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Unraveling a 70-year-old Taxonomic Puzzle: Redefining the Genus *Ikedosoma* (Annelida: Echiura) on the Basis of Morphological and Molecular Analyses

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After a long-standing taxonomic confusion, the echiurid genus *Ikedosoma* Bock, 1942, endemic to Japan and surroundings, is redefined on the basis of morphological and molecular analyses of many new Japanese materials and some museum specimens. The re-examination of a syntype of *I. elegans* (Ikeda, 1904), the type species of the genus, first revealed that its oblique muscle layer is continuous throughout and never fasciculate between longitudinal muscle bands, unlike those described in the definitions that have prevailed for ca. 70 years, making this genus indistinguishable from *Listriolobus* Spengel, 1912. Two Japanese species of *Ikedosoma*, *I. elegans* and *I. gogoshimense* (Ikeda, 1904), which were thus redefined, had also been poorly defined in the past to the point of being nearly indistinguishable from each other, largely due to incomplete descriptions and poor collections. Molecular phylogenetic analyses using 18S and 28S ribosomal RNA, histone H3, and cytochrome *c* oxidase subunit I (COI) genes clearly confirmed the distinction between these two species, their monophyletic origin, and their distinction from *L. sorbillans* (Lampert, 1883). The genus *Ikedosoma* thus validated is morphologically distinguishable from *Listriolobus* by the absence of a rectal caecum. *Ikedosoma elegans* and *I. gogoshimense* also differ in the disposition of gonoduct pairs. The third known species, *I. qingdaoense* Li, Wang and Zhou, 1994, from Qingdao, North China, lacks information on oblique muscle layers, which makes even its generic affiliation uncertain.

Key words: Annelida, Echiura, Echiuridae, Thalassematinae, *Ikedosoma*, revision, taxonomy, molecular phylogeny

INTRODUCTION

For taxonomists, solving taxonomic puzzles is often more interesting than simply describing new species, although both processes undoubtedly contribute to our better understanding of biodiversity. Taxonomic puzzles, or *incertae sedis*, include uncertain taxonomic identities caused by various factors, ranging from incomplete original descriptions, to lack of name-bearing types, to subsequent misidentifications, to cryptic species issues. In the present study, we resolved a taxonomic puzzle in echiurans, mainly caused by incomplete descriptions and misinterpretations that have prevailed for ca. 70 years.

Echiurans (spoon worms) are a group of marine coelomate invertebrates, which present a cylindrical trunk and a nonretractable proboscis. There are ca. 165 known species of echiurans (Biseswar, 2012). This group has been ranked as a phylum since 1939 (for further details, see Fisher, 1946); however, recent molecular phylogenetic and phylogenomic analyses strongly indicate that these organisms belong to the phylum Annelida (McHugh, 1997; Struck et al., 2007, 2011; Kvist and Siddall, 2013; Weigert et al., 2014).

Echiurans are still awaiting taxonomic revision, as exemplified in the revision by Nishikawa (2002) in which the order Heteromyota Fisher, 1946 and its only family Ikedidae Bock, 1942 were abolished for being established on misunderstood morphological peculiarities. Thus, echiurans are now divided into two orders: Echiuroinea Bock, 1942 (with two families: Echiuridae Quatrefages, 1847 and Bonelliidae Lacaze-Duthiers, 1858) and Xenopneusta Fisher, 1946 (with a single family: Urechidae Monro, 1927). *Ikeda taenioides* (Ikeda, 1904), the type species of the still valid genus *Ikeda* Wharton, 1913, is now included in the family Echiuridae; however, Nishikawa (2002) suggested its possible relationship with the family Bonelliidae. This suggestion has been validated using molecular methods (Lehrke, 2012; Goto et al., 2013), giving new insights into overall echiuran systematics.

Another taxonomic problem is related to the still less-known echiurid genus *Ikedosoma* Bock, 1942, with three described species, endemic to Japanese and adjacent waters. As described below in the Historical Background section, this problem arises from the fact that this genus was established exclusively on the basis of incomplete descriptions of the species concerned.

Here, we revise the definition of the genus *Ikedosoma* and its two Japanese species on the basis of detailed examination of a syntype and many newly collected specimens. We also conducted molecular phylogenetic analyses using nuclear 18S ribosomal RNA (hereafter, 18S), 28S ribosomal RNA (28S), histone H3 (H3), and the mitochondrial

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cytochrome *c* oxidase subunit I (COI) genes. Molecular information has been shown to be a powerful tool for the delimitation of species and a reliable support for phylogenetic hypotheses. This did hold in our present taxonomic studies of echiurans with fewer diagnostic morphological characters.

HISTORICAL BACKGROUND AND TAXONOMIC PROBLEMS

Three species belong to the genus *Ikedosoma*: *I. elegans* (Ikeda, 1904) and *I. gogoshimense* (Ikeda, 1904) (so far recorded from the Japanese coast) as well as *I. qingdaoense* Li, Wang and Zhou, 1994 (from Quingdao, North China). On the other hand, *I. pirotansis* Menon and DattaGuputa, 1962 from Pirotan Island, Gulf of Kutch, India, is now included in the genus *Ikeda* (Nishikawa, 2002).

Ikeda's (1904) original descriptions of *I. elegans* (originally described as *Thalassema elegans*) and *I. gogoshimense* (as *T. gogoshimense*) were brief; however, the syntypes of the former were redescribed in detail by Ikeda (1907). Since its original and subsequent descriptions, *I. elegans* has not been fully described taxonomically, mainly due to a lack of further specimens. On the other hand, the internal morphology of *I. gogoshimense* remains uncertain; its original description only mentioned that the species "shows… an essential agreement with *Thalassema elegans*…, the agreement may be said to be complete… It therefore seems unnecessary to give a description of the anatomy" (Ikeda, 1904, p. 67); however, this description also noted the tendency to present more gonoducts in males than in females. Some differences in the habitus and ecology between these two species, mentioned in the original descriptions, will be considered below.

In his comprehensive taxonomic revision of echiurans, Bock (1942) established the genus *Ikedosoma* for *T. elegans* Ikeda, 1904 and placed it within the subfamily Thalassematinae Forbes and Goodsir, 1841 of Echiuridae. This reclassification was based on peculiarities regarding gonoduct number and arrangement in *T. elegans*. According to Ikeda's (1904, 1907) descriptions, up to 27 gonoducts could be observed; males usually had more gonoducts than females, and they were often clustered (e.g., two or more gonoducts gathering together). Soon afterward, Fisher (1946) considered it as a valid genus, and his "key to genera" explicitly described the genus *Ikedosoma* as having an oblique muscle layer fasciated between longitudinal muscle bands (LMB), typically like that observed in the genus *Ochetostoma* Rüppell and Leuckart, 1828. At that time, Fisher (1946) cited Ikeda's (1907, p. 50) description that "the circular muscle fibers form more or less regularly arranged transverse bundles," followed by the sentence that "This structure of the body wall closely approximates that of *Ochetostoma*…" (Fisher, 1946, p. 224). Therefore, it seems clear that Fisher regarded Ikeda's "transverse bundles" as oblique muscle fascicles, which we show to have been a misunderstanding in the present study. Fisher (1946) also transferred *T. gogoshimense* to the genus *Ikedosoma* on the basis of some similarities in the internal morphology between *T. gogoshimense* and *I. elegans,* as stated above. Following this, Fisher (1946) distinguished *Ikedosoma* from *Ochetostoma* mainly by the more and often clustered gonoducts (at least in male) in the former. Fisher also noted the difference between the two genera in the position of the vascular ring vessel; however, the validity of this character remains uncertain to distinguish between the two genera. His amended definition of the genus *Ikedosoma*, as often lacking the vessel feature, has been followed by subsequent authors till date (Stephen and Edmonds, 1972; DattaGupta and Menon, 1976; Nishikawa, 1992; Edmonds, 2000).

Very recently, our re-examination of the *T. elegans* syntype deposited in the Zoological Collection of the University Museum, University of Tokyo (UMUTZ), has revealed that its oblique muscle layer is rather continuous, even between LMB, and never fasciculate, in contrast to Fisher's amended definition. Furthermore, according to Sato (1934), all the 18 specimens of *I. gogoshimense* he examined showed no sexual difference in the gonoduct number. Subsequently, *Ikedosoma* may not be distinguishable from *Listriolobus* Spengel, 1912, which also has a banded longitudinal muscle layer and a continuous oblique muscle layer and does not present sexual dimorphism in the gonoduct number (Fisher, 1949; Nishikawa, 2004). Therefore, the validity of *Ikedosoma* needs to be re-examined.

The brief original description of *I. gogoshimense* also remains contentious. In addition, the absence of name-bearing type specimens makes the existence of this species uncertain, despite which it has often been used for embryological and cytological studies (Sawada and Ochi, 1962; Ochi, 1963, 1976; Sawada and Noda, 1963; Sawada et al., 1975). In summary, the total number of gonoducts, if considered as a presumptive diagnostic feature, cannot be used for distinction between *I. gogoshimense* and *I. elegans* (six to 32 in the former and 13 to 27 in the latter); thus, the taxonomic distinction between these two species remains doubtful.

MATERIALS AND METHODS

Materials

From the UMUTZ echiuran collection, we examined a specimen of *T. elegans*, labeled as "Type specimen," which we assume corresponds to the "Specimen D" of the syntypes in Ikeda (1907), judging from its sex (female) and characteristic number and arrangement of gonoducts. The specimen was helpful for identifying the other five specimens of *I. elegans*, including two specimens long kept at our laboratory (one from the Takasu sandy flat, Okayama Pref., Seto Inland Sea and the other from a sandy flat of Ikarise Islet at the entrance of the Lake Hamana, Shizuoka Pref., Enshûnada Sea) and three newly collected specimens from the Ikarise in July 2012 and 2013 for morphological and molecular analyses (Fig. 1).

As described above, the taxonomic identity of *I. gogoshimense* remains unclear. Therefore, we collected a total of 41 individuals for morphological and molecular analyses during 2010 to 2013 from the type localities, Gogoshima Island in the Seto Inland Sea and Moroiso Inlet of Misaki in Sagami Bay, as well as from three other localities in the Seto Inland Sea (Ushimado and Takasu, Okayama Pref. and Hachi-no-higata, Hiroshima Pref.; Fig. 1). No inconsistencies were found after careful comparisons of their external appearance, including living coloration, ecology (particularly the size and shape of fecal pellets), and their internal structure, with the original description. These specimens were then identified as *I. gogoshimense*. We also examined 14 specimens deposited in the Toyama Science Museum (TOYA), Tohoku University Museum (TUM), National Museum of Nature and Science, Tsukuba (NSMT), as well as the ones long kept at our laboratory.

We also collected an individual of *Ochetostoma erythrogrammon* Rüppell and Leuckart, 1828 and two individuals of *Listriolobus*

sorbillans (Lampert, 1883) from Sesoko, Okinawa Island (26°38′N, 127°52′E) and Kabira Bay, Ishigaki Island (24°27′N, 124°08′E), Ryukyu Islands, respectively, for molecular analyses.

The collected individuals were first anesthetized with menthol. Small pieces were then taken from the proboscis and fixed with 99.5% ethanol for molecular analyses. The rest of the specimen was fixed in ca. 10% seawater formalin and stored in 70% ethanol. All *Ikedosoma* specimens are deposited in NSMT and the remains at the Laboratory of Taxonomy, Toho University (LTTU).

We also aimed to examine the name-bearing type and other specimens of *I. qingdaoense*, which were described to be deposited in the Qingdao Marine Product Museum, China by Li et al.

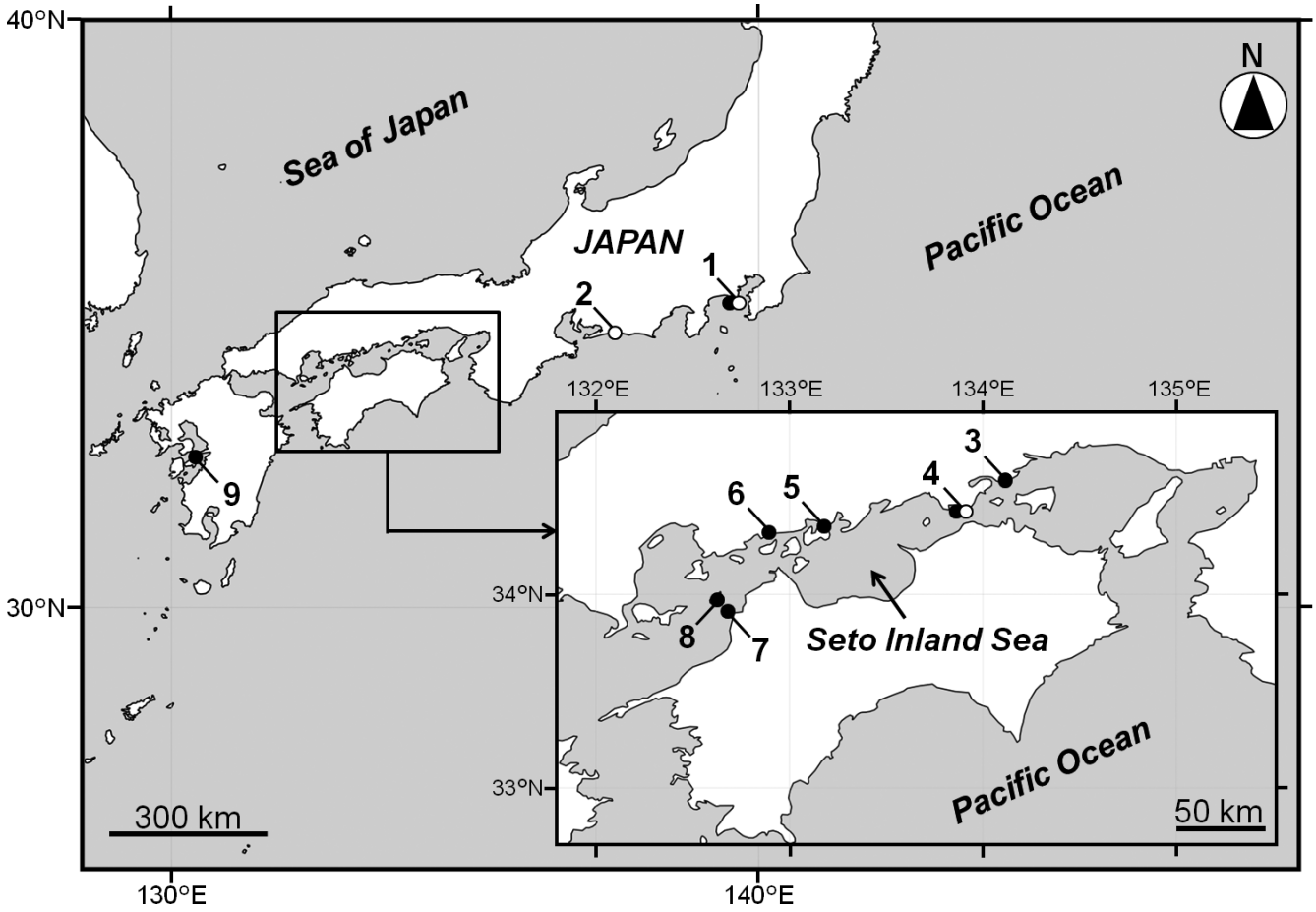

Fig. 1. Localities of *Ikedosoma elegans* (indicated by open circles) and *I. gogoshimense* (solid circles) used in the present study. Both species were recorded from Misaki (locality 1) and Takasu (4). Numbers indicate the localities as follows: 1, Misaki, Kanagawa Pref.; 2, Ikarise Islet, Shizuoka Pref.; 3, Ushimado, Okayama Pref.; 4, Takasu, Okayama Pref.; 5, Onomichi Bay, Hiroshima Pref.; 6, Hachi-no-higata, Hiroshima Pref.; 7, Gogoshima Island, Ehime Pref.; 8, Nakajima Island, Ehime Pref.; and 9, Ôikejima Island, Kumamoto Pref.

Table 1. List of PCR and cycle sequencing (CS) primers used in the present study for 18S, 28S, H3, and COI genes.

Gene	Primer name	Primer sequence (in 5'–3' direction)	Direction	Reaction	Sources
18S	G01	CACCTGGTTGATCCTGCCAG	Forward	PCR & CS	Saunders and Kraft (1994)
	G07	AGCTTGATCCTTCTGCAGGTTCACCTAC	Reverse	PCR & CS	Saunders and Kraft (1994)
	Echi_18S-1R	ACKACGAGCTTTTTRACTGCARC	Reverse	PCR & CS	Present study
	Echi_18S-2F	GGTAATWCCAGCTCCARTAG	Forward	PCR & CS	Present study
	Echi_18S-2R	GAGDTTYCCCGYGTTGAGTC	Reverse	PCR & CS	Present study
	Echi_18S-3F	GCTGAAACTTRAAGGAATTGACGGA	Forward	PCR & CS	Present study
28S	28S D1F	ACCCSCTGAAYTTAAGCAT	Forward	PCR & CS	Colgan et al. (2003)
	28S D3	GACGATCGATTTGCACGTCA	Reverse	PCR & CS	Vonnemann et al. (2005)
	28S D2F	CCCGTCTTGAAACACGGACCAAGG	Forward	CS	Vonnemann et al. (2005)
	28S C2R	ACTCTCTCTTCAAAGTTCTTTTC	Reverse	CS	Vonnemann et al. (2005)
H3	H3F	ATGGCTCGTACCAAGCAGACVGC	Forward	PCR & CS	Colgan et al. (1998)
	H3R	ATATCCTTRGGCATRATRGTGAC	Reverse	PCR & CS	Colgan et al. (1998)
COI	Echi_cox1L	ACTCAACAAACCACAAAGACATTGG	Forward	PCR & CS	Present study
	Echi_cox1H	TAKACYTCWGGRTGSCCAAARAATCA	Reverse	PCR & CS	Present study
	COI-E	TATACTTCTGGGTGTCCGAAGAATCA	Reverse	PCR & CS	Bely and Wray (2004)
	polyLCO	GAYTATWTTCAACAAATCATAAAGATATTGG	Forward	PCR & CS	Carr et al. (2011)
	polyHCO	TAMACTTCWGGGTGACCAAARAATCA	Reverse	PCR & CS	Carr et al. (2011)

(1994) and Zhou et al. (2007). However, we have not succeeded in finding such a specimen, and its present whereabouts are unknown (H. Zhou, pers. comm.).

Morphological examination

Observations, dissections, and drawings were made using a stereoscopic microscope. The trunk length (TL) and proboscis length (PL) were measured using a digital caliper, and the number of LMB was counted. Trunk musculature was examined with the aid of a light box. The type of gametes found in the gonoducts was used to identify the specimens' sex. The general terminology used here follows that of Stephen and Edmonds (1972) and Nishikawa (2004).

DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from the tissue samples using NucleoSpin Tissue (Macherey-Nagel) following the manufacture's protocol. 18S (ca. 1800 bp), 28S (ca. 1000 bp), H3 (ca. 350 bp), and COI (ca. 700 bp) partial genetic sequences were amplified by polymerase chain reaction (PCR) with the primers listed in Table 1. Each PCR was performed in a 10-μl reaction volume containing 2.2 μl of distilled H_2O (Millipore), 5 μl 2 × Gflex Buffer (TaKaRa), 0.8 μl of each primer (5 μM), 0.2 μl of Tks Gflex DNA polymerase (TaKaRa), and 1 μl of template on LifeTouch Thermal Cycler (Bioer Technology) for 30–35 cycles, with the following thermal cycle profile: preheating at 94°C for 1 min, denaturing at 98°C for 10 s, annealing from 48°C to 54°C for 15 s, and extension at 68°C for 30 s. To confirm that amplifications were successful, 2-μl aliquots of PCR products were visualized with electrophoresis on 1.5% TAE agarose gel stained with ethidium bromide for 30 min or more. Before sequencing, successful PCR products were cleaned using ExoStar (GE Healthcare), consisting of exonuclease I and alkaline phosphatase. Direct sequencing of the purified DNA products was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit in a 3130xl Genetic Analyzer (Life Technologies) or by Fasmac (Atsugi, Kanagawa, Japan). The obtained sequences were assembled and edited using ATGC (GENETYX). All sequences were deposited in DDBJ/EMBL/GenBank (accession nos. AB967985–968034).

Phylogenetic analyses

Additional Echiuridae and Urechidae sequences were obtained from DDBJ/EMBL/GenBank. Nearly all sequences extracted from GenBank were described by Goto et al. (2013). A total of 13 echiuran species were used for the present analyses: nine species of Thalassematinae of Echiuridae [*Arhynchite* (one species), *Listriolobus* (one species), *Thalassema* (one species), *Ikedosoma* (two species), *Ochetostoma* (four species)], and four species [Echiurinae of Echiuridae (one species) and Urechidae (three species)] as outgroups following Goto et al.'s (2013) tree topology. For further information, refer to Table 2.

The H3 and COI sequences were aligned using Mesquite Version 2.75 (Maddison and Maddison, 2011) taking into account each codon position. 18S and 28S sequences were aligned using MAFFT version 7 with L-INS-i (Katoh et al., 2005; Katoh and Standley, 2013) and subsequently trimmed using Gblocks ver. 0.91b with "With Half" allowed gap positions (Castresana, 2000). The final length of the aligned sequences was 1736 bp for 18S, 972 bp for 28S, 327 bp for H3, and 579 bp for COI.

We prepared five datasets (individual 18S, 28S, H3, and COI sequences, and a concatenated sequence of all of them) for analyses and set three partitions (first, second, and third codon positions) for H3 and COI or eight partitions (18S, 28S, and codon-specific positions of H3 and COI) for the concatenated sequence. Phylogenetic trees were constructed using maximum likelihood (ML) and Bayesian inference (BI). ML phylogenies were obtained

Table 2. List of species used for phylogenetic analyses in the present study with the registration number of the voucher specimens, collection locality or sources, and DDBJ/EMBL/GenBank accession numbers.

Species	Abbreviation	Voucher	Locality or Sources	Accession no.			
				18S	28S	H3	COI
Arhynchite pugettensis	—	—	Passamaneck and Halanych (2006)	AY210441	AY210455	—	—
Ikedosoma elegans	Ie-1	NSMT-Ec 112	Ikarise Islet, Shizuoka Pref.	AB967985	AB967998	AB968009	AB968022
Ikedosoma elegans	Ie-2	NSMT-Ec 113	Ikarise Islet, Shizuoka Pref.	AB967986	AB967999	AB968010	AB968023
Ikedosoma elegans	Ie-3	NSMT-Ec 114	Ikarise Islet, Shizuoka Pref.	AB967987	AB968000	AB968011	AB968024
Ikedosoma gogoshimense	Ig-1	NSMT-Ec 130	Ushimado, Okayama Pref.	AB967988	AB968001	AB968012	AB968025
Ikedosoma gogoshimense	Ig-2	NSMT-Ec 133	Takasu, Okayama Pref.	AB967989	AB968002	AB968013	AB968026
Ikedosoma gogoshimense	Ig-3	NSMT-Ec 154	Gogoshima Island, Ehime Pref.	AB967990	AB968003	AB968014	AB968027
Ikedosoma gogoshimense	Ig-4	NSMT-Ec 131	Takasu, Okayama Pref.	AB967991	AB968004	AB968015	AB968028
Ikedosoma gogoshimense	Ig-5	NSMT-Ec 138	Hachi-no-higata, Hiroshima Pref.	AB967992	—	AB968016	AB968029
Ikedosoma gogoshimense	Ig-6	NSMT-Ec 139	Hachi-no-higata, Hiroshima Pref.	AB967993	AB968005	AB968017	AB968030
Ikedosoma gogoshimense	Ig-7	NSMT-Ec 116	Misaki, Kanagawa Pref.	AB967994	—	AB968018	AB968031
Ikedosoma gogoshimense	Ig-8	—	Goto et al. (2013)	AB771456	AB771470	AB771481	AB771491
Listriolobus sorbillans	Ls-1	LTTU-Y098	Kabira Bay, Ishigaki Island	AB967995	AB968006	AB968019	AB968032
Listriolobus sorbillans	Ls-2	LTTU-Y099	Kabira Bay, Ishigaki Island	AB967996	AB968007	AB968020	AB968033
Listriolobus sorbillans	Ls-3	—	Goto et al. (2013)	AB771470	AB771471	AB771482	AB771492*
Ochetostoma erythrogrammon	Oe-1	LTTU-Y100	Sesoko, Okinawa Island	AB967997	AB968008	AB968021	AB968034
Ochetostoma erythrogrammon	Oe-2	—	Goto et al. (2013)	AB771458	Ab771472	AB771483	AB771493
Ochetostoma sp. 1	—	—	Goto et al. (2013)	AB771459	AB771473	AB771484	—
Ochetostoma sp. 2	—	—	Goto et al. (2013)	AB771460	—	AB771485	—
Ochetostoma sp. 3	—	—	Goto et al. (2013)	AB771461	—	AB771486	AB771494
Thalassema owstoni	—	—	Goto et al. (2013)	AB771462	AB771474	AB771474	AB771495
Outgroups							
Echiurus echiurus	—	—	Goto et al. (2013)	AB771455	AB771469	—	—
Urechis caupo	—	AToL000328	Andrade et al. (2012)	JF509727	JF509731	JF509712	JF509718
Urechis unicinctus	—	—	Goto et al. (2013)	AB771464	AB771476	—	AB771497
Urechis sp. 1	—	—	Goto et al. (2013)	AB771465	AB771477	—	AB771498

* This sequence has been registered under the bivalve name of "*Basterotia* sp. RG-2012" in DDBJ/EMBL/GenBank, but it obviously came from *Listriolobus sorbillans* (see Goto et al., 2013, Table S1).

using the partitioned method as implemented in RAxML-VI-HPC (Stamatakis, 2006) and its graphical interface raxmlGUI version 1.3 (Silvestro and Michalak, 2012). As recommended in the RAxML-VI-HPC manual (Stamatakis, 2006), the general time reversible model with sites following a discrete gamma distribution (GTR + Γ: Tavaré, 1986; Yang, 1993) for each partition was used without incorporating a proportion of invariant sites. Rapid bootstrap (BS) analysis was conducted with 1000 replicates (–f a option). BI and Bayesian posterior probabilities (BPP) were estimated using MrBayes version 3.2.2 (Altekar et al., 2004; Ronquist et al., 2012). The substitution models for each partition were calculated on the basis of the Bayesian Information Criterion (Schwarz, 1978), as implemented in Kakusan4 (Tanabe, 2011). The selected models were as follows: for 18S, K2P model (Kimura, 1980) + Γ; for 28S and the third codon of COI, GTR + Γ; for the first and second codon of H3, JC69 model (Jukes and Cantor, 1969) + homogeneous; for the third codon of H3, HKY85 model (Hasegawa et al., 1985) + Γ; for the first codon of COI, SYM model (Zharkikh, 1994) + Γ; for the second codon of COI, F81 (Felsenstein, 1981) + Γ. Two independent runs for four Markov chains were performed over 10 million generations. The trees were sampled every 100 generations. Based on the parameter estimates and convergence estimated using Tracer version 1.5 (Rambaut and Drummond, 2009), the first 10,001 trees were discarded as burn-in. A consensus of sampled trees was computed, and the posterior probability for each interior branch was obtained. Pairwise genetic distances [± standard deviation (SD)] were calculated on the basis of the COI sequence using uncorrected *p*-distance and Kimura's two parameter (K2P) model (Kimura, 1980), as implemented in MEGA5.2 (Tamura et al., 2011).

RESULTS

Morphological examination

Detailed examination of the internal morphology of the present materials clearly revealed that both *I. elegans* and *I. gogoshimense* had a continuous oblique muscle layer, instead of forming distinct fascicules, even between LMB. In addition, they completely lacked the interbasal muscle and rectal caecum. Tables 3 and 4 show the anomalies found in the number and arrangement of gonoducts from the usual patterns (six postsetal pairs for *I. elegans* and three postsetal pairs for *I. gogoshimense*): e.g., the six usual pairs present plus two additional gonoducts, one added to the left of the fourth pair and another added to the right of the fifth pair, without the left one of the sixth gonoduct [indicated as "L4(2)R5(2)L6(0)"]. The three usual postsetal pairs were also often present, but with one additional gonoduct posterior to the third pair on the right [as "R4(1)"]. Occasionally, the three usual pairs were present with three additional gonoducts, two paired and situated anterior to the first pair and the remaining added to the right one of the first pair, without the left one of the third pair [as "L0(1)R0(1)R1(2) L3(0)"]. As shown in these tables, the number of gonoduct pairs varied from five to six in *I. elegans* and from three to five in *I. gogoshimense*. The total number of gonoducts ranged from 10 to 13 in the former and six to 13 in the latter. The number of gonoducts and variability considerably overlapped between the two species; therefore, these numbers have been proved useless to distinguish these species. Instead, we found a new stable difference in the distribution of gonoduct pairs between these two: the longitudinal distance between the adjacent pairs was almost constant, with all pairs occupying the anterior one-third of the trunk in *I. gogoshimense*, while the distance became longer toward the posterior section, with the posterior-most pairs being located in the middle of the trunk in *I. elegans*.

Phylogenetic analyses

The 18S, 28S, H3, and COI alignments contained 99, 226, 53, and 258 variable sites, of which 56, 157, 31, and 243 sites were parsimony-informative. The mean base composition was 23.1% (A), 27.2% (C), 26.2% (C), and 23.5% (T).

We found a remarkable difference in the COI sequence of *I. gogoshimense* between the data of Goto et al. (2013) (see above) and our own data. The obtained trees (Fig. 2, only ML trees are shown) revealed that in each of the 18S,

Table 3. Variation in the number and disposition of gonoducts in *Ikedosoma elegans* in the present study; for further details, see text.

Specimen (sex)	Disposition of gonoducts
UMUTZ-Echi-15 (female)	R1(2) (= 13 in total)
NSMT-Ec 111 (unknown)	L6(0)R6(0) (= 10)
NSMT-Ec 112 (male)	L4(2)R5(2)L6(0) (= 13)
NSMT-Ec 113 (male)	L5(2) (= 13)
NSMT-Ec 114 (male)	R6(0) (= 11)
NSMT-Ec 115 (unknown)	Usual* (= 12)

* Usual pattern is indicated as L1(1)R1(1)L2(1)R2(1)L3(1)R3(1) L4(1)R4(1)L5(1)R5(1)L6(1)R6(1).

Table 4. Variation in the number and disposition of gonoducts in *Ikedosoma gogoshimense* in the present study; for further details, see text.

Sex	Disposition pattern of gonoducts									
	Usual* (= 6 in total)	L0(1)L1(0) (= 6)	L0(1) (= 7)	L1(2) (= 7)	L2(2) (= 7)	L4(1) (= 7)	R1(2) (= 7)	R2(2) (= 7)	R4(1) (= 7)	L0(1)R0(1) (= 8)
Male	8		2		1	1			2	1
Female	6	1			1			1	1	
Unknown	17			1	1		1		1	
Total	31	1	2	1	3	1	1	1	4	1

Sex	Disposition pattern of gonoducts								
	L0(1) L4(1) (= 8)	L2(2) L4(1) (= 8)	R0(1) R2(2) (= 8)	L0(1)R0(1) R1(2)L3(0) (= 8)	L0(1)R3(2) R4(1) (= 9)	R1(2)L4(1) R4(1) (= 9)	L0(1)R0(1) R1(2)L2(2) (= 10)	L0(1)R0(1) L1(2)R1(2) L2(2)R2(2)R4(1) (= 13)	Total
Male			1			1		1	18
Female	1						1		12
Unknown		1		1	1	1			25
Total	1	1	1	1	1	2	1	1	55

* Usual pattern is indicated as L1(1)R1(1)L2(1)R2(1)L3(1)R3(1).

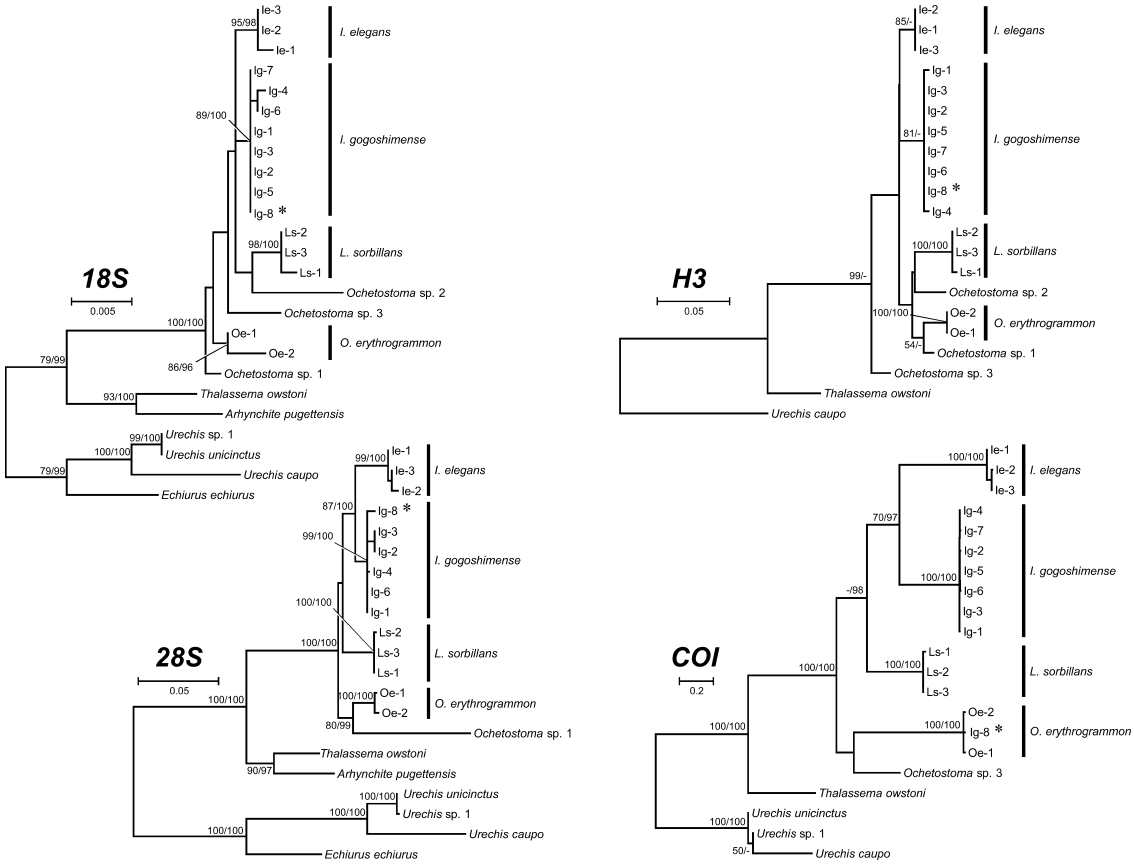

Fig. 2. Maximum-likelihood (ML) tree based on nuclear 18S, 28S, H3, and mitochondrial COI sequences. Numbers above branches represent the level of bootstrap support (BS) for ML analysis and Bayesian posterior probabilities (BPP). BS higher than 50% and BPP higher than 95% are shown. Data from Goto et al. (2013) of *Ikedosoma gogoshimense* (abbreviated as Ig-8) are marked with an asterisk.

28S, and H3 data sets, the downloaded sequence (AB771456, AB771470, and AB771481, respectively) was nested within our newly determined ones (AB967988–967994, AB968001–968005, and AB968012–968018, respectively), while the COI sequence (AB771491) was nested within those of *O. erythrogrammon* (AB771493 and AB968034) instead of *I. gogoshimense*. This discrepancy may be attributable to mitogenomic introgression or to human errors such as contamination or lack of quality control. Therefore, we excluded the COI sequence registered as AB771491 from our concatenated dataset.

The gene-concatenated ML tree demonstrated that the ingroup taxa could be divided into two major clades: A and B (Fig. 3). BI analysis produced an identical tree topology (data not shown). Clade A was composed of seven taxa (*I. elegans*, *I. gogoshimense*, *L. sorbillans*, *O. erythrogrammon*, and *Ochetostoma* species1–3) supported by 100% probabilities in BS and BPP. In Clade A, *I. elegans* and *I. gogoshimense* formed reciprocal monophyletic branches with high probabilities (BS = 100%, BPP = 100%). Furthermore, the monophyletic nature of these two *Ikedosoma* species was recovered with high support values (BS = 85%, BPP = 99%); however, *L. sorbillans* was not included therein. The average genetic divergence of the COI sequence between two *Ikedosoma* species, *I. elegans* and *I. gogoshimense* (excluding AB771491, shown above), was 23 ± 0.3% in *p*-distance (27.9 ± 0.5% in K2P). The average genetic diver-

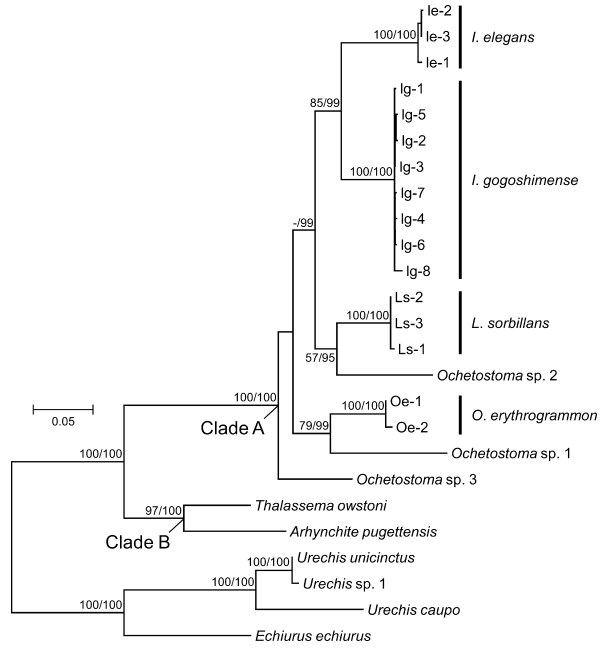

Fig. 3. Maximum-likelihood (ML) tree based on concatenated sequences of nuclear 18S, 28S, H3, and mitochondrial COI genes. Numbers above branches represent the level of bootstrap support (BS) for ML analysis and Bayesian posterior probabilities (BPP). BS higher than 50% and BPP higher than 95% are indicated.

gences within *I. elegans* and *I. gogoshimense* were 1.9 ± 1.3 and 0.8 ± 0.2% in *p*-distance (1.9 ± 1.4 and 0.8 ± 0.2% in K2P), respectively. Within the four *Ochetostoma* species used here, only *Ochetostoma* sp. 2 was contained in a subclade composed of *Ikedosoma* spp. and *L. sorbillans* with high BI support (BPP = 99%). On the other hand, internal relationships were not resolved by ML. Thus, the genus *Ochetostoma* was not shown to be monophyletic. Clade B was composed of *Arhynchite pugettensis* and *Thalassema owstoni* supported by high probabilities (BS = 97%, BPP = 100%).

DISCUSSION

Our molecular analyses revealed that *I. elegans* and *I. gogoshimense* form a distinct monophyletic clade, which does not include *L. sorbillans*. Furthermore, these two *Ikedosoma* species are morphologically distinguishable from all known species of *Listriolobus* owing to the absence of a rectal caecum. Table 5 shows a comparison of all known genera currently assigned to the subfamily Thalassematinae of Echiuridae, with some character states and authorships updated since Stephen and Edmonds (1972). Thus, *Ikedosoma* Bock, 1942 is regarded as a valid genus with the amended diagnosis shown below. A rectal caecum is a small blind sac of unknown function, projected from the end of the postsiphonal part of the intestine (Stephen and Edmonds, 1972). The caecum has often been ill described, chiefly owing to its vulnerability. The present study may show the significance of this organ in generic diagnosis; therefore, more attention should be paid to it.

Our molecular and morphological analyses also revealed the validity of both *I. elegans* and *I. gogoshimense*. Unfortunately, we were unable to examine the third species of this genus, *I. qingdaoense*. Its previous descriptions lack any information regarding the oblique muscle layer; therefore, even its generic affiliation remains unclear. Hence, this species is treated as "Species of uncertain status" in the Taxonomy section below.

Our molecular analyses did not provide evidence of monophyly in the genus *Ochetostoma*, despite its fasciculation of the innermost oblique muscle layer between LMB, unique among Thalassematinae (Stephen and Edmonds, 1972; Biseswar, 1988a; see also Table 5). Thus, the generic assignment of the *Ochetostoma* species 1–3 specimens requires reconfirmation for further discussion.

TAXONOMY

Ikedosoma Bock, 1942

(Japanese name: Yume-yumushi-zoku)

Ikedosoma Bock, 1942: 10–18 (type species by original designation: *Thalassema elegans* Ikeda, 1904).

Amended diagnosis

PL as or longer than its TL, without bifurcation at tip. Longitudinal muscle layer of trunk wall showing regular thickening into LMB. Oblique muscle layer continuous and never fasciculate between LMB. Gonoducts three to seven pairs, often with some anomalies. Interbasal muscle absent. Rectal caecum undetectable.

Ikedosoma elegans (Ikeda, 1904)

(Japanese name: Yume-yumushi)
(Figs. 4A–B and 5; Table 3)

Thalassema elegans Ikeda, 1904: 65–66; Ikeda, 1907: 47–55, pl. 1, fig. 4, pl. 4, figs. 48–49; Sato, 1935: 15, fig. 16; Satô, 1939: 356.
Ikedosoma elegans: Bock, 1942: 10–18; Fisher, 1946: 224; Stephen and Edmonds, 1972: 461–462, figs. 57D–E.
Anelassorhynchus mucosus: Nishikawa, 2007: 180 (partim).

Material examined

Syntype: UMUTZ-Echi-15, one female, TL ca. 215 mm, PL ca. 180 mm, LMB 11, Moroiso Inlet, Misaki, Kanagawa Pref., Sagami Bay, Japan, 21.vi.1902, coll. I. Ikeda, corresponding to the "Specimen D" of Ikeda (1907, p. 54).

Nontype specimens: NSMT-Ec 111, one of unknown sex, TL 70 mm, PL 130 mm, LMB eight, intertidal sandy or muddy flat of Ikarise Islet at the entrance of Lake Hamana, Shizuoka Pref., Enshûnada Sea (34°41′04″N, 137°35′59″E), 12.v.2002, coll. T. Nishikawa (TN), S. Kimura, and T. Kimura, referred to as *Anelassorhynchus mucosus* (Ikeda,

Table 5. Comparison of all known genera currently assigned to the subfamily Thalassematinae.

Genus	Longitudinal musculature	Oblique musculature	Gonostomal lips	Number of gonoduct pairs	Rectal caecum	Position of gonostome	Sources
Ikedosoma Bock, 1942	banded	continuous	spirally coiled	3–7	absent	basal	Present study
Listriolobus Spengel, 1912	banded	continuous	spirally coiled	2–4	present	basal	Nishikawa (2004)
Ochetostoma Rüppell and Leuckart, 1828*	banded	fasciculate	spirally coiled	2–7	present or absent	basal	Biseswar (1988a)
Lissomyema Fisher, 1946	banded	continuous	fan-shaped	2–3	unknown	basal	Fisher (1946)
Anelassorhynchus Annandale, 1922	continuous	continuous	spirally coiled	1–5	present or absent	basal	DattaGupta (1974); Biseswar (1984)
Thalassema Pallas, 1774	continuous	continuous	not spirally coiled	1–3	present or absent	basal	Biseswar (1988b); Nishikawa (1998)
Arhynchite Satô, 1937	continuous	continuous	leaf-like	1	present or absent	basal	Tanaka and Nishikawa (2013)
Ikeda Wharton, 1913	continuous or banded	continuous	simple, without modification	unpaired and numerous (up to 400 in total)	absent	distal	Nishikawa (2002)

* Authorships of the genus *Ochetostoma* and its type species *O. erythrogrammon* have been wrongly attributed to "Leuckart and Rüppell."

1904) by Nishikawa (2007). NSMT-Ec 112, one male, TL 160 mm, PL > 65 mm (25 mm + a 40 mm fragment), LMB 11, intertidally from Ikarise (see above), 1.vii.2012, coll. T. Unagami and M. Tanaka (MT). NSMT-Ec 113, one male, TL 100 mm, PL > 105 mm (55 mm + a 50 mm fragment), LMB 12, do., 7.vii.2013, coll. T. Unagami and MT. NSMT-Ec 114, one male, TL 103 mm, PL > 57 mm (only an anterior fragment), LMB 12, same collection data as above. NSMT-Ec 115, one of unknown sex, TL 120 mm, PL 90 mm, LMB 10, Takasu sandy flat, off Ohjigatake, Okayama Pref., Seto Inland Sea (34°27′N, 133°52′E), 28.v.1975, coll. TN.

Description

Trunk colored deep wine red, when alive, with white narrow longitudinal lines corresponding to LMB (Fig. 4A). Proboscis yellow to orange with green pigment distally (Fig. 4A, B). These colorations faded to pale yellow or beige after fixation with formalin.

In preserved specimens, TL ranging from 70 to 215 mm (n = 6) and PL ranging from 90 to 180 mm (n = 3). Proboscis elongated and truncated in its anterior extremity (Figs. 4A, B, 5A). Trunk wall rather thin and covered with numerous papillae, particularly prominent (up to ca. 700 μm in height) at posterior end. Trunk musculature composed of outermost circular, middle longitudinal, and innermost continuous oblique layers (Fig. 5B, C). LMB variable from eight to 12 (n = 6). When contracted, circular layer often weakly fasciculate to form transverse bands. Paired ventral setae of usual form, without interbasal muscle. Five to six pairs of gonoducts, all situated posterior to the setae. Each pair often clustered; total number of gonoducts ranging from 10 to 13 (n = 6) (Table 3). Longitudinal distance between adjacent pairs increasing toward posterior section, with posterior-most gonoducts being located in middle of trunk. Gonostome proximal, with its lips elongated and spirally coiled.

Alimentary canal long and convoluted, filled with sand grains. Anterior part, or foregut, fastened to ventral trunk wall by sheet-like mesentery, almost straight along ventral nerve cord, and divided into pharynx, esophagus, gizzard, and crop. Behind foregut, intestine fastened to trunk wall through numerous thread-like mesenteries and divided into presiphonal, siphonal, and postsiphonal parts. Presiphonal and postsiphonal parts covered with ciliated groove. Rectal caecum absent.

Vascular system composed of dorsal, neurointestinal, ventral, and ring vessels. Dorsal vessel attached throughout entire length of crop and connected to dorsomedian part of ring vessel. Ring vessel incompletely encircling posterior end of crop. Ventral vessel running along almost entire length of ventral nerve cord and terminating at posterior end of postsiphonal intestine near anus. Neurointestinal vessel linked to ventral vessel at level of first pair of gonoducts, bifurcated into large loop, and terminating on each side of ring vessel.

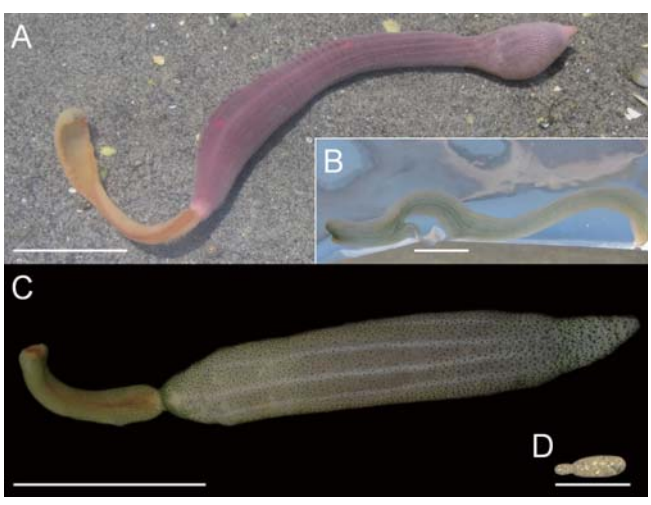

Fig. 4. Photographs of two *Ikedosoma* live specimens. **(A)** *I. elegans* (NSMT-Ec 113) from Ikarise Islet, proboscis incomplete, ventral view. **(B)** Anterior fragment of the proboscis of the specimen in **(A)** showing greenish pigmentation, ventral view. **(C)** *I. gogoshimense* (NSMT-Ec 116) from Moroiso Inlet, ventral view. **(D)** A fecal pellet of *I. gogoshimense* immediately after evacuation from the specimen in **(C)**. Scales: **(A)** and **(C)**, 5 cm; **(B)** and **(D)**, 1 cm.

Fig. 5. A syntype (UMUTZ-Echi-15) of *Thalassema elegans* Ikeda, 1904. **(A)** Whole body with the label probably written by Dr. Iwaji Ikeda. **(B)** Dorsal view of the opened trunk showing the musculature, using a light box. **(C)** Magnified trunk wall showing the two longitudinal muscle bands (marked by an asterisk) and the continuous oblique muscle layer in between. Scales: **(A)** 5 cm; **(B)** 3 cm.

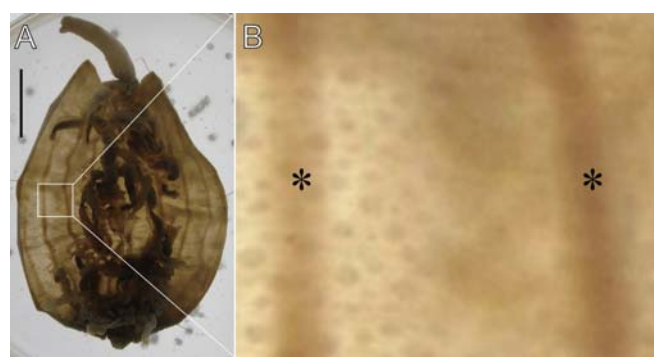

Fig. 6. A dissected specimen of *Ikedosoma gogoshimense* (NSMT-Ec 116), collected intertidally from Moroiso Inlet. **(A)** Opened trunk, using a light box, dorsal view. **(B)** Magnified trunk wall showing the two longitudinal muscle bands (indicated with an asterisk) and the continuous oblique muscle layer in between. Scale: **(A)**, 3 cm.

Paired simple anal vesicles, ca. one-third of TL in well-preserved specimens, basally fastened to trunk wall by a few mesenteries, and entirely covered in numerous microscopic ciliated funnels.

Biological notes

Ikedosoma elegans was recorded to inhabit a deep (up to ca. 1.2 m), vertical, or somewhat oblique burrow, similar to the description of *Ikeda taenioides*, by Ikeda (1904, 1907). In the sandy flat in the Ikarise Islet in 2012 and 2013, we collected few specimens of *I. elegans* from burrows reaching ca. 50 cm in depth; however, the openings and the shape could not be ascertained. Similar to the results of the surveys by Ikeda (1907) in Moroiso, no proboscises or traces were found protruding or radiating from burrows. No symbionts have been found in the burrows (Ikeda, 1904, 1907; present study).

Remarks

The original and subsequent syntype descriptions were reported nearly 100 years ago. We managed to collect five new specimens of *I. elegans* from two new localities: one from the Pacific coast in central Japan and the other from the Seto Inland Sea; however, we did not succeed in collecting specimens from the type locality (Misaki) and its vicinity.

Ikeda (1907) examined four syntypes (three males and one female) and concluded that this species exhibits a sexual difference in the total number of gonoducts, i.e., up to 27 in males versus 13 in females. In the present study, however, we found only 11 or 13 gonoducts in total in the three newly collected males (Table 3). Consequently, it is highly probable that the number is much variable among individuals and this variation is unrelated to sexual dimorphism.

According to Ikeda's (1907) subsequent description of *I. elegans*, "in Specimen A, the first pair of segmental organs [gonoducts] lies just behind the ventral hooks [setae]; whereas in all the other specimens I have found it situated in front of the hooks" (p. 55). However, Ikeda's "Specimen D," re-examined in the present study, showed an anterior-most pair of gonoducts actually behind the ventral setae, similar to "Specimen A." Ikeda's description cited above may possibly represent an individual variation in the arrangement of gonoducts, although the pair was invariably located behind the setae in all the examined specimens in the present study.

Distribution

This species is thought to be endemic to the Japanese coast (Fig. 1). It is found on intertidal sandy to muddy flats of Moroiso Inlet, Misaki, Kanagawa Pref., Sagami Bay (type locality: Ikeda, 1904, 1907), of Ikarise Islet, Lake Hamana, Shizuoka Pref., Enshûnada Sea (present study), and of Takasu, Okayama Pref., Seto Inland Sea (present study).

***Ikedosoma gogoshimense* (Ikeda, 1904)**

(Japanese name: Gogoshima-yumushi)
(Figs. 4C–D, 6, and 7; Table 4)

Thalassema gogoshimense Ikeda, 1904: 66–67, pl. 1, fig. 19; Sato, 1934: 251–252, figs. 7 and 8; Sato, 1935: 16, figs. 17 and 18; Satô, 1939: 356.

Ikedosoma gogoshimense: Fisher, 1946: 224; Stephen and Edmonds, 1972: 462.

Anelassorhynchus mucosus: Nishikawa, 1992: 307, pl. 62, fig. 4.

Material examined

Nontype specimens: NSMT-Ec 116, one female, TL 89 mm, PL 26 mm, LMB 11, Moroiso Inlet, intertidal flat near Misaki Marine Biological Station (MMBS), The University of Tokyo, Kanagawa Pref., Sagami Bay (35°09′28″N, 139°36′44″E), 4.vi.2012, coll. H. Kohtsuka. NSMT-Ec117–118, two females, TL 37 and 54 mm, PL 12 and 18 mm, LMB 9 and 10, Moroiso Inlet, 4 m deep, near MMBS (do.),

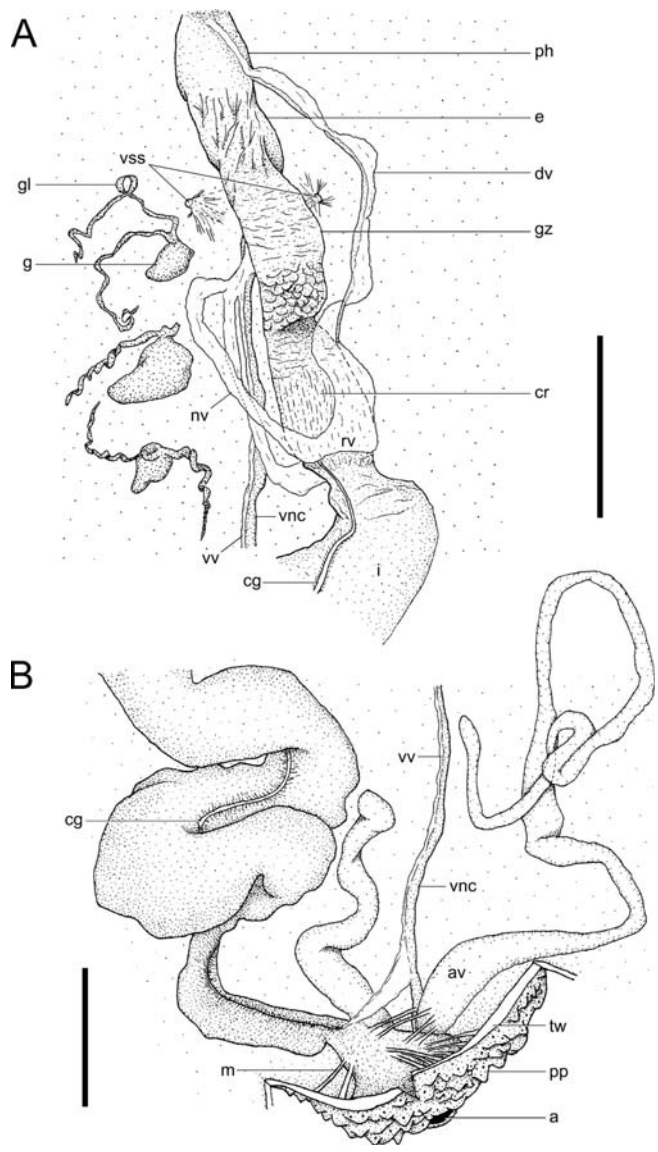

Fig. 7. A dissected trunk of *Ikedosoma gogoshimense* (NSMT-Ec 136), unknown sex, collected from Hachi-no-higata sandy flat, dorsal view, with right gonoducts and most of mesenteries omitted for clarity. **(A)** Anterior part. **(B)** Posterior part. Abbreviations: a, anus; av, anal vesicle; cg, ciliated groove; cr, crop; dv, dorsal vessel; e, esophagus; g, gonoduct; gl, gonostomal lip; gz, gizzard; i, intestine; m, mesentery; nv, neurointestinal vessel; ph, pharynx; pp, papillae; rv, ring vessel; tw, trunk wall; vnc, ventral nerve cord; vss, ventral setal sac; and vv, ventral vessel. Scale: 5 mm.

24.iv.2013, coll. H. Kohtsuka and M. Sekifuji. NSMT-Ec 119, one female, TL 41 mm, PL 15 mm, LMB 10, Ushimado, Okayama Pref., Seto Inland Sea, 5.vi.1985, coll. K. Ito, the photographed specimen of *Anelassorhynchus mucosus* (Ikeda, 1904) in Nishikawa (1992, pl. 62, Fig. 4). NSMT-Ec 120–130, three males + one female + six of unknown sex, TL 44–67 mm, PL 15–26, LMB 9 to 10, intertidal, near Ushimado Marine Laboratory, Okayama University, Okayama Pref., Seto Inland Sea (34°36′34″N, 134°08′33″E), 21.v.2011, coll. MT and TN. NSMT-Ec 131–133, one male + two specimens of unknown sex, TL 40–63 mm, PL 1.5–2.4 mm, LMB eight to nine, Takasu sandy flat, off Ohjigatake, Okayama Pref., Seto Inland Sea (34°27′N, 133°52′E), 20.v.2011, coll. MT and TN. NSMT-Ec 134, one specimen of unknown sex, TL 58 mm, proboscis absent, LMB nine, Kagawa Pref. (exact locality unknown), vii.1975, collector unknown. TUM Echiurida 2-9, one male + one specimen of unknown sex, TL 35 and 50 mm, PL 10 and 13 mm, LMB 9 and 10, Onomichi Bay, Hiroshima Pref., Seto Inland Sea, 7.iii.1931, coll. Takashi Gamô. NSMT-Ec 135, one female, TL 72 mm, PL 26 mm, LMB 9, Hachi-no-higata sandy flat at the mouth of Kamogawa River, Hiroshima Pref., Seto Inland Sea (34°19′25″N, 132°53′53″E), 28.vi.2010, coll. TN. NSMT-Ec 136–147, three males + one female + eight specimens of unknown sex, TL 31–50 mm, PL 11–23 mm, LMB eight to nine, do., 18.v.2011, coll. MT and TN. NSMT-Pol R58, one male + three specimens of unknown sex, TL 33–64 mm, PL 10–21 mm, LMB 9 to 10, Funakoshi, Gogoshima Island, Ehime Pref., Seto Inland Sea, 19.iii.1950. NSMT-Ec 148–158, five males + four females + one specimen of unknown sex, TL 43–87 mm, PL 13–44 mm, LMB 8 to 10, intertidal, Gogoshima Island, Ehime Pref., Seto Inland Sea (33°55′N, 132°41′E), coll. MT and K. Shibata. NSMT-Ec 159–163, two males + three specimens of unknown sex, TL 33–47 mm, PL 8–14 mm, LMB 8–10, Nakajima Island, Ehime Pref., Seto Inland Sea, 10.iii.2005, coll. G. Itani. TOYA-Iv-IV2118, one female, TL 56 mm, proboscis absent, LMB 10, Ôikejima Island, Kumamoto Pref., Yatsushiro Sea, coll. N. Nunomura.

Description

Trunk colored pale reddish green, when alive, with white narrow longitudinal lines corresponding to LMB (Fig. 4C). Proboscis yellow to orange. Trunk and proboscis covered with dark green spots. These colorations faded to pale yellow or beige after fixation with formalin; however, dark green spot often remained unfaded.

In preserved specimens, TL ranging from 31 to 89 mm (n = 55) and PL from 8 to 44 mm (n = 50). Proboscis elongated and truncated in its anterior extremity (Fig. 4C). Trunk wall thick and covered with numerous papillae, particularly prominent (up to ca. 1 mm in height) at posterior end (Fig. 7B). Trunk musculature composed of outermost circular, middle longitudinal, and innermost continuous oblique layers (Fig. 6A, B). LMB variable from eight to 11 (n = 55). Paired ventral setae of usual form, without interbasal muscle (Fig. 7A). Gonoducts usually in three pairs and situated behind ventral setae (Fig. 7A) but with some anomalies in number (up to 13 in total) and disposition (up to five pairs, rarely with anteriormost pair in front of ventral setae) (Table 4). Longitudinal distance between adjacent pairs almost constant with all pairs occupying anterior one-third of trunk (Fig. 7A). Gonostome proximal with its lips elongated and spirally coiled (Fig. 7A).

Alimentary canal long and convoluted, filled with sand grains and spheroidal fecal pellets, ca. 10 mm long (Fig. 4D, 7A–B). Anterior part, or foregut, fastened to ventral trunk wall by sheet-like mesentery, almost straight along ventral nerve cord, and divided into pharynx, esophagus, gizzard, and crop (Fig. 7A). Behind foregut, intestine fastened to trunk wall through numerous thread-like mesenteries and divided into presiphonal, siphonal, and postsiphonal parts (Fig. 7A, B). Presiphonal and postsiphonal parts covered with ciliated groove (Fig. 7A, B). Rectal caecum absent (Fig. 7B).

Vascular system composed of dorsal, neurointestinal, ventral, and ring vessels (Fig. 7A, B). Dorsal vessel attached half to entire length of crop and connected to dorsomedian part of ring vessel (Fig. 7A). Ring vessel incompletely encircling posterior end of crop (Fig. 7A). Ventral vessel running along almost entire length of ventral nerve cord and terminating at posterior end of postsiphonal intestine near anus (Fig. 7A, B). Neurointestinal vessel linked to ventral vessel at level of first or second pair of gonoducts, terminating on each side of ring vessel usually in bifurcation with large loop (Fig. 7A). In two specimens (NSMT-Ec 124 and 139), however, neurointestinal vessel not bifurcated.

Paired simple anal vesicles, only one-sixth of TL to more than twice TL, probably depending on fixed conditions, basally fastened to trunk wall by a few mesenteries (Fig. 7B), and entirely covered in numerous microscopic ciliated funnels.

Biological notes

Ikedosoma gogoshimense inhabits an L-shaped burrow, the vertical part of which is ca. 10 cm in depth (Kawaguti, 1971; Goto et al., 2011; Nishikawa, 2012). The burrow entrance is scattered with many fecal pellets (Ikeda, 1904; Kawaguti, 1971; Arakawa, 1971; Goto et al., 2011; Nishikawa, 2012). The fecal pellets are spheroidal, ca. 1 cm in length, each with a small, twisted protrusion (Fig. 4D; Arakawa, 1971; Nishikawa, 2012). *Basterotia gouldi* (Bivalvia: Sportellidae), *Macromphalus tornatilis* (Gastropoda: Vanikoridae), and *Pinnixa* sp. (Malacostraca: Pinnotheridae) have been found in burrows of this species and reported as symbionts (Goto et al., 2011).

This species was once abundantly found and collected in great numbers to be used as fish bait in the Seto Inland Sea (Satô, 1939), called by local people as “Inuyu,” “Inui,” “Inukouju,” or “Modoki” (Mori et al., 1932; Sato, 1934; Ishikawa, 1938). However, its density has largely declined recently, mostly because of overexploitation as fish bait (Nishikawa, 2007, 2012), as in the case of *Arhynchite hayaoi* (Tanaka and Nishikawa, 2013).

Remarks

Table 6 shows a comparison between two species of *Ikedosoma*: *I. gogoshimense* and *I. elegans*. *Ikedosoma gogoshimense* can be distinguished from *I. elegans* by the following: (1) presence of dark green spots over the trunk and proboscis in living specimens, (2) the constant longitudinal distance between the two adjacent pairs of gonoducts, and (3) the gonoducts occupying the anterior one-third of the trunk. In addition, *I. gogoshimense* shows some ecological peculiarities such as the spheroidal large fecal pellets

Table 6. Comparison between the two Japanese species of the genus *Ikedosoma*.

Species	Maximal length of trunk (mm) in life	Maximal length of proboscis (mm) in life	Number of longitudinal muscle bands	Coloration in life		Gonoducts				Sources
				Trunk	Proboscis	Number of pairs	Total number	Longitudinal distance between adjacent pairs	Occupying	
I. elegans	ca. 350	ca. 400	8–12	Deep wine red	Yellow to orange with green pigment distally	5–7	10–27	Longer in a more posterior position	Anterior half of trunk	Ikeda (1907); present study
I. gogoshimense	ca. 150	ca. 105	8–11	Pale reddish green with dark green spots	Yellow to orange with dark green spots	3–5	4–32	Almost invariable	Anterior one-third of trunk	Ikeda (1904); Sato (1934); present study

scattered around the openings of the burrows (no such pellets are found in *I. elegans* burrows) and the shallower burrows (up to 15 cm instead of up to 1.2 m in *I. elegans*).

In the original description, Ikeda (1904) noted that the number of gonoducts in *I. gogoshimense* markedly differed between sexes: three pairs (six in total) in females and three to four pairs in males. Male gonoducts are also often clustered, resulting in up to 32 gonoducts in total. In our examination of 55 specimens, the total number varied from six to 13 in three to five pairs. More than half of these 55 specimens showed a constant number of gonoducts (three pairs, six in total) regardless of sex (Table 4). The same observation was already described by Sato (1934) for the specimens from Onomichi Bay and Gogoshima Island, the Seto Inland Sea. Consequently, it is highly probable that the number of gonoducts is variable among individuals but unrelated to sexes, similar to *I. elegans*, as described above. In this context, Ikeda's (1904) male with 32 gonoducts, invariably clustered in each position of four pairs, may represent extreme intraspecific variation, possibly even different species. Further materials will help solve this problem.

Distribution

This species is thought to be endemic to the Japanese coast (Fig. 1). It is found mainly on intertidal sandy to muddy flats of Moroiso Inlet, Misaki, Kanagawa Pref. in Sagami Bay (one of the type localities: Ikeda, 1904; present study), in the Seto Inland Sea of Ushimado, Okayama Pref. (Nishikawa, 1992; present study), of Takasu, Okayama Pref. (present study), of Onomichi Bay, Hiroshima Pref. (Sato, 1934); of Hachi-no-higata, Hiroshima Pref. (present study), of Gogoshima Island, Ehime Pref. (the other type locality: Ikeda, 1904; present study), of Nakajima Island, Ehime Pref. (present study); and of Kagawa Pref., details unknown (present study), and in Yatsushiro Sea of Ôikejima Island, Kumamoto Pref. (present study).

Species of uncertain status

Ikedosoma qingdaoense Li, Wang and Zhou, 1994

Ikedosoma qingdaoense Li, Wang and Zhou, 1994: 207–208, fig. 2; Wang et al., 1995: 31; Zhou et al., 2007: 149–151, fig. 86.

Remarks

Ikedosoma qingdaoense was first described on the basis of six specimens collected from Huiquan Bay, Qingdao. It seems natural that the original description followed Fisher's (1946) definition of the genus *Ikedosoma*. However, if the oblique muscle layer of *I. qingdaoense* can be regarded to be fasciculate, the species may belong to *Ochetostoma*. Unfortunately, the description lacked any references to the muscle layer, and the original and other specimens of *I. qingdaoense* are no longer available for re-examination. Therefore, the generic affiliation of the present species remains unknown.

Besides its doubtful generic affiliation, according to the original description of *I. qingdaoense* this species was distinguishable from *I. elegans* by the number of LMB (12 instead of 10) and by the position of gonoducts (behind the ventral setae in the former instead of only the anterior-most pair in front of the setae in the latter). However, the original or subsequent descriptions and the present studies of *I. elegans* clearly show that the number of LMB in *I. elegans* ranges from eight to 12, covering the number found in *I. qingdaoense*. Furthermore, Ikeda's (1907) subsequent description of *I. elegans* also shows that the anterior-most pair of gonoducts may be located in front of the ventral setae, although it was never detected in the material referred to in the present study. Therefore, *I. qingdaoense* appears to be also similar to most, if not all, specimens of *I. elegans* in that all gonoducts are situated behind the setae.

Thus, the abovementioned differences between these two species cannot be validated. Moreover, they also resemble each other in the variable longitudinal distance between adjacent gonoduct pairs (see fig. 2 of Li et al., 1994 for *I. qingdaoense*). Future comparisons are required between Japanese and Chinese populations to clarify their species identities.

Distribution

Known only from Qingdao, China, on intertidal sandy to muddy flats of Huiquan Bay (type locality: Li et al., 1994) and Xuejiadao, Jiaozhou Bay (Wang et al., 1995; Zhou et al., 2007).

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