPER 1982). *Euglesa milium* has been considered to be one of the most easily identifiable species within Sphaeriidae due to its subtrapezoidal shell shape, elongated perisiphonal suture, and ventrally positioned adductor muscles, although variations have been detected in shell outlines and surface sculptures (ODHNER 1929, KORNIUSHIN 1996, KORNIUSHIN & GLAUBRECHT 2002, MOUTHON & AUDIBERT 2023). These variations include two forms: “unioides” characterised by a long anterior end and straight ventral margin (WESTERLUND 1873,

HOLOPAINEN & KUIPER 1982) and “pulchelloides” distinguished by pronounced striations, that have been identified as intraspecific forms of *E. milium* (KUIPER 1942, MOUTHON & AUDIBERT 2023). Shell and anatomical characters of this species have been examined by ODHNER (1929), DYDUCH-FALNIOWSKA (1983), and KORNIUSHIN (1996) using European and Russian specimens, and summarised by KORNIUSHIN & GLAUBRECHT (2002).

Recently, the authors discovered pea mussels with features similar to *E. milium* in Japan. In this study, we investigated the morphological and genetic characteristics of these specimens and report this species from Japan for the first time.

MATERIAL AND METHODS

SPECIMEN PREPARATION AND MORPHOLOGICAL EXAMINATION

Specimens were collected from two localities in Hokkaido, northern Japan on 15 July 2025 (locality No. 1) and 16 May 2022 (locality No. 2) during surveys targeting sphaeriid species (Fig. 1; Table 1). The specimens were prepared by boiling for three

seconds in water at 95 °C referring to FUKUDA et al. (2008). The shells were dried, and the whole soft bodies were preserved in 99% ethanol. Newly collected specimens were deposited at the Zoological Collection of Kyoto University, Japan with the following voucher numbers: KUZ Z6379, one specimen from locality No. 1 used for shell, anatomical, and genetic examinations; Z6380, five specimens from

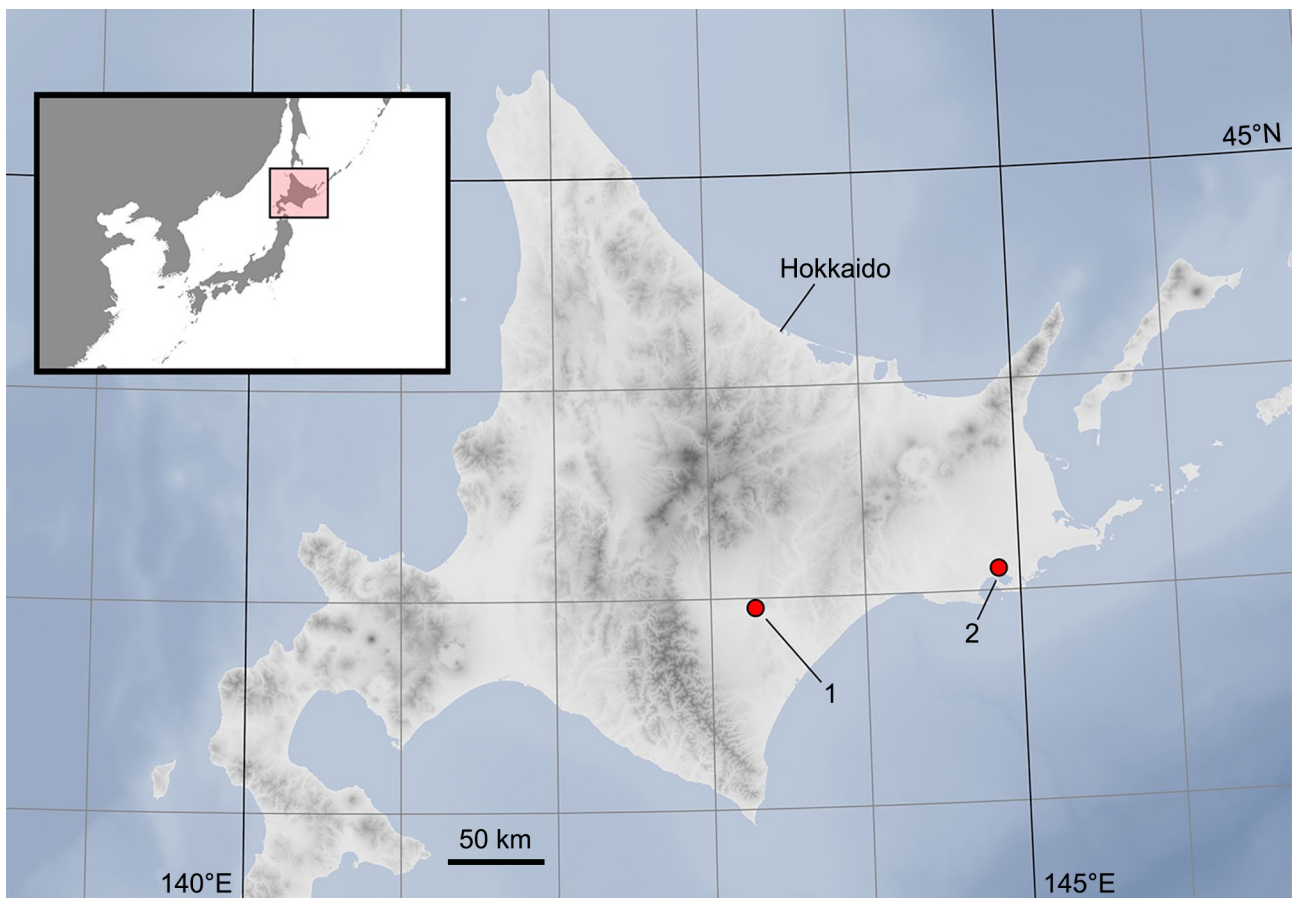


Fig. 1. Map representing the collection localities of *Euglesa milium* in Hokkaido, Japan. The map was drawn based on the data from REUTER et al. (2007) and AMANTE & EAKINS (2009)



locality No. 1 used for examinations of both shell and anatomy, or shell alone; Z6381, one specimen from locality No. 2 used for shell and genetic examinations; Z6382, three specimens from locality No. 2 used for shell examination.

Shells of six specimens from locality No. 1 and four specimens from locality No. 2 were examined using a stereomicroscope and scanning electron

microscope (SEM). Soft bodies were dissected only in three specimens from locality No. 1 because of poor storage condition of soft bodies from locality No. 2. Shells and soft bodies were observed using a YS05T stereomicroscope (Micronet Inc., Japan) and photographed using a SwiftCam SC1003-CK camera (SwiftCam Technologies Group Co., Ltd., Hong Kong) attached to the stereomicroscope.

Table 1. Specimen list of the present molecular analyses with collection localities, accession numbers of International Nucleotide Sequence Database (INSD), and references. Locality information of GURALNICK (2005) referred by BESPALAYA et al. (2024) because a supplemental online appendix of GURALNICK (2005), which includes specimen information, was unavailable. The parenthesised numbers in the locality indicate the locality numbers

Locality	INSD Accession Nos		Reference
	16S	28S	
Japan: Hokkaido Pref., Kato-gun, Otofuke-cho, Tokachigawa-onsen (No. 1)	LC919957	LC919959	This study
Japan: Hokkaido Pref., Akkeshi-gun, Akkeshi-cho, Sannushi (No. 2)	LC919956	LC919958	This study
Germany: Heiliges Meer (No. 3)	AY093564		LEE & FOIGHIL (2003)
USA: Michigan (No. 4)	AF152028		LEE & FOIGHIL (2003)
USA: 1/6 mi. from Hessie Rd./4th July trail juncture in pond, Colorado (No. 5)	AY957844, AY957852		GURALNICK (2005)
USA: Echo Lake, Clear Creek County, Colorado (No. 6)	AY957847		GURALNICK (2005)
USA: Eldora. Hessie Pond, Colorado (No. 7)	AY957848		GURALNICK (2005)
USA: Hessie Pond – Eldora, Colorado (No. 8)	AY957851, AY957853		GURALNICK (2005)
USA: Hessie Pond, Colorado (No. 9)	AY957841		GURALNICK (2005)
USA: In marsh area adjacent to first Chicago Lake, Colorado (No. 10)	AY957849		GURALNICK (2005)
USA: Lost Lake, Boulder, Colorado (No. 11)	AY957854		GURALNICK (2005)
USA: Pond 1/4 mi. from Jasper Lake toward Devil's Thumb Lake, Colorado (No. 12)	AY957846		GURALNICK (2005)
USA: pond 1/4 mi. from Jasper Lake towards Devil's thumb lake Boulder, Colorado (No. 13)	AY957842, AY957843		GURALNICK (2005)
USA: Pond 1/6 mile from Hessie Road Juncture, Colorado (No. 14)	AY957840, AY957850		GURALNICK (2005)
USA: Pond just east of Guanella Pass (11,510 ft) Mount Evans, Colorado (No. 15)	AY957855		GURALNICK (2005)
USA: Sprague Lake, Colorado (No. 16)	AY957845		GURALNICK (2005)
USA: Montana, Yellowstone National Park, Swan Lake (No. 17)	ON563525	ON783900	BESPALAYA et al. (2024)
Russia: Irkutsk region, near Vitim Nature Reserve, Danno lake (No. 18)	ON563637		BESPALAYA et al. (2024)
Russia: Irkutsk region, Vitim Nature Reserve, Gnilaya Kuria (No. 19)	ON563629		BESPALAYA et al. (2024)
Russia: Kamchatka, Ust-Kamchatsky District, biostation "Raduga", Azabachye lake (No. 20)	ON563567		BESPALAYA et al. (2024)
Russia: Nenets Autonomous Area, Bolshezemelskaya tundra, Vashutkin Lakes, Diyaty lake (No. 21)	ON563521		BESPALAYA et al. (2024)
Russia: Nenets Autonomous Area, Nes village, Niznee Maglinskoe lake (No. 22)	ON563590	ON783939	BESPALAYA et al. (2024)
Russia: Yakutia, Srednekolymsk, lake next to the oil depot (No. 23)	ON563647, ON563648	ON783962	BESPALAYA et al. (2024)
Russia: Yakutia, Ust-Yansky District, Ust-Kuyga village (No. 24)	ON563600–ON563602		BESPALAYA et al. (2024)
Russia: Krasnoyarsk Territory (No. 25)	PQ032444, PQ032445		BESPALAYA et al. (2025)
Russia: Commander Islands (No. 26)	PQ032465		BESPALAYA et al. (2025)

Table 2. Information on primers used for PCR amplification and sequencing, and PCR conditions in this study

Primer	Direction	Sequence 5'–3'	Reference for primer	PCR condition
16S rRNA				
16Sar-sph	Forward	CGCGCCTGTTTATCAAAAACATCG	SAWADA et al. (2026)	98 °C 2 min, (98 °C 10 s, 52 °C 15 s, 68 °C 35 s) × 35, 68 °C 5 min
16Sbr-sph	Reverse	TCACGCCGGTCTGAACTCAGATC	SAWADA et al. (2026)	
28S rRNA				
28S-sph2F	Forward	CCAATGTGGGTGGTAAACTC	This study	98 °C 2 min, (98 °C 10 s, 62 °C 15 s, 68 °C 35 s) × 35, 68 °C 5 min
D6R	Reverse	CCAGCTATCCTGAGGGAAACTTCG	PARK & FOIGHIL (2000)	

SEM observations were conducted using a JSM-6510LV scanning electron microscope (JEOL Ltd., Japan) after platinum coating using a JEC-3000FC (JEOL Ltd.). Morphological characters were examined following the methods of KORNIUSHIN (1998), MANSUR & MEIER-BROOK (2000), and KORNIUSHIN & GLAUBRECHT (2002, 2006). The following shell measurements and indices were also obtained and calculated from digital images according to KUIPER (1983), ITUARTE (1996), and KORNIUSHIN (2001), using ImageJ v1.51 (SCHNEIDER et al. 2012): commissural index (commissural height/shell height; HK/H), convexity index (shell width/height; W/H), height index (shell height/length; H/L), hinge height ratio (hinge height/shell height; Hih/H), hinge length ratio (hinge length/shell length; Hil/L), ligament length, ligament-length index (ligament length/shell length; Li/L), shell length, shell height, and shell width.

GENETIC ANALYSIS

Genomic DNA was extracted from one specimen from each of localities No. 1 and No. 2, following OKAMOTO et al. (2006). Fragments of the mitochondrial 16S rRNA (16S) and nuclear 28S rRNA (28S) genes were amplified using polymerase chain reaction (PCR). PCR was performed using a T100 Thermal Cycler (Bio-Rad Laboratories, Inc., USA)

RESULTS

MORPHOLOGICAL DESCRIPTION

Shell (Figs 2–9, 12–26)

Shell small [shell length 2.8 ± 0.1 mm (average \pm standard deviation), shell height 2.3 ± 0.1 mm, shell width 1.9 ± 0.3 mm], thin, rounded subtrapezoidal, moderately low (H/L 0.81 ± 0.01), moderately inflated (W/H 0.80 ± 0.01). Anterior margin protruded strongly in locality No. 1, weakly in No. 2 (Figs 2–9, 12–15). Posterior margin rounded, slightly

and TaKaRa Ex Premier DNA Polymerase (Takara Bio, Japan). The primer sets and PCR conditions shown in Table 2 were used based on SAITO et al. (2022) and BESPALAYA et al. (2024). Cycle sequencing reactions were performed as described by SAWADA et al. (2021). The sequences obtained in this study were deposited in the International Nucleotide Sequence Database (INSD) Collaboration through the DNA Databank of Japan (LC919956–LC919959).

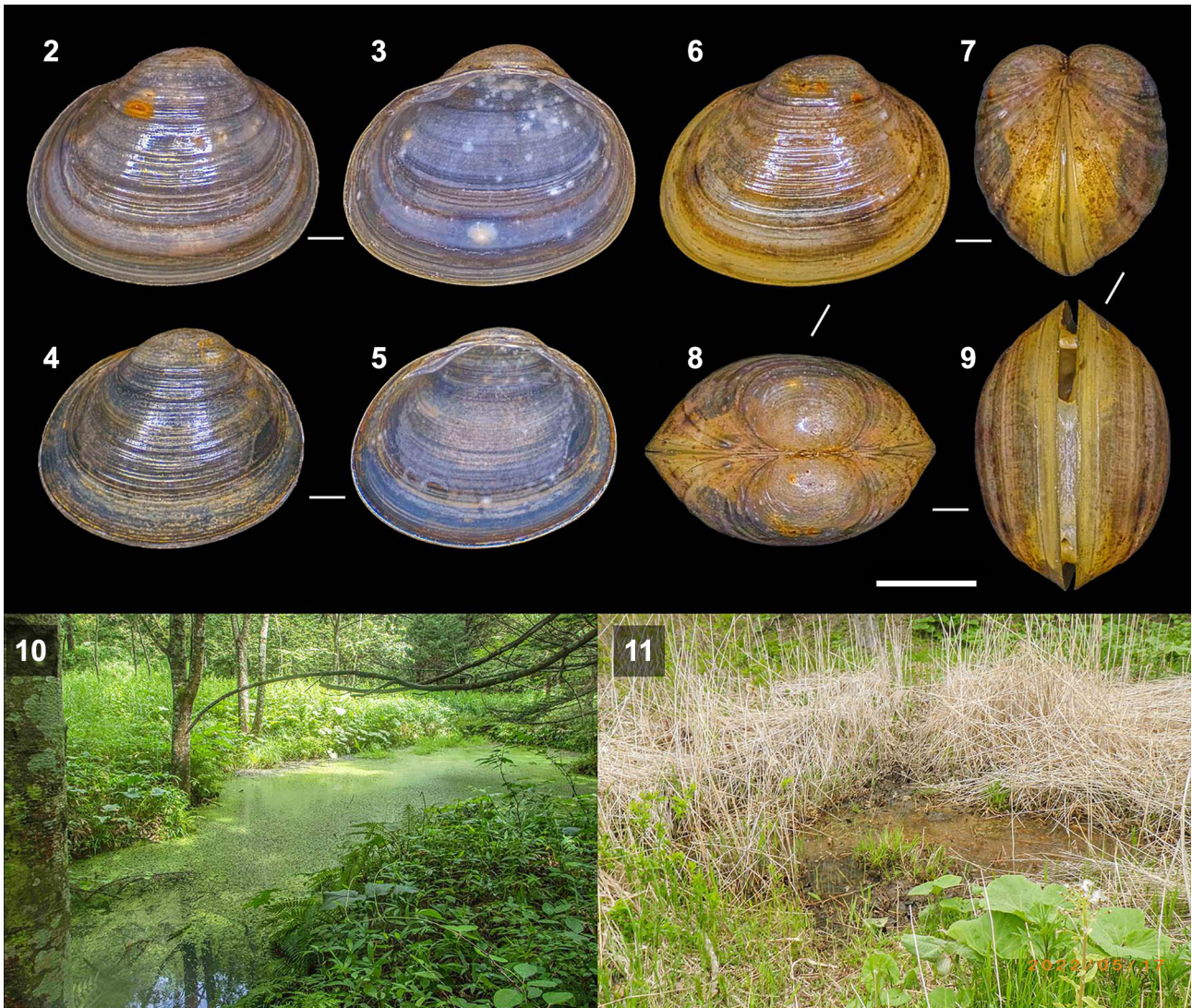
Haplotype relationships between the Japanese specimens and *E. milium* from Germany, Russia, and USA (Table 1) were estimated using 16S sequences provided by LEE & FOIGHIL (2003), GURALNICK (2005), and BESPALAYA et al. (2024, 2025) with statistical parsimony (CLEMENT et al. 2000) using PopART v1.7 (LEIGH & BRYANT 2015). In addition, genetic variations in the 28S sequences were visually evaluated using MEGA v11 (TAMURA et al. 2021). Before analyses, the sequences were aligned using MAFFT v7 (KATO et al. 2019) for each gene with the LINS-I strategy. The non-conserved positions of each gene were trimmed using Gblocks v0.91b (CASTRESANA 2000). A setting of minimum number of sequences for a flank position was changed to “50% of the number of sequences +1”. The other parameters were set to default values. The trimmed lengths of the 16S and 28S sequences were 440 and 400 bps, respectively.

truncated. Dorsal margin has slightly to moderately sloping shoulders. Posterior half of dorsal margin smoothly curved, slightly angular at anterior half. Ventral margin curved slightly in locality No. 1, strongly in No. 2. Umbo subcentral, shifted posteriorly, angled posteriorly, located at approximately 60% of shell length, broad, moderately swollen toward dorsal side (HK/H 0.92 ± 0.01). Sculpture on outer surface distinct, irregular, with concentric ribs outside umbonal region (Figs 16–17). Fine radial folds visible on umbonal region (Fig. 24). Concentric

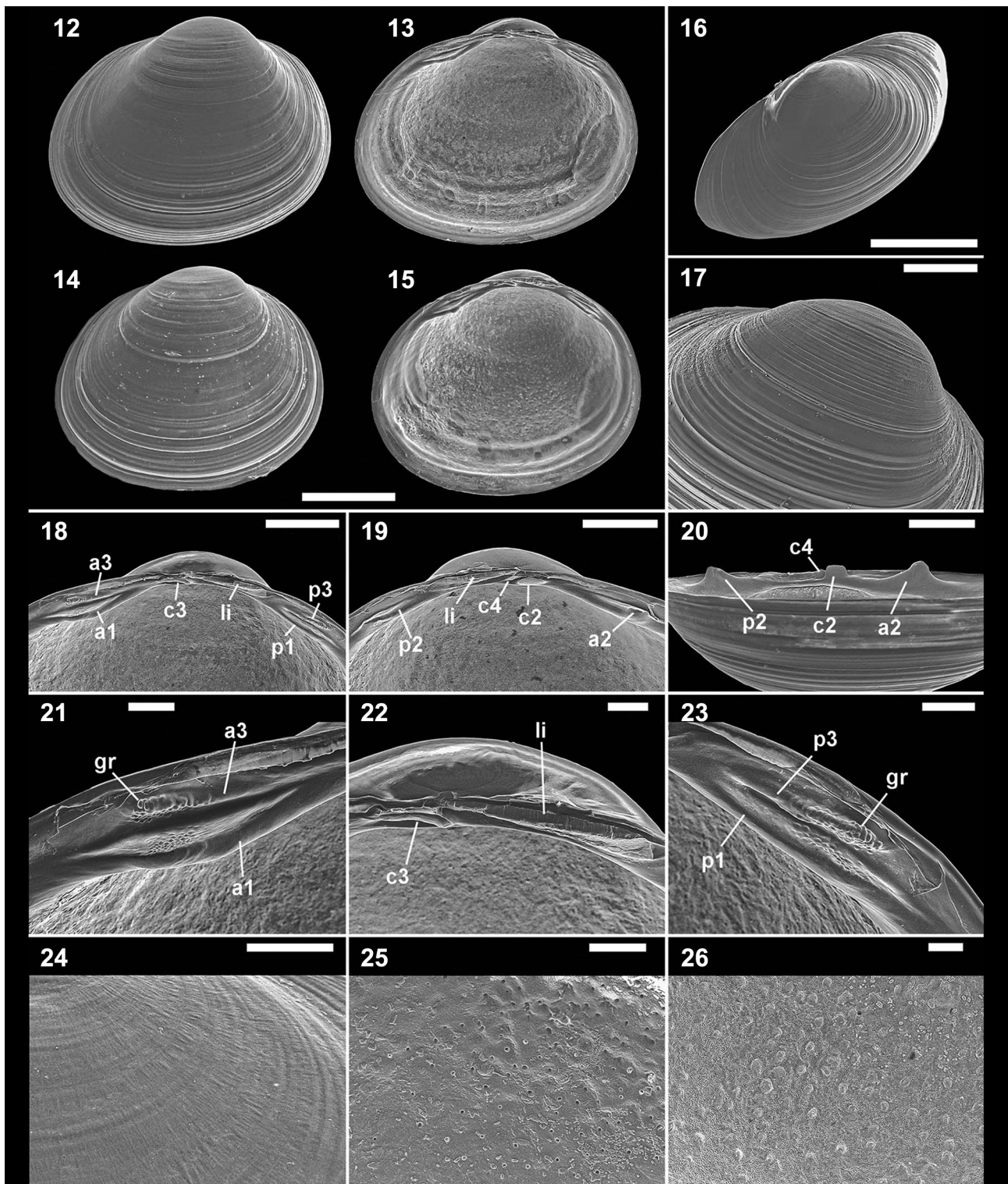
folds of periostracum absent. Outer surface glossy, coloured bluish grey to brownish grey with irregular broad brown concentric bands (Figs 2, 4). Inner surface mat, coloured grayish blue with irregular broad brown concentric bands and irregular milky white spots (Figs 3, 5). Shell visible yellowish brown before separating beige-coloured soft body (Figs 6–9). Pores distinguishable on inner shell surface, dense in umbonal region (approximately 50–80 μm between pores), sparse at other ones, approximately 5 μm in diameter (Fig. 25). Dimples present in one specimen from locality No. 1, small, dense, visible around centre of inner shell surface, approximately 30 μm in diameter (Fig. 26). Scars of inner radial muscles obscure, separated from pallial line at anterior portion, combining with posteriorly (Figs 13, 15).

Hinge (Figs 18–23)

Hinge plate narrow (H_{ih}/H 0.037 \pm 0.007), relatively long (H_{il}/L 0.53 \pm 0.01). Inner left cardinal tooth (c2) long, thin, strongly arched dorsoventrally (Fig. 19), strongly protruding laterally (Fig. 20). Outer left cardinal (c4) long, thin, almost parallel to c2 at anterior half, weakly arched at posterior, or almost straight entirely. Right cardinal tooth (c3) long, thin, anterior half straight, posterior end weakly curved, slightly thickened (Fig. 22). Left lateral teeth (a2, p2) long, thick (Fig. 19), with nearly central blunt cusps, strongly protruding laterally (Fig. 20). Right lateral teeth (a1, a3, p1, p3) long, thick with small grains at central portions (Figs 21, 23). Ligament not introverted, narrow, relatively long (L_i/L 0.18 \pm 0.01), not protruding externally (Fig. 22).



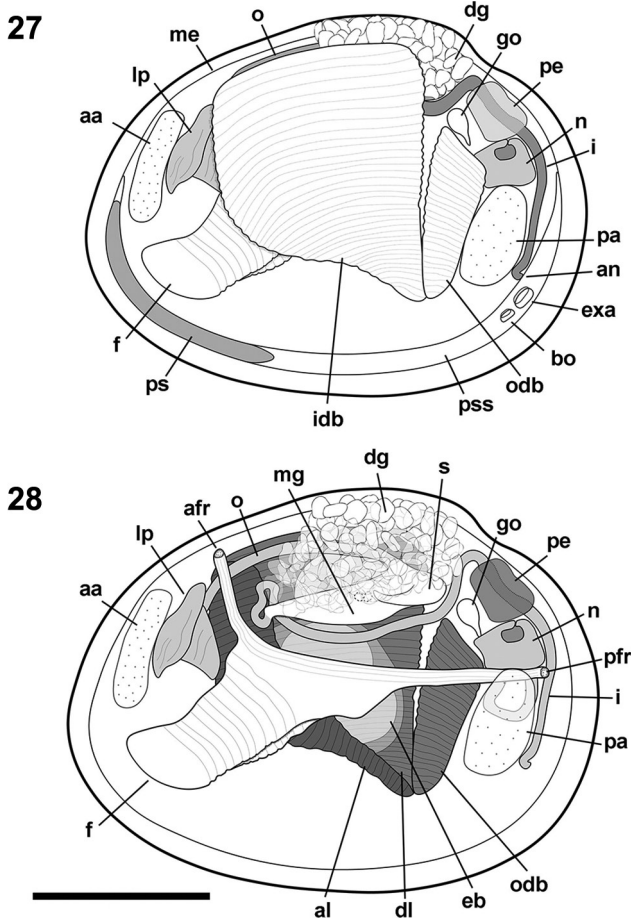
Figs 2–11. Specimens and habitats of *Euglesa milium*: 2–5 – empty shells from localities No. 1 (2–3) and No. 2 (4–5); 6–9 – specimens from locality No. 1 before separating soft bodies from lateral (6), posterior (7), dorsal (8), and ventral (9) views, scale bar 1 mm; 10–11 – habitat of localities No. 1 (10) and No. 2 (11)



Figs 12–26. Shell morphologies of *Euglesa milium*: 12–13, 16–19, 21–23 – locality No. 1; 14–15, 20, 24–26 – locality No. 2. 12–16 – shell from lateral (12–15) and dorsal (16) views; 17 – shell surface; 18–20 – hinge plates of the right (18) and left valves from the lateral (19) and ventral (20) views; 21–23 – anterior (21) and posterior (23) lateral and cardinal (22) teeth and ligament of the right valve; 24 – outer surface of umbonal region; 25 – inner surface of the umbonal region with dense pores; 26 – inner surface of central region of shell with dense dimples. Abbreviations: a1 – inner anterior lateral tooth of the right valve; a2 – anterior lateral tooth of the left valve; a3 – outer anterior lateral tooth of the right valve; c2 – inner cardinal tooth of the left valve; c3 – outer cardinal of the right valve; c4 – outer cardinal tooth of the left valve; gr, grain; li – ligament; p1 – inner posterior lateral tooth of the right valve; p2 – posterior lateral tooth of the left valve; p3 – outer posterior lateral tooth of the right valve. Scale bars: 1 mm (12–16), 0.5 mm (17–20), 0.1 mm (21–26)

Mantle cavity (Figs 27, 28)

Foot narrow, elongated, with deep groove on its ventral side and shallow lines on lateral sides, connected to anterior and posterior foot retractors. Anterior foot retractor adheres shell near labial palp. Distal portion of posterior foot retractor locates between posterior adductor muscle and nephridia. Digestive organs located dorsally along foot retractor. Labial palps at the anterodorsal of mantle cavity connect to oesophagus. Most of outer lateral surface of stomach, midgut, and digestive gland covered by large ctenidia. Intestine originating from midgut

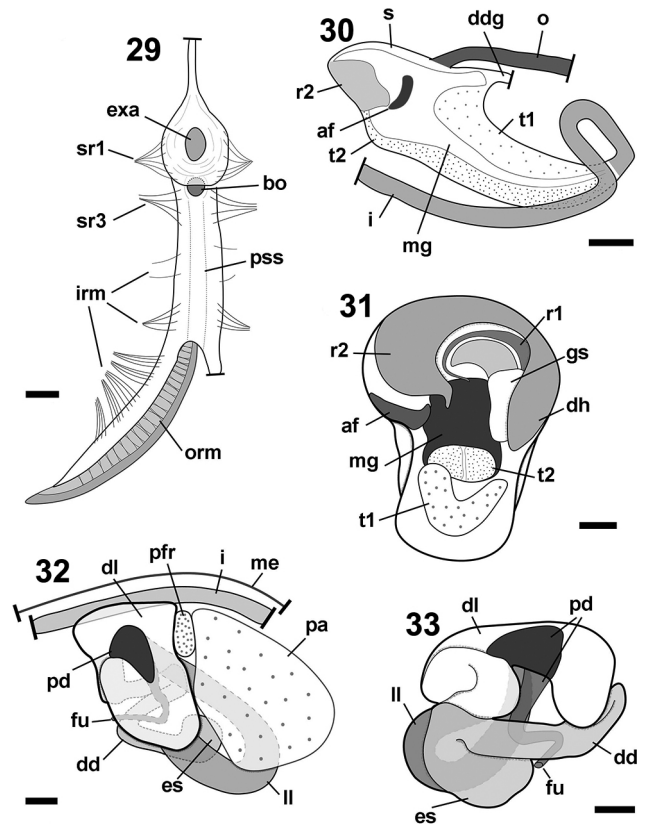


Figs 27–28. Schematic illustrations of the mantle cavity of *Euglesa milium* after removing the left mantle lobe (27) and inner morphology after removing the left ctenidium (28). Abbreviations: aa – anterior adductor muscle; afr – anterior foot retractor; al – ascending lamella of inner demibranch; an – anus; bo – branchial opening; dg – digestive gland; dl – descending lamella of inner demibranch; eb – embryo; exa – exhalant siphon; f – foot; go – gonad; i – intestine; idb – inner demibranch; lp – labial palp; me – mantle edge; mg – midgut; n – nephridium; o – oesophagus; odb – outer demibranch; pa – posterior adductor muscle; pe – pericardium; ps – pedal slit; pfr – posterior foot retractor; pss – perisiphonal suture; s – stomach. Scale bar 1 mm

coiled, running along ventral side of stomach, curving sharply at dorsal margin of mantle cavity, penetrating through pericardium, passing dorsal sides of nephridia and posterior adductor muscle, reaching anus on upper side of exhalant siphon. Gonad surrounded by intestine, pericardium, and nephridia.

Mantle edge (Figs 27, 29)

Only exhalant siphon present with one pair of retractors (sr1). Branchial opening present, with one pair of lower retractors (sr3). Upper branchial retractors (sr2) absent. Lower retractor fibres of st3 connected with perisiphonal suture. Perisiphonal su-



Figs 29–33. Schematic illustrations of the organs of *Euglesa milium*: 29 – mantle edge; 30 – digestive system around the stomach from the right lateral view; 31 – inner structure of the stomach from the dorsal view after removing the stomach roof and style sac in the midgut; 32–33 – left nephridium from the lateral (32) and coronal views (33). Abbreviations: af – anterior fold; bo – branchial opening; dd – distal duct; ddg – digestive gland duct; dh – dorsal hood; dl – dorsal lobe; es – excretory sac; exa – exhalant siphon; fu – funnel; gs – gastric shield; i – intestine; irm – bundles of inner radial mantle muscles; ll – lateral loop; me – mantle edge; mg – midgut; o – oesophagus; orm – bundles of inner radial mantle muscles; pa – posterior adductor muscle; pd – pericardial duct; pfr – posterior foot retractor; pss – perisiphonal suture; s – stomach; r1, r2 – fold of stomach wall; sr1, sr3 – siphonal retractors; t1 – major typhlosole; t2 – minor typhlosole. Scale bars 0.2 mm (29–30), 0.1 mm (31–33)

ture prominently elongated (almost same length as pedal slit), its posterior end corresponds to branchial opening. Pedal slit short. Inner radial mantle muscles long, thin, organised in five to six rather strong bundles, converging medially. Bundles of equally developed, strongly organised around pedal slit, weakly organised around in central perisiphonal suture. Outer radial mantle muscles short. Peripheral mantle edge thickened.

Ctenidium and brood pouch (Figs 27, 28)

Inner and outer demibranches present. Inner demibranch large, occupying a large portion of mantle cavity surface, with ascending and descending lamellae. Ascending lamella relatively low, approximately one-third height of descending one. Outer demibranch relatively large, with only one lamella, its anterior edge placed at eighth to tenth filaments of the inner one. Brood pouch developing along edge of ascending lamella, occupying the greatest part of ctenidium in later stages of development with 1–4 embryos per brood pouch. Seven filaments involved in formation of brood pouch.

Digestive system (Figs 28, 30, 31)

Mouth locates at base of outer lamellae of labial palps. Outer palps with anteriorly projecting angles, forming deep V-shaped incision between palps. Ventral surface of outer lips and dorsal surface of inner lips ridged. Oesophagus nearly uniform in thickness, running along dorsal edge of mantle cavity, between dorsal elevation of digestive glands, connecting to dorsal side of stomach. Stomach almost totally embedded in digestive glands, not separated

from midgut, stretched posteriorly, with narrow sorting area and dorsal hood on its roof. Anterior fold not elevated. Dorsal hood connects to posterior ridge of stomach wall (r2). Anterior ridge (r1) runs alongside posterior one (r2) until centre of stomach. Major typhlosole (t1) thick, elevated, forming one U-shaped loop on anterior stomach wall, directed to openings of digestive gland ducts. Minor typhlosole (t2) turning posteriorly at its proximal end. Intestine coil simple, with one loop.

Nephridium (Figs 32, 33)

Nephridium open type, with pericardial duct visible from dorsal lobe. Pericardial duct has two loops, forming S-shape, its tip forming narrow funnel. Pericardial duct curves sharply between dorsal lobe, connecting to lateral loop. Lateral loop well visible from dorsal side of nephridium after removing posterior adductor muscle, separated from dorsal lobe by loop of pericardial tube, opening to ventral portion of dorsal lobe. Anterior portion of lateral loop completely covered by dorsal lobe. Dorsal lobe rectangular, broad type, its length less than width, connecting to distal duct at its dorsal portion toward ventral direction of nephridium. Distal duct has three loops, opening to excretory sac at basal portion. Excretory sac relatively small, not extended anteriorly.

HABITAT

Living animals were found in a small pool approximately 1 m deep along a small mountainous stream at locality No. 1 (Fig. 10). At locality No. 2, specimens were obtained from a small marsh approximately 10 cm deep in the grass near the national

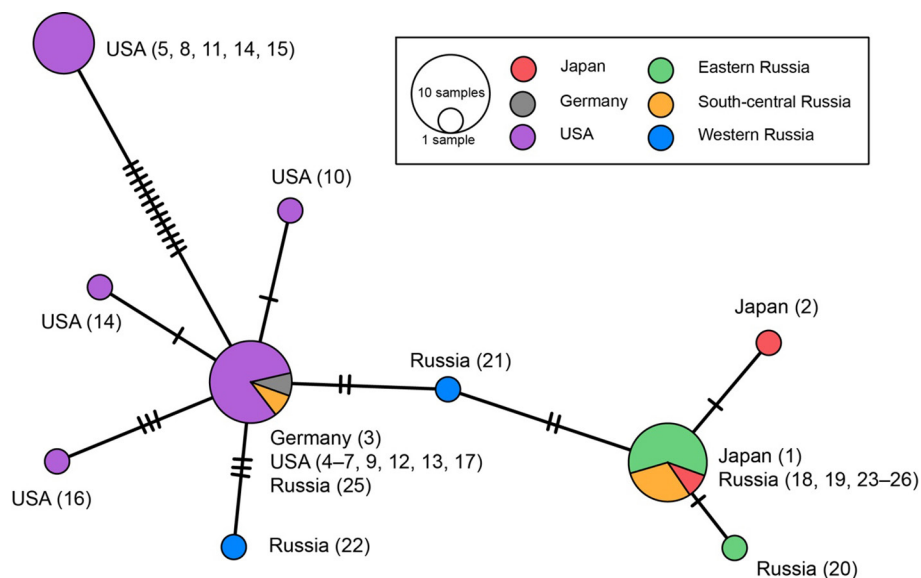


Fig 34. Statistical parsimony network showing the relationships between the 16S haplotypes detected in *Euglesa milium*. Each connection indicates a single mutation, and the hatch marks represent missing intermediate haplotypes. The numbers in parentheses indicate the locality numbers listed in Table 1

highway (Fig. 11). No other sphaeriid species was found at locality No. 1, whereas *E. japonica* (Pilsbry & Y. Hirase, 1908) was observed at locality No. 2.

GENETIC RELATIONSHIPS

A single mutation was detected between the 440 bp 16S sequences obtained from the two Japanese

specimens (Fig. 34). The sequences from locality No. 1 completely matched in aligned positions with those from Eastern and South-central Russia (localities No. 18, 19, 23–26). Japanese sequences were separated from those of Western Russia, Germany, and the USA by 2–8, 4–5, and 4–18 steps, respectively. The 400 bp 28S sequences obtained from five Japanese, Russian, and USA specimens were identical.

DISCUSSION

Euglesa milium is distinguished from other sphaeriid species by its typical subtrapezoidal shell shape, elongated perisiphonal suture, and ventrally positioned adductor muscles (ODHNER 1929). The states of the shell and anatomical characters of *E. milium* were summarised by KORNIUSHIN & GLAUBRECHT (2002), except for the digestive glands. The Japanese specimens possessed the typical shell and mantle characteristics of *E. milium*, showing variations in shell outline shapes between the two localities. The states of the examined characters of the shell, hinge, mantle edge, ctenidium, brood pouch, and nephridium were consistent with those of previous descriptions, although the anatomical characteristics were examined at only one locality. The overall characteristics of the digestive system and internal stomach structure were also similar to other *Euglesa* species (MANSUR & MEIER-BROOK 2000, KORNIUSHIN & GLAUBRECHT 2002). Distinct discrepancies were detected only in the form of the dorsal lobe of the nephridium: square type (ODHNER 1929), broad type (KORNIUSHIN & GLAUBRECHT 2002, this study). This may have been caused by the different developmental stages of the specimens, as indicated by KORNIUSHIN (1998).

The haplotype network analysis of 16S detected that the Japanese specimens had identical or similar haplotypes to geographically close (Eastern Russian) specimens, in addition to South-central Russian ones. The 28S sequences of the Japanese, Russian, and USA specimens were identical in the aligned positions. These haplotypic characteristics were consistent with the morphological similarities among the specimens from Japan and other regions, although only partial sequences of two genes were analysed in one specimen at each locality. Based on the morphological and haplotypic similarities, the examined Japanese specimens were identified as *Euglesa milium*. Among the forms detected in this species, Japanese specimens correspond to the typical form based on their relatively rounded ventral margin and irregular striations on the shell surface (WESTERLUND 1873, KUIPER 1942, MOUTHON & AUDIBERT 2023). A Japanese name of this species is proposed here as

“Hoppe-mameshijimi”, which refers to the distinctly protruded anterior margin of shell.

Approximately 25 Sphaeriidae species have been recorded from Japan (MORI 1938a, AKIYAMA & USUI 2017). *Euglesa milium* is one of the most easily identifiable species within the family, and can be distinguished from most other Japanese species based on a combination of shell and anatomical characteristics (ODHNER 1929, KORNIUSHIN & GLAUBRECHT 2002, KILEEN et al. 2004). Among the Japanese species, *E. subtruncata* (Malm, 1855), which was described as a subspecies *E. s. alta* (Mori, 1938) (Hanataka-mameshijimi in Japanese) from Japan, is probably most morphologically similar to *E. milium*. The elongated perisiphonal suture is shared by *E. milium* and *E. subtruncata* (ODHNER 1929). However, *E. milium* does not possess an oblique shell characteristic of *E. subtruncata*, and the ventrally positioned adductor muscles of the former species distinguish the two species. The less rectangular form of *E. milium* (Fig. 4) can also be confused with *E. nitida* (Jenyns, 1832) (Tsuya-mameshijimi), whereas the absence of umbonal grooves in a shell and elongated perisiphonal suture clearly identify *E. milium* (ODHNER 1929, MORI 1938b, KILEEN et al. 2004).

The classification of Japanese Sphaeriidae has long stagnated, whereas phylogenetic analyses based on samples from wide areas of Japan have recently detected numerous Japanese clades (SAITO et al. 2022). Although a subsequent study determined the scientific names of many of these clades (BESPALAYA et al. 2024), taxonomic work based on the revision of the type series or topotypes has not been conducted. Given the lack of study on Sphaeriidae in Japan, it is not surprising that *E. milium* with Holarctic distribution has been overlooked. The discovery of this species also suggests that other sphaeriids, whose geographic distributions include this region, have also been overlooked, and that the species richness of Japanese Sphaeriidae has been underestimated. Therefore, an integrative taxonomy based on the re-examination of type specimens and exhaustive taxon sampling is required for Japanese Sphaeriidae.



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REFERENCES

- AKIYAMA Y. B., USUI T. 2017. The first record of *Pisidium casertanum* (Bivalvia, Sphaeriidae) in Esashi Town, Hokkaido. *Bulletin of the Okhotsk Museum ESASHI* 7–8: 35–38.
- AMANTE C., EAKINS B. W. 2009. ETOPO1 1 Arc-minute global relief model: procedures, data sources and analysis. NOAA technical memorandum NESDIS NGDC-24. National Geophysical Data Center, NOAA. <https://doi.org/10.7289/V5C8276M>
- BESPALAYA Y., BULAKHOVA N., GOFAROV M., KONDAKOV A., TOMILOVA A., BERMAN D. 2020. Occurrence of the mollusc species *Euglesa globularis* (Clessin, 1873) in North-East Asia (Magadan, Russia) with data on dispersal mechanism and vectors. *Limnologia* 85: 125832. <https://doi.org/10.1016/j.limno.2020.125832>
- BESPALAYA Y. V., AKSENOVA O. V., AKSENOV A. S., SOKOLOVA S. E., KROPOTIN A. V., TRAVINA O. V., KHREBTOVA I. S., KONDAKOV A. V., ELISEEVA T. A., ZUBRII N. A., BUTORINA E. V. 2025. Taxonomic and genetic diversity of freshwater mollusks of Bering Island (the Commander Islands, Kamchatka Krai, Russia). *Inland Water Biology* 18(4): 837–848. <https://doi.org/10.1134/S1995082925600620>
- BESPALAYA Y. V., VINARSKI M. V., AKSENOVA O. V., BABUSHKIN E. S., GOFAROV M. Y., KONDAKOV A. V., KONOPLEVA E. S., KROPOTIN A. V., MABROUKI Y., OVCHANKOVA N. B., PALATOV D. M., SOKOLOVA S. E., SHEVCHENKO A. R., TRAVINA O. V., TAYBI A. F., SOBOLEVA A. A., ZUBRII N. A., BOLOTOV I. N. 2024. Phylogeny, taxonomy, and biogeography of the Sphaeriinae (Bivalvia: Sphaeriidae). *Zoological Journal of the Linnean Society* 201(2): 305–338. <https://doi.org/10.1093/zoolinnean/zlad139>
- CASTRESANA J. 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. *Molecular Biology and Evolution* 17(4): 540–552. <https://doi.org/10.1093/oxfordjournals.molbev.a026334>
- CLEMENT M., POSADA D., CRANDALL K. A. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9(10): 1657–1659. <https://doi.org/10.1046/j.1365-294x.2000.01020.x>
- CLEWING C., BÖSSNECK U., OHEIMB P. V. VON, ALBRECHT C. 2013. Molecular phylogeny and biogeography of a high mountain bivalve fauna: The Sphaeriidae of the Tibetan Plateau. *Malacologia* 56(1&2): 231–252. <https://doi.org/10.4002/040.056.0213>
- DYDUCH-FALNIOWSKA A. 1983. Shell microstructure and systematics of Sphaeriidae (Bivalvia, Eulamellibranchiata). *Acta Zoologica Cracoviensia* 26(9): 251–296.
- FUKUDA H., HAGA T., TATARA Y. 2008. Niku-nuku: a useful method for anatomical and DNA studies on shell-bearing. *Zoosymposia* 1: 15–38. <https://doi.org/10.11646/zoosymposia.1.1.5>
- GRAF D. L. 2013. Patterns of freshwater bivalve global diversity and the state of phylogenetic studies on the Unionoida, Sphaeriidae, and Cyrenidae. *American Malacological Bulletin* 31(1): 135–153. <https://doi.org/10.4003/006.031.0106>
- GRAF D. L., CUMMINGS K. S. 2026. The freshwater mussels (Unionoida) of the world (and other less consequential bivalves). MUSSEL Project Web Site. Available online at <http://www.mussel-project.net/> (accessed 9 February 2026).
- GURALNICK R. P. 2005. Combined molecular and morphological approaches to documenting regional biodiversity and ecological patterns in problematic taxa: a case study in the bivalve group *Cyclocalyx* (Sphaeriidae, Bivalvia) from western North America. *Zoologica Scripta* 34(5): 469–482. <https://doi.org/10.1111/j.1463-6409.2005.00205.x>
- HOLOPAINEN I. J., KUIPER J. G. J. 1982. Notes on the morphometry and anatomy of some *Pisidium* and *Sphaerium* species (Bivalvia, Sphaeriidae). *Annales Zoologici Fennici* 19(2): 93–107.
- IYAMA H. 2008. Morphological review of *Pisidium kawamurae* *hukuiense* Mori, 1938 and *Pisidium japonicum* Pilsbry and Hirase, 1908 (Bivalvia: Sphaeriidae). *Venus* 66(3–4): 161–167. https://doi.org/10.18941/venus.66.3-4_161
- IYAMA H., TAKAHASHI S. 2004. Occurrence of *Pisidium conventus* aff. *akkesiense* in Gunma Prefecture, Japan (Bivalvia: Sphaeriidae). *Venus* 62(3–4): 111–116. https://doi.org/10.18941/venus.62.3-4_111
- INABA O., KAYANE S., IKEZAWA H., IYAMA H. 2011. *Pisidium* (Bivalvia: Sphaeriidae) collected from Ibaraki Prefecture, Japan. *Chiribotan* 41(2): 89–92.
- ITUARTE C. 1996. Argentine species of *Pisidium* Pfeiffer, 1821, and *Musculium* Link, 1807 (Bivalvia: Sphaeriidae). *The Veliger* 39: 189–203.
- KATOH K., ROZEWICKI J., YAMADA K. D. 2019. MAFFT online service: multiple sequence alignment, inter-



- active sequence choice and visualization. *Briefings in Bioinformatics* 20(4): 1160–1166.
<https://doi.org/10.1093/bib/bbx108>
- KEBAPÇI Ü. 2023. Cyrenoidea Ve Sphaerioidea. In: YILDIRIM Z. (ed.). Türkiye Yumuşakçaları Tatlısu Çiftçenellileri (Bivalvia) 1. Cilt. Iksad publishing house, Ankara, pp. 65–92 [in Turkish].
- KERNEY M. 1999. Atlas of the land and freshwater molluscs of Great Britain and Ireland. Harley Books, Colchester.
- KILEEN I., ALDRIDGE D., OLIVER G. 2004. Freshwater bivalves of Britain and Ireland. Field Studies Council, Telford.
- KORNIUSHIN A. V. 1996. Dvustvorchatyje molluski nadsemejstva Pisidioidea palearktiki: fauna, sistematika, filogenija. Schmalhausen Institute of Zoology, Kiev [in Russian].
- KORNIUSHIN A. V. 1998. A comparative investigation of nephridia in fingernail and pill clams (Bivalvia Pisidioidea). *Malacological Review, Supplement 7*: 53–63.
- KORNIUSHIN A. 2001. Taxonomic revision of the genus *Sphaerium* sensu lato in the Palaearctic Region, with some notes on the North American species (Bivalvia: Sphaeriidae). *Archiv für Molluskenkunde* 129: 77–122.
<https://doi.org/10.1127/arch.moll/129/2001/77>
- KORNIUSHIN A. V., GLAUBRECHT M. 2002. Phylogenetic analysis based on the morphology of viviparous freshwater clams of the family Sphaeriidae (Mollusca, Bivalvia, Veneroidea). *Zoologica Scripta* 31(5): 415–459.
<https://doi.org/10.1046/j.1463-6409.2002.00083.x>
- KORNIUSHIN A. V., GLAUBRECHT M. 2006. Anatomy and reproduction of viviparous *Pisidium* (*Parapisidium*) *reticulatum* Kuiper, 1966: implications for the phylogeny of Sphaeriidae (Mollusca: Bivalvia: Heterodonta). *Organisms Diversity & Evolution* 6(3): 185–195.
<https://doi.org/10.1016/j.ode.2005.09.003>
- KUIPER J. G. J. 1942. Beiträge zur Ökologie der niederländischen Pisidien. *Basteria* 7(1–2): 23–40.
- KUIPER J. G. J. 1968. Die spätpleistozänen Pisidien des ehemaligen Aschersiebener Sees. *Archiv für Molluskenkunde* 98(1–2): 23–38.
- KUIPER J. G. J. 1983. The Sphaeriidae of Australia. *Basteria* 47(1–4): 3–52.
- KUIPER J. G. J. 2009. Fossil records of Palaearctic *Pisidium* species in tropical Africa (Bivalvia, Sphaeriidae). *Zoologische Mededelingen* 83(10): 593–594.
- KURODA T. 1930. Chapter 3. Nantai dobutsu (Mollusks). In: TANAKA A. (ed.). *Nihon Kita Arupusu Kosho No Kenkyu*. (Limnology of the North Japan Alps). Shinano Kyoiku Kai Kita Azumino Bukai, Omachi, pp. 621–626 [in Japanese].
- LEE T. 2019. Sphaeriidae Deshayes, 1855 (1820). In: CUMMINGS K., LYDEARD C. (eds). *Freshwater Mollusks of the World. A Distribution Atlas*. Johns Hopkins University Press, Baltimore, pp. 197–201.
<https://doi.org/10.1353/book.66164>
- LEE T., FOIGHIL D. O. 2003. Phylogenetic structure of the Sphaeriinae, a global clade of freshwater bivalve molluscs, inferred from nuclear (ITS-1) and mitochondrial (16S) ribosomal gene sequences. *Zoological Journal of the Linnean Society* 137(2): 245–260.
<https://doi.org/10.1046/j.1096-3642.2003.00047.x>
- LEIGH J. W., BRYANT D. 2015. POPART: full-feature software for haplotype network construction. *Methods in Ecology and Evolution* 6(9): 1110–1116.
<https://doi.org/10.1111/2041-210X.12410>
- MANSUR M. C. D., MEIER-BROOK C. 2000. Morphology of *Eupera* Bourguignat 1854, and *Byssanodonta* Orbigny 1846 with contributions to the Phylogenetic systematics of Sphaeriidae and Corbiculidae (Bivalvia: Veneroidea). *Archiv für Molluskenkunde* 128: 1–59.
<https://doi.org/10.1127/arch.moll/128/2000/1>
- MORI S. 1933. On the classification of Japanese *Sphaerium*. *The Venus* 4(3): 149–158. [in Japanese]
https://doi.org/10.18941/venusomsj.4.3_149
- MORI S. 1935a. On the Classification of Japanese *Sphaerium* (2). *The Venus* 5(4): 202–206. [in Japanese]
https://doi.org/10.18941/venusomsj.5.4_202
- MORI S. 1935b. Description of a new subspecies of *Pisidium* from Etorohu Island. *The Venus* 5(4): 194–195.
https://doi.org/10.18941/venusomsj.5.4_194
- MORI S. 1936. On the classification of Japanese *Sphaerium* (3). *The Venus* 6(2): 108–110. [in Japanese]
https://doi.org/10.18941/venusomsj.6.2_108
- MORI S. 1937. On the classification of Japanese *Sphaerium* (4). *The Venus* 7(4): 170–172.
https://doi.org/10.18941/venusomsj.7.4_170
- MORI S. 1938a. Molluscan fauna of the limestone caves in Miyako-zima of the Ryuku Islands. II. Descriptions of two new molluscs, *Cochliopopsis basiangulata* n.g., n. sp. and *Pisidium* (*Neopisidium*) *cavernicum* n. sp. *Biogeographica* (Tokyo) 3: 110–114.
- MORI S. 1938b. Classification of Japanese *Pisidium*. *Memoirs of the College of Science, Kyoto Imperial University, Series B* 14: 255–278.
- MOUTHON J., AUDIBERT C. 2023. Gros plan sur quelques formes ou variétés de quatre espèces d'*Euglesa* (Bivalvia, Sphaeriidae). *Folia Conchylologica* 68: 3.
- ODHNER N. 1929. Die Molluskenfauna des Tåkerns. Utgiven av K Svenska Vetenskaps Akademien 8: 1–130.
- OKAMOTO T., MOTOKAWA J., TODA M., HIKIDA T. 2006. Parapatric distribution of the lizards *Plestiodon* (Formerly *Eumeces*) *laticutatus* and *P. japonicus* (Reptilia: Scincidae) around the Izu Peninsula, central Japan, and its biogeographic implications. *Zoological Science* 23(5): 419–425.
<https://doi.org/10.2108/zsj.23.419>
- OOYAGI A., IEYAMA H. 2017. *Pisidium* (Bivalvia: Sphaeriidae) collected from Shimokita Peninsula, Aomori Prefecture, Japan. *Chiribotan* 47(1–4): 87–89.
- PARK J.-K., FOIGHIL D. Ó. 2000. Sphaeriid and corbiculid clams represent separate heterodont bivalve radiations into freshwater environments. *Molecular Phylogenetics and Evolution* 14(1): 75–88.
<https://doi.org/10.1006/mpev.1999.0691>
- PILSBRY H. A., HIRASE Y. 1908. New land and fresh-water Mollusca of the Japanese Empire. *Proceedings of the Academy of Natural Sciences of Philadelphia* 60: 31–32.



- REUTER H. I., NELSON A., JARVIS A. 2007. An evaluation of void-filling interpolation methods for SRTM data. *International Journal of Geographical Information Science* 21(9): 983–1008.
<https://doi.org/10.1080/13658810601169899>
- SAITO T., FUJIMOTO K., UCHIDA S., YAMAZAKI D., HIRANO T., SANO I., YE B., KAGAWA O., SHARIAR M. S., DO V. T., MORII Y., PROZOROVA L., CHIBA S. 2022. Uncovering overlooked diversity using molecular phylogenetic approach: A case of Japanese sphaeriid clams (Bivalvia: Sphaeriidae). *Molecular Phylogenetics and Evolution* 173: 107508.
<https://doi.org/10.1016/j.ympev.2022.107508>
- SAWADA N., KAMEDA Y., FUKUDA H. 2026. *Cavernacmella dentata* n. sp. (Caenogastropoda: Truncatelloidea: Assimineidae) endemic to the Sekigahara Limestone Cave, central Honshū, Japan. *Molluscan Research* 46(1): 49–67.
<https://doi.org/10.1080/13235818.2025.2592643>
- SAWADA N., HWANG C.-C., HARL J., NAKANO T. 2021. Integrative taxonomy of insular land snails of the genus *Sicradiscus* Páll-Gergely, 2013 (Gastropoda, Plectopylidae) with description of a new species. *Journal of Zoological Systematics and Evolutionary Research* 59(8): 1798–1815.
<https://doi.org/10.1111/jzs.12553>
- SCHNEIDER C. A., RASBAND W. S., ELICEIRI K. W. 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 9(7): 671–675.
<https://doi.org/10.1038/nmeth.2089>
- STAROBOGATOV Y. I., BOGATOV V. V., PROZOROVA L. A., SAENKO E. M. 2004. Molluscs. In: TSALOLIKHIN S. J. (ed.). *Key to the Freshwater Invertebrates of Russia and Adjacent Lands*. Vol. 6 (Molluscs, Polychaetes, Nemerteans). Nauka, Saint-Petersburg, pp. 11–492.
- STELFOX A. W., KUIPER J. G. J., MCMILLAN N. F., MITCHELL G. F. 1972. *The Late-Glacial and Post-Glacial Mollusca of the White Bog*, Co. Down. *Proceedings of the Royal Irish Academy. Section B: Biological, Geological, and Chemical Science* 72: 185–207.
- TAMURA K., STECHER G., KUMAR S. 2021. MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution* 38(7): 3022–3027.
<https://doi.org/10.1093/molbev/msab120>
- WELTER-SCHULTES F. W. 2012. *European non-marine molluscs, a guide for species identification*. Planet Poster Editions, Göttingen.
- WESTERLUND C. A. 1873. *Fauna molluscorum terrestrium et fluviatilium Sveciae, Norvegiae et Daniae*. I. *Landmolluskerna*. v.1-2. Adolf Bonnier, Stockholm.
- WESTERLUND C. A. 1883. *Von der Vega-Expedition in Asien gesammelte Binnenmollusken*. *Nachrichtenblatt der Deutschen Malakozoologischen Gesellschaft* 15(3-4): 48–59.

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