Spherillo obscurus (Budde-Lund, 1885)とS. dorsalis (Iwamoto, 1943)の再定義，および，種同定のためのDNAマーカーの開発

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Redefinitions of *Spherillo obscurus* (Budde-Lund, 1885) and *S. dorsalis* (Iwamoto, 1943) (Crustacea: Oniscidea: Armadillidae), with DNA markers for identification

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Abstract The species definition of *Spherillo obscurus* (Budde-Lund, 1885) was redefined based on observations of syntype and specimens collected from a broad-leaved forest in The University of Tokyo (Hongo Campus), Japan. The species definition of *S. dorsalis* (Iwamoto, 1943) was also modified based on the specimens collected from a broad-leaved forest in Yokohama National University, Japan. Partial DNA sequences of mitochondrial cytochrome oxidase subunit I and 12S and 16S ribosomal RNA and of nuclear 18S and 28S ribosomal RNA were determined for identifying these species.

Key words: *Armadillo*, mitochondrial DNA, nuclear DNA, *Sphaerillo*, *Venezillo*

Introduction

The family Armadillidae is one of the most diversified families of terrestrial isopods (suborder Oniscidea), and includes about 600 species worldwide (Taiti *et al.*, 1998; Schmalfuss, 2004). However, the taxonomy of Armadillidae is very confusing, and thus a comprehensive revision of this family is essential to accurately gauge the true diversity of terrestrial isopods (Taiti *et al.*, 1998).

Budde-Lund (1885) described the first armadillid species as *Armadillo obscurus* Budde-Lund, 1885 based on specimens collected from Yokohama, Kanagawa Prefecture, Japan. In 1943, Iwamoto (1943) described *Armadillo dorsalis* Iwamoto, 1943, also from Yokohama. Thus far, a total of 33 armadillid species have been reported from Japan (Saito *et al.*, 2000; Nunomura, 2011). The original description of *A. obscurus*, however, is insufficient, because Budde-Lund (1885) mainly described morphological characters that defined taxa at the genus or family levels. Moreover, the type specimens of *A. dorsalis* have never been found (Nunomura, 2003), so the definitions of both species are still vague. This fact also causes doubts about the taxonomic statuses of armadillid species described after the two species. Thus, the species characters of *A. obscurus* and *A. dorsalis* must be redefined to encourage the taxonomic study of terrestrial isopods.

As a result of the comprehensive taxonomic studies of terrestrial isopods in Japan by N. Nunomura, most of the armadillid species in Japan were moved from the genus *Armadillo* to *Sphaerillo* (Nunomura, 1990), *Venezillo* (Nunomura, 1999a), and *Spherillo* (Nunomura, 2011). In this Introduction, however, we use the genus *Armadillo* for armadillid species in Japan for convenience. Nunomura (1990) investigated the specimens of *A. obscurus* deposited in the Berlin Museum, Germany, and the Zoologisches Museum of Universität Humburg, Germany. These specimens were collected from Uweno (probably Ueno in Tokyo Metropolis) and Moji (possibly an area in Fukuoka Prefecture), respectively; Nunomura (1990) redescribed *A. obscurus* based on the specimens collected from Uweno Park. The specimens described by Budde-Lund (1885), however, were collected from Yokohama, and were though to be deposited in the Museum für Naturkunde, Humboldt University, Germany, and

the Zoological Museum, University of Copenhagen, Denmark, as syntype (Jeppesen, 2000). Thus, the redefinition of *A. obscurus* requires examination of the syntype.

Nunomura (1990) also tried to redefine *A. dorsalis* based on specimens collected from Obama, Fukui Prefecture, because the type specimens of this species have never been found (Nunomura, 2003). According to his description, the morphology of the pleopod 1 exopodite of *A. dorsalis* is low triangular, and this morphological character has been considered important to distinguishing between *A. obscurus* and *A. dorsalis* (Nunomura, 1999b). However, the figure in Iwamoto (1943) suggested that the pleopod 1 exopodite of *A. dorsalis* from Yokohama is high triangular and perhaps more similar to that of *A. obscurus* in Nunomura (Fig. 142I in Nunomura, 1990) than the figures of *A. dorsalis* in Nunomura (Fig. 143I in Nunomura, 1990; Fig. 2Q in Nunomura, 2003). Thus, the specific definition of *A. dorsalis* by Nunomura (1990, 2003) might be mistaken. *Armadillo dorsalis* sensu Nunomura (1990, 2003) has been reported from Ibaraki to Osaka Prefectures (Nunomura, 2011), and is known to occupy Sankeien Garden at Yokohama (N. Nunomura, person. comm.). We were able to collect specimens of *A. dorsalis* sensu Nunomura (1990, 2003) from Sankeien Garden and examine whether this species conforms to the definition of *A. dorsalis* by Iwamoto (1943).

The second and third authors of this paper are studying the distribution and genetic divergence of Armadillidae in the Kanto region, central Japan. In the course of their work, they discovered at least two armadillid species in Kanto, and neither conformed to the description of *A. dorsalis* sensu Nunomura (1990, 2003).

The aim of this study was to redefine the specific characters of *A. obscurus* and *A. dorsalis*. To clarify the definitions of these species, we examined the syntype of *A. obscurus*, the specimens of *A. dorsalis* sensu Nunomura (1990, 2003) collected from Sankeien Garden, and two armadillid species found in Tokyo and Yokohama, Japan.

Materials and Methods

Samples

We collected samples of armadillid species from The University of Tokyo and Ueno Park in Tokyo and from Yokohama National University, Kamoike Park, Commonwealth War Cemetery Yokohama, and Sankeien Garden in Yokohama. Specimens were collected with aspirators from leaf litter and/or from beneath decaying logs and were preserved in 99.5% ethanol at room temperature until use. We also examined the syntype of *A. obscurus* deposited in the Museum für Naturkunde, Humboldt University (MNHU 6757). Of the specimens examined in this study, 3 male of *Spherillo obscurus* (KMNH-IvR 500698–500700), 3 males of *Spherillo dorsalis* (KMNH-IvR 500695–500697), and 2 males of *Spherillo* sp. collected from Sankeien Garden in Yokohama (KMNH-IvR 500701, 500702) were deposited in the collection of Kitakyushu Museum of Natural History and Human History (KMNH-IvR), Japan. The other specimens are deposited in the personal collection (SK).

Morphological study

The syntype of *A. obscurus* is in poor condition and we were only able to examine the pereonites 1 and 2, the pleopod 1 endopodite, the pleopod 2 endo- and exopodites, and the telson. One vial of the syntype (MNHU 6757) contained more than 2 individuals. Morphological observations were made using a stereoscope SZH (Olympus, Japan) and a microscope Eclipse E400 (Nikon, Japan) and specimens that were mounted temporarily on slides. The noduli laterales were also examined from scanning electron microscope (SEM) images. The specimens for SEM were dried at room temperature, coated with gold, and photographed using a SEM JCM-5100 (JEOL, Japan). Color photos were produced from multi-focused montage images using a digital microscope VHX-2000 (KEYENCE Corporation, Japan). The original figures of pleopods 1 and 2 of *A. dorsalis* described by Iwamoto (1943; Figs. 24D, E) were traced from an original print using the vector graphics editor Inkscape (http://inkscape.org/). Permission to trace and print the original figures was obtain from Yokendo Co. Ltd, Japan. The specimens used for the morphological study are described in Material examined.

DNA extraction and PCR amplification

Total DNA was prepared by using a Qiagen DNeasy Blood and Tissue Kit, according to the manufacturer's protocol (Qiagen, Germany). Mitochondrial cytochrome oxidase subunit I (COI), mitochondrial 12S and 16S ribosomal RNA (r RNA) genes, and nuclear 18S and 28S rRNA genes were used as molecular markers. Primers used for polymerase chain reactions (PCRs) are listed in Table 1. PCRs were carried out in 20-μl reaction volumes with Ex Taq (Takara Bio, Japan). The cycle program comprised an initial denaturation step at 94°C for 3 min; followed by 30 cycles

Table 1. PCR primers used in this study.

Genes	Primer	Sequences (5′ to 3′)	Source
Forward			
COI	LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer *et al.* (1994)
12S	12Sai	AAACTAGGATTAGATACCCTATTAT	Palumbi (1996)
16S	16Sar-int-sf	GCCGCAGTATHCTRACTGTGCT	Parmakelis *et al.* (2008)
18S	18Sforward	TACCTGGTTGATCCTGCCAG	Maraun *et al.* (2009)
28S	D3A	GACCCGTCTTGAAACACGGA	Litvaitis *et al.* (1994)
Reverse			
COI	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer *et al.* (1994)
12S	12Sbi	AAGAGCGACGGGCGATGTGT	Palumbi (1996)
16S	16Sbr	CCGGTCTGAACTCAGATCACGT	Klossa-Kilia *et al.* (2006)
18S	18S614r	TCCAAC TACGAGCTTTTTAACC	Maraun *et al.* (2009)
28S	D3B	TCGGAAGGAACCAGCTACTA	Litvaitis *et al.* (1994)

Table 2. Species, locality and DDBJ accession numbers.

Species	Locarity	DDBJ sccession no.				
		COI	12S	16S	18S	28S
S. obscurus	Ueno Park, Taito-ku, Tokyo	AB861896	-	AB861909	AB861916	AB861923
S. obscurus	The University of Tokyo, Bunkyo-ku, Tokyo	AB861900	AB861906	AB861913	AB861920	AB861926
S. obscurus	The University of Tokyo, Bunkyo-ku, Tokyo	AB861901	AB861907	AB861914	AB861921	AB861927
S. dorsalis	Kamoike Park, Tsuzuki-ku, Yokohama, Kanagawa	AB861897	AB861903	AB861910	AB861917	AB861924
S. dorsalis	Commonwealth War Cemetery Yokohama, Hodogaya-ku, Yokohama, Kanagawa	AB861898	AB861904	AB861911	AB861918	-
S. dorsalis	Yokohama National Uiversity, Hodogaya-ku, Yokohama, Kanagawa	AB861899	AB861905	AB861912	AB861919	AB861925
Spherillo sp.	Sankeien Garden, Naka-ku, Yokohama, Kanagawa	AB861902	AB861908	AB861915	AB861922	AB861928

- shows fragments that were not deteced.

of 1 min at 94°C, 1 min at 44–48°C, and 1 min at 72°C; and a 7-min extension at 72°C. PCR products were purified using a QIAquick PCR Purification Kit (Qiagen) and directly sequenced by Macrogen Japan (Japan) using the same primer sets used for PCR. Pairwise genetic distances were estimated by the Kimura 2-parameter model (K2P; Kimura, 1980) using MEGA 5 (Tamura *et al.*, 2011). Details of the DNA samples and the DDBJ accession numbers of sequences are described in Table 2.

Results

Morphological study

Taxonomic Account

Genus *Spherillo* Dana, 1853

[Japanese name: Koshibiro-dangomushi-zoku]

***Spherillo obscurus* (Budde-Lund, 1885)**

[Japanese name: Tôkyô-koshibiro-dangomushi]

(Figs. 1–6)

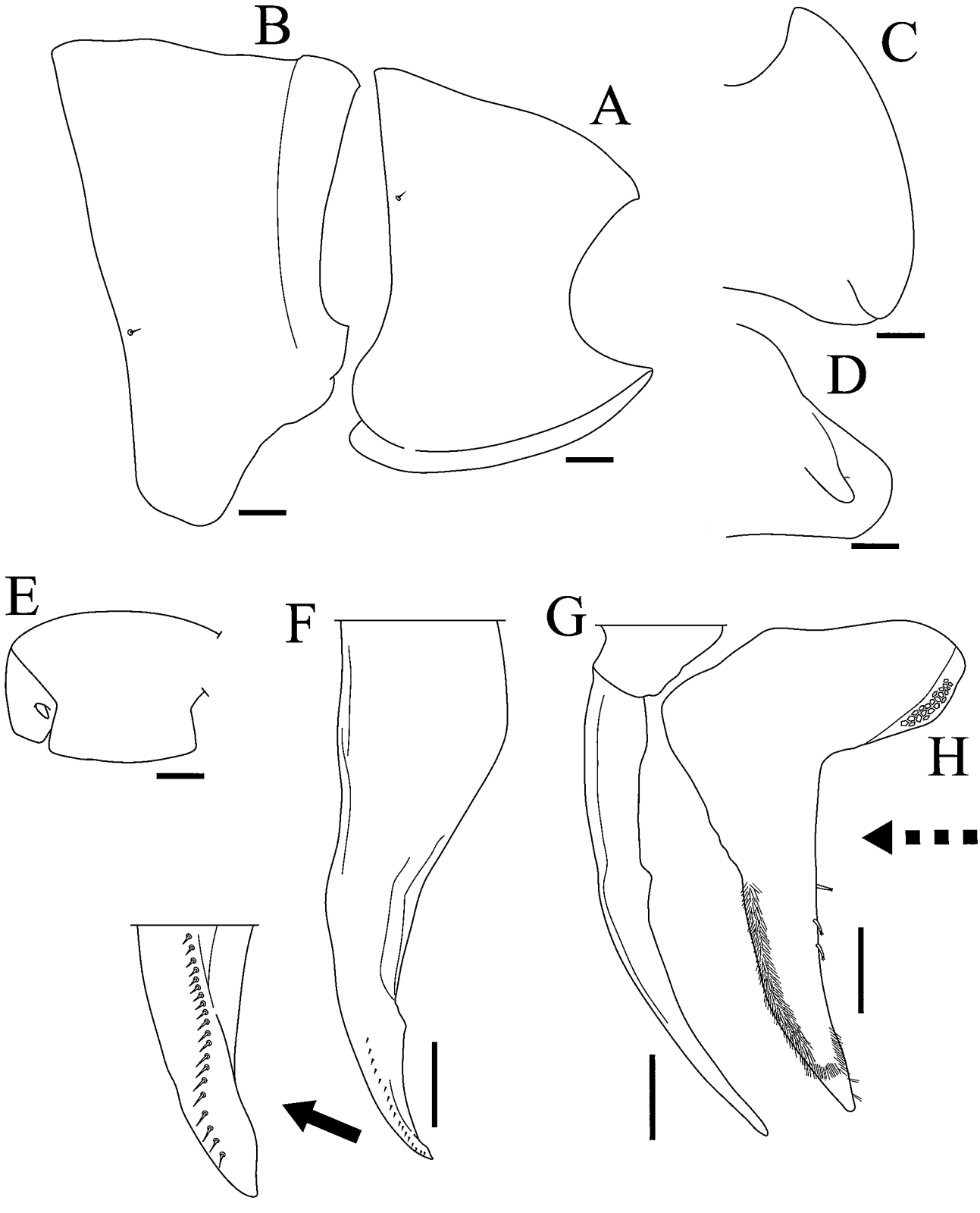

Fig. 1. *Spherillo obscurus*, syntype, MNHU 6757. A: pereonite 1, lateral view; B: pereonite 2, lateral view; C: pereonite 1, ventral view; D: pereonite 2, ventral view; E: uropod and telson, dorsal view; F: pleopod 1 endopodite; G: pleopod 2 endopodite; H: pleopod 2 exopodite, arrow indicates distal part. Scale bars: 200 μm.

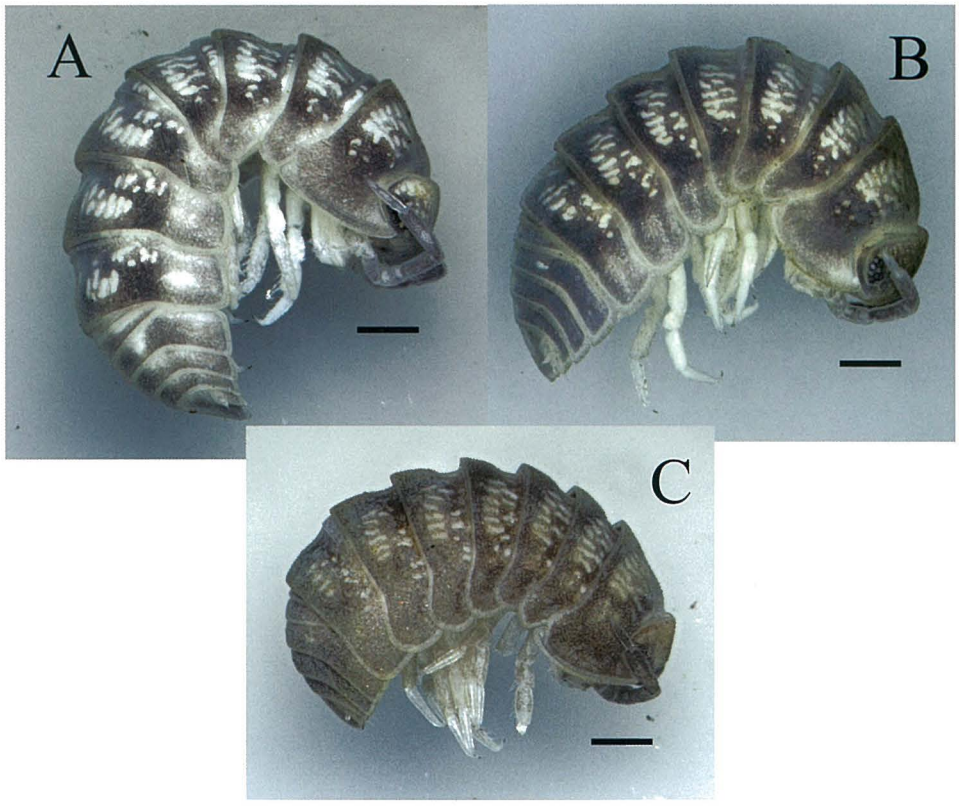

Fig. 2. Lateral view of male specimens in 99.5% ethanol. A: *Spherillo obscurus*, KMNH-IvR 500700, collected from The University of Tokyo; B: *S. dorsalis*, KMNH-IvR 500695, collected from Yokohama National University; C: *Spherillo* sp. (*S. dorsalis* sensu Nunomura), KMNH-IvR 500701, collected from Sankeien Garden. Scale bars: 1 mm.

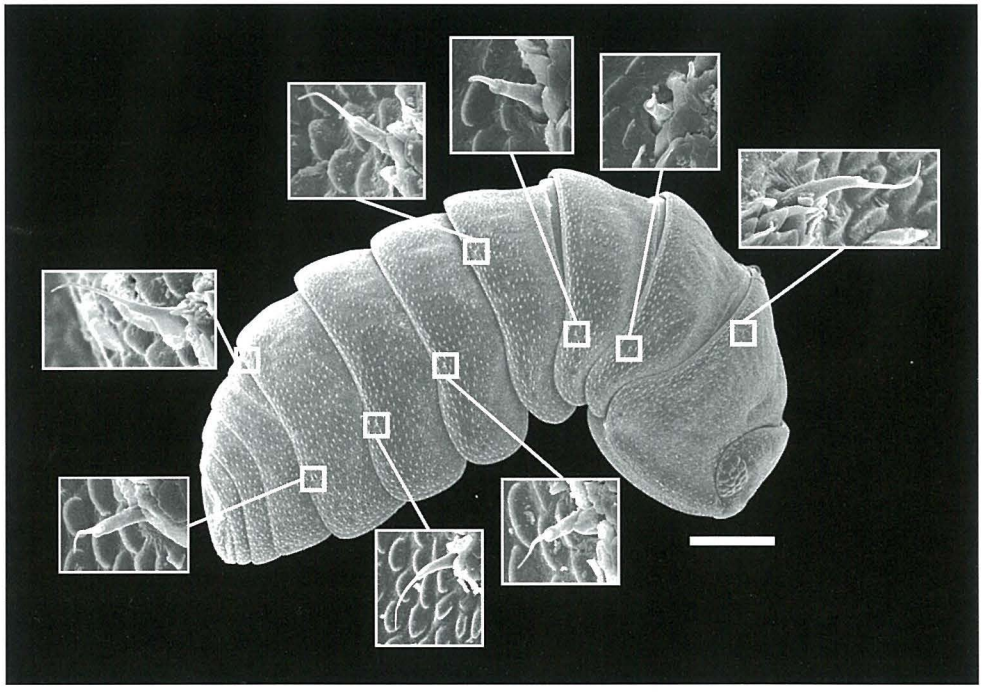

Fig. 3. Scanning electron microscope photographs of noduli laterales (in quadrangular frames) and body (lateral view) of *Spherillo obscurus* (SK ve-3298, male) collected from The Univerisity of Tokyo. Antennae 2 and all pereiopods were removed. Scale bar: 500 μm.

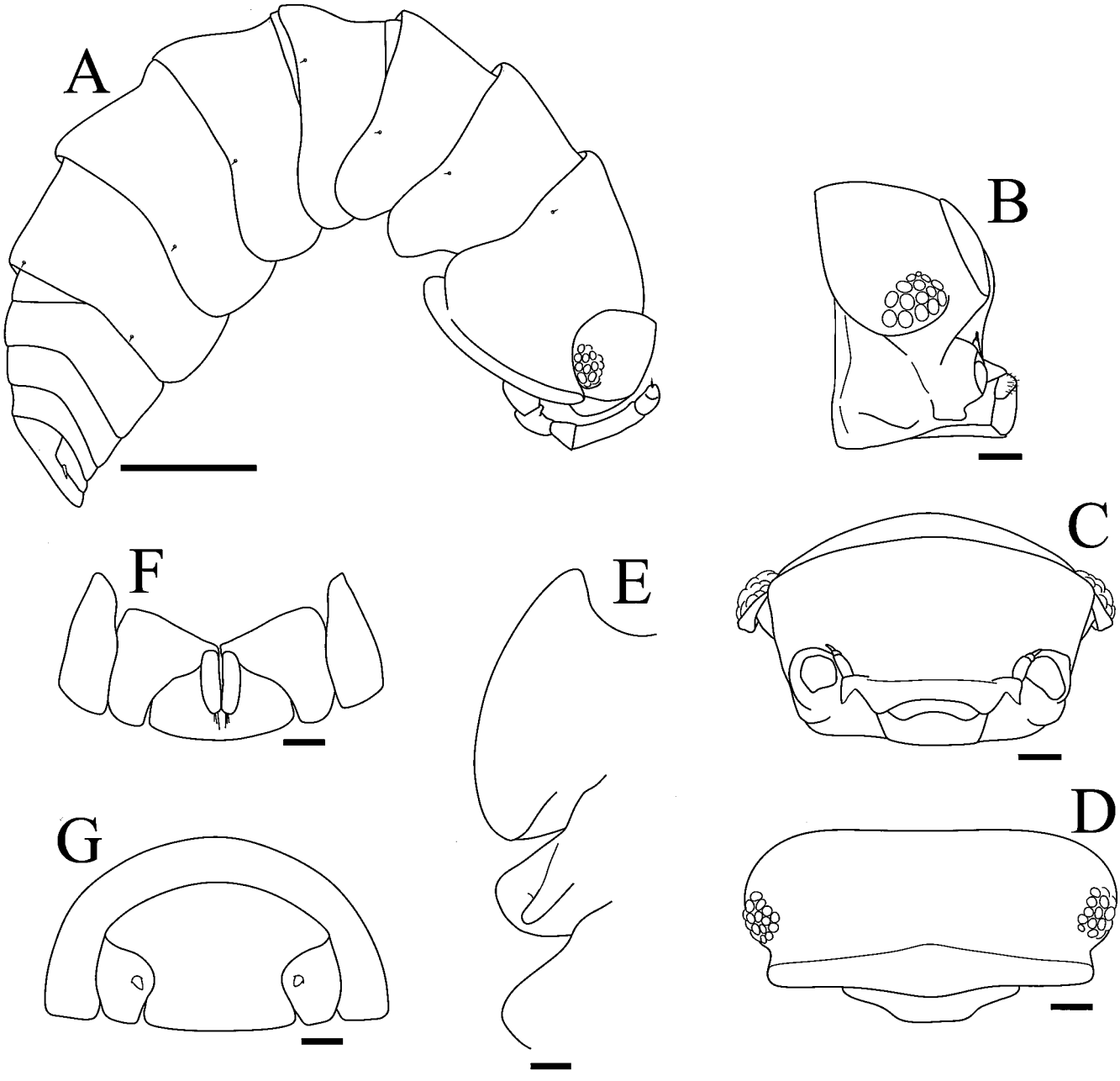

Fig. 4. *Spherillo obscurus*, male, KMNH-IvR 500699, collected from The University of Tokyo. A: Body, lateral view; B: cephalon, lateral view; C: cephalon, frontal view; D: cephalon, dorsal view; E: pereonites 1 and 2, ventral view; F: uropods and telson, ventral view; G: pleonite 5, uropods and telson, dorsal view. Scale bars: 1 cm in A, 200 μm in B–G.

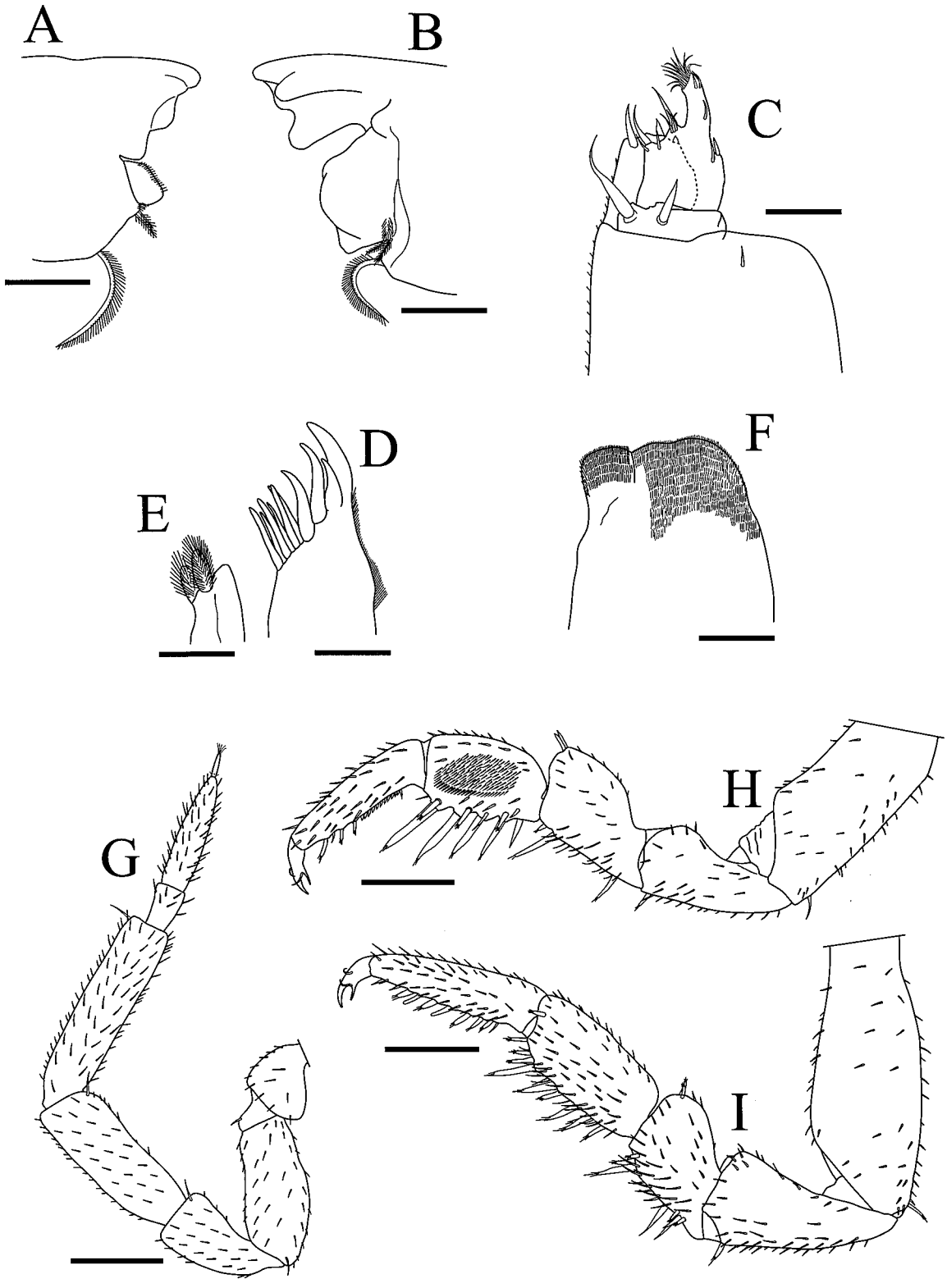

Fig. 5. *Spherillo obscurus*, male, collected from The University of Tokyo. A: Right mandible, KMNH-IvR 500698; B: left mandible, KMNH-IvR 500698; C: maxilliped, KMNH-IvR 500699; D: outer endite of first maxilla, KMNH-IvR 500699; E: inner endite of first maxilla, KMNH-IvR 500699; F: second maxilla, KMNH-IvR 500699; G: second antenna, KMNH-IvR 500699; H: pereiopod 1, frontal view, KMNH-IvR 500699; I: pereiopod 7, frontal view, KMNH-IvR 500699. Scale bars: 100 μm in A–F, 300 μm in G–I.

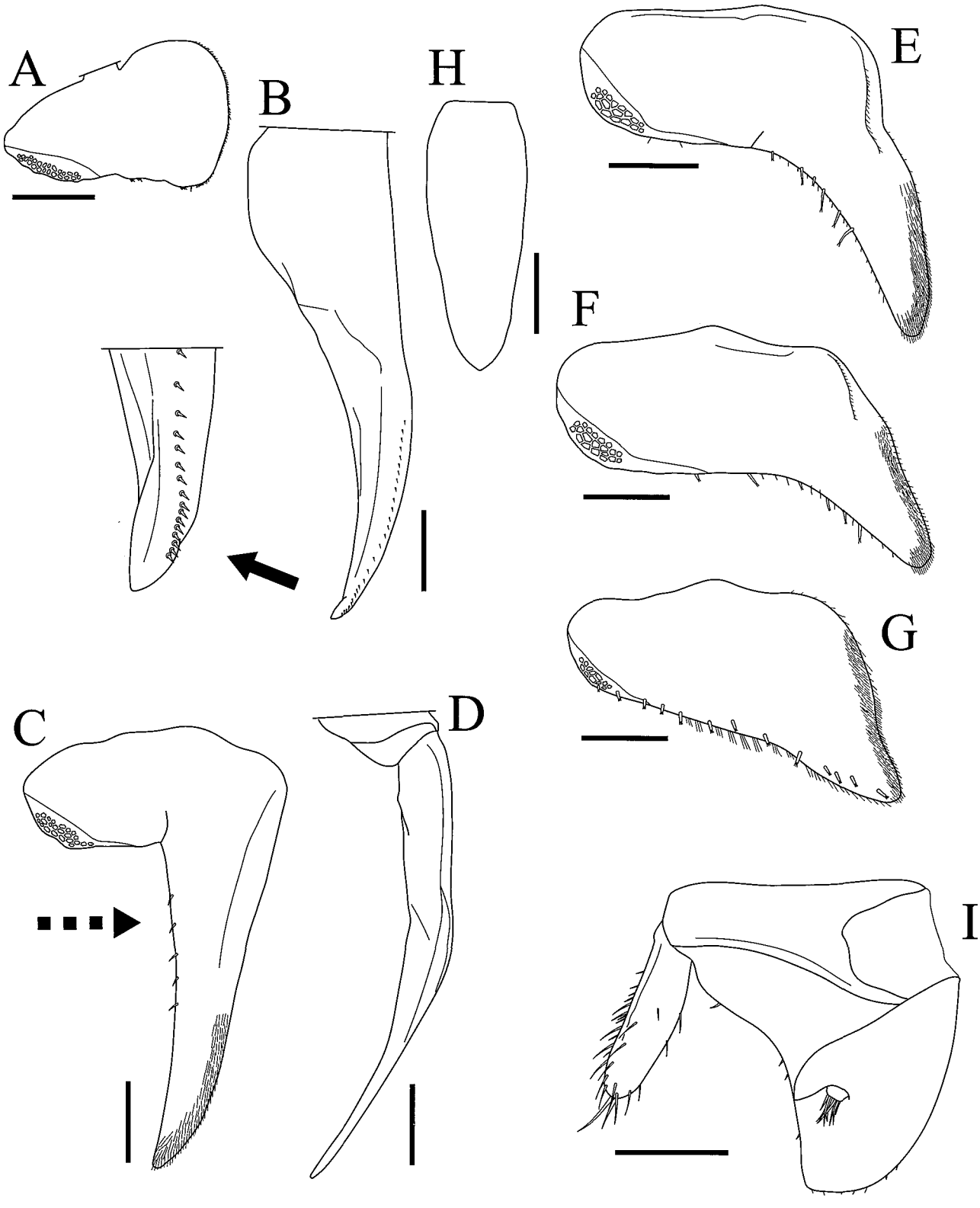

Fig. 6. *Spherillo obscurus*, male, KMNH-IvR 500699, collected from The University of Tokyo. A: Pleopod 1 exopodite; B: pleopod 1 endopodite; C: pleopod 2 exopodite, arrow indicates distal part; D: pleopod 2 endopodite; E: pleopod 3 exopodite; F: pleopod 4 exopodite; G: pleopod 5 exopodite; H: genital papilla (ventral shield); I: uropod, dorsal view. Scale bars: 200 μm.

Material examined. Syntype of *Armadillo obscurus*: MNHU 6757, 2 males?, Yokohama, collected by Martens Allata, dissected. Non types: KMNH IvR 500698–500700, 3 males, forest floor in broad-leaved forest (mainly Fagaceae spp.), The University of Tokyo (Hongo Campus) at Tokyo (N35.7125, E139.7614), 1 August 2013, collected by Yasuto Kanazawa.

Description. Syntype of *Armadillo obscurus*: Body color white. One nodulus lateralis per side on pereonites 1 and 2; that on pereonite 1 further from lateral margin than that on pereonite 2 (Figs. 1A, B). Pereonite 1 with lateral margin not grooved; schisma of pereonite 1 deep with inner lobe almost equal to outer lobe; inner lobe of schisma protruding posteriorly relative to outer lobe (Figs. 1A, C). Pereonite 2 with an oblique lobe on ventral surface (Fig. 1D). Telson hour-glass-shaped (Fig. 1E). Pleopod 1 endopodite with apical part bent outwards (Fig. 1F). Pleopod 2 endopodite slender, without special modification (Fig. 1G); pleopod 2 exopodite with long triangular distal part (arrow in Fig. 1H); distal part relatively slender, its outer base almost a right angle (Fig. 1H).

Specimens collected from The University of Tokyo: Body color black with yellowish spots on dorsal surface, distinctly white on lateral margin in absolute ethanol (Fig. 2A). One nodulus lateralis per side on pereonites 1–6, 2 noduli laterales on pereonite 7; those on pereonites 1 and 4 further from lateral margin than those on pereonites 2, 3, 5 and 6 (Figs. 3, 4A). Eyes with about 15 ommatidia (Figs. 3, 4A, B). Cephalon with frontal shield separated and bent over vertex; frontal lamina not protruding beyond vertex (Figs. 4B–D). Pereonite 1 with schisma deep; inner lobe almost equal to outer lobe (Figs. 4A, E). Pereonite 2 with an oblique lobe on ventral surface (Fig. 4E). Telson hour-glass-shaped, wider basally than distally; distal margin straight (Figs. 4F, G). Left mandible with larger lacinia mobilis than right mandible; right lacinia mobilis covered with minute setae; pars molaris unbranched (Figs. 5A, B). Maxilliped with endite rectangular bearing 3 spines on distal margin; maxilliped palp with basal article bearing 2 long setae, distal article with apical turf of small setae (Fig. 5C). First maxilla consisting of inner and outer endites; outer endite with 4 + 6 simple setae; inner endite with 2 stout plumose setae (Figs. 5E, D). Second maxilla apically bilobate; inner lobe smaller (Fig. 5F). Second antenna with 2 flagella (Fig. 5G). Pereiopod 1 with antennal brush on carpus; propodus with 10 short setae on basal half of inner margin; carpus with 3–5 long stout setae on inner margin; merus with 2 long setae on apical and basal sides of inner margin (Fig. 5H). Pereiopod 7 without special modification (Fig. 5I). Pleopod 1–5 exopodites monospiracular; pleopod 1 exopodite high triangular, inner margin longer than outer one; pleopod 1 endopodite with apical part bent outward (Figs. 6A, B). Pleopod 2 exopodite with long triangular distal part (arrow in Fig. 6C), more slender than in *S. dorsalis*; outer base of distal part almost a right angle; pleopod 2 endopodite slender, apical part bent outward (Figs. 6C, D). Pleopod 3 and 4 exopodites with triangular part on posterior inner corner; pleopod 5 exopodite triangular (Figs. 6E–G). Genital papilla with fusiform genital shield (Fig. 6H). Uropodal protopod trapezoidal, with a short exopodite beneath a small tooth (Fig. 6I).

Remarks. Observation of syntype of *A. obscurus* showed that the nodulus lateralis on pereonite 1 inserts further from the lateral margin than the one on pereonite 2 and that the distal part of the pleopod 2 exopodite is slender and its outer base is almost right anglular. These morphological characters are consistent with those of the armadillid species collected from The University of Tokyo. Thus we considered the specimens collected from The University of Tokyo to be *A. obscurus*. The syntype and the specimens collected from The University of Tokyo have morphological characters that are identical to the definition of the genus *Spherillo*: cephalon with frontal lamina does not protrude over vertex, an oblique tooth-like lobe lies on the ventral surface of pereonite 2, a short exopodite inserts beneath a transversal tooth on the protopod of uropod, first maxilla inner endite bears 2 stout plumose setae, monospiraculars are found on all pleopod exopodites; although the inner lobe on pereonite 1 schisma is almost equal in length to that of the outer lobe (Kwon and Taiti, 1993). Thus, this species can be assigned to the genus *Spherillo*. Based on the specimens collected from The University of Tokyo, we redefine *S. obscurus* as follows: 1) the noduli laterales on pereonites 1 and 4 insert further from the lateral margin than those on pereonites 2, 3, 5 and 6, but the setae on pereonites 2 and 3 infrequently insert further from the lateral margin; 2) the carpus of pereiopod 1 bears 3–5 stout setae on its inner margin; 3) the merus of pereiopod 1 usually bears 2 long setae on its inner margin; 4) the distal part on pleopod 2 exopodite is slender and its outer base is almost right angular; 5) the lateral sides of fresh specimens are distinctively pale in absolute ethanol.

Spherillo dorsalis (Iwamoto, 1943)

[Japanese name: Seguro-koshibiro-dangomushi]

(Figs. 2, 7–10)

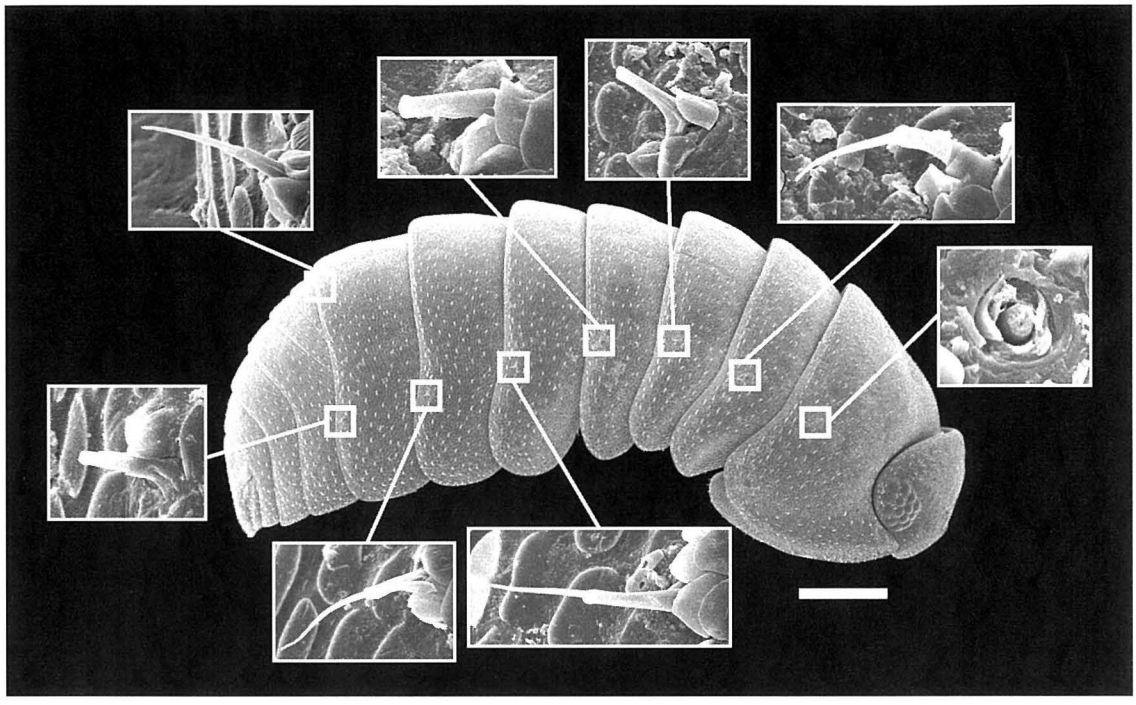

Fig. 7. Scanning electron microscope photographs of noduli laterales (in quadrangular frames) and body (lateral view) of *Spherillo dorsalis* (SK ve-2315, male) collected from Yokohama National University. Antennae 2 and all pereiopods were removed. Scale bar: 500 μm.

Material examined. Non types: KMNH IvR 500695–500697, 3 males, forest floor in broad-leaved forest (mainly Fagaceae spp.), Yokohama National University at Yokohama, Kanagawa Pref. (N35.47268, E139.589619), 3 June 2010, collected by Atori Kurita.

Description. Specimens collected from Yokohama National University. Body color black with yellowish spots on dorsal surface, pale or yellowish on lateral margins (Fig. 2B). One nodulus lateralis per side on pereonites 1–6, 2 noduli laterales on pereonite 7; all more or less at same distance from lateral margin except for inner one on pereonite 7 (Figs. 7, 8A). Eyes with about 15 ommatidia (Figs. 7, 8A, B). Cephalon with frontal shield; profrons slightly depressed in middle (Figs. 8B–D). Pereonite 1 with schisma; inner lobe almost equal to outer one (Figs. 8A, E). Pereonite 2 with a large ventral tooth (Fig. 8E). Telson hour-glass-shaped; distal margin straight (Figs. 8F, G). Mandible, maxilliped, first and second maxillae, and second antenna identical to ones of *S. obscurus* (Figs. 9A–G). Pereiopod 1 with 3–5 long setae on inner margin of carpus; merus with 4 setae on inner margin (Fig. 9H). Pereiopod 7 with normal congeneric morphology (Fig. 9I). Pleopods 1–5 similar to those of *S. obscurus* (Figs. 10A–G); except pleopod 2 exopodite with long triangular part on distal margin (arrow in Fig. 10C); distal part thicker than in *S. obscurus* (Fig. 10C). Genital papilla with fusiform genital shield (Fig. 10H). Uropod trapezoid; a small exopodite inserted on dorsal surface, beneath a short tooth (Fig. 10I).

Remarks. Iwamoto (1943) described that body of *A. dorsalis* was yellowish-brown laterally, and the bodies of specimens collected from Yokohama National University were pale or yellowish on the sides. Moreover, Iwamoto's figures of the pleopod 1 and 2 exopodites were very similar to those of specimens collected from Yokohama National University (Fig. 11; Figs. 24D, E in Iwamoto, 1943): pleopod 1 exopodite is high triangular and the distal part on pleopod 2 exopodite is relatively thick. Thus, we assigned the specimens collected from Yokohama National University to *A. dorsalis* sensu Iwamoto (1943). However, this species is clearly distinguishable from '*dorsalis*' sensu Nunomura (1990, 2003) in body color and in the morphology of pereiopod 1 and pleopod 1 exopodite (see Discussion). The specimens collected from Yokohama National University have all the

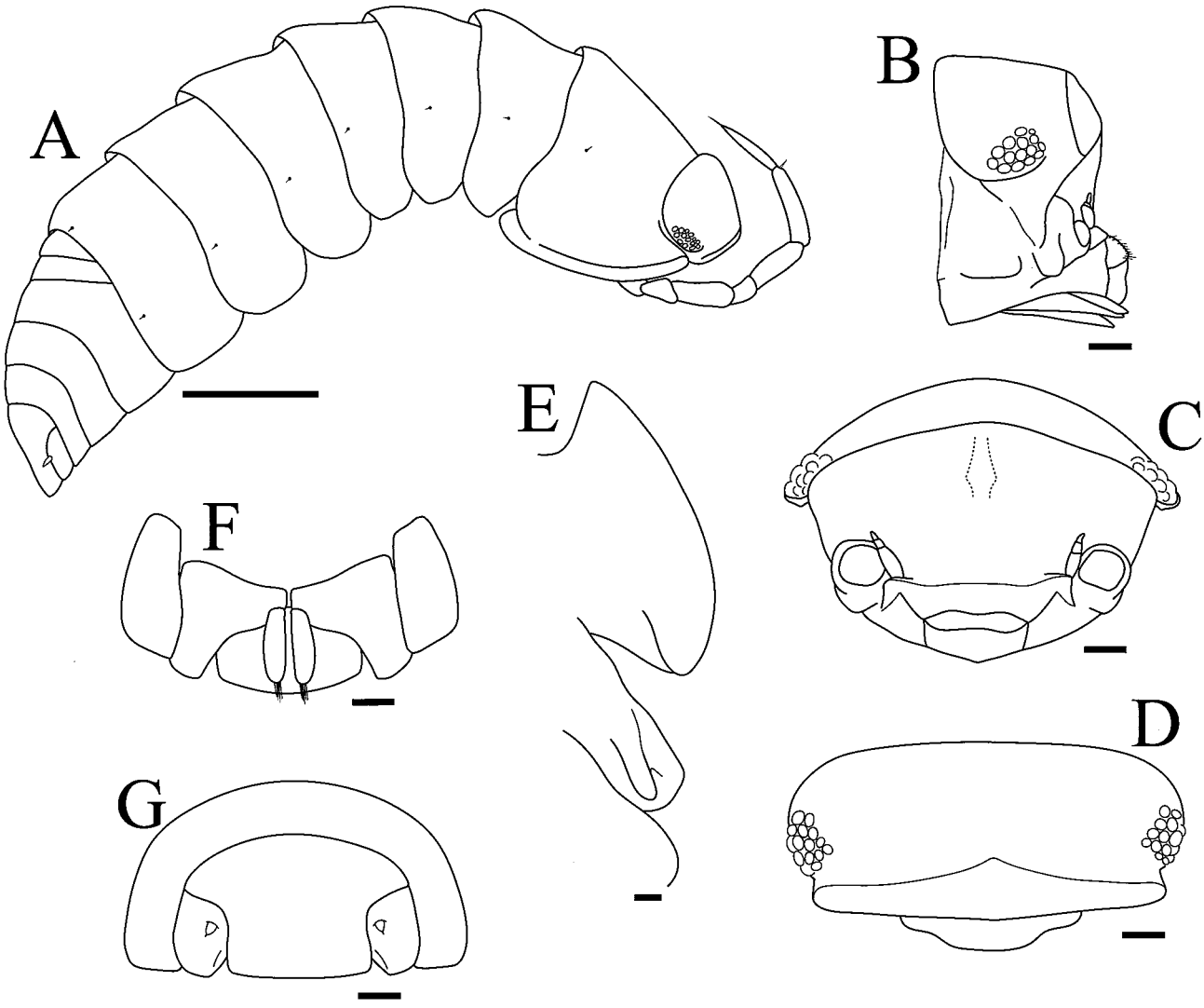

Fig. 8. *Spherillo dorsalis*, male, KMNH-IvR 500697, collected from Yokohama National University. A: Body, lateral view; B: cephalon, lateral view; C: cephalon, frontal view; D: cephalon, dorsal view; E: pereonites 1 and 2, ventral view; F: uropods and telson, ventral view; G: pleonite 5, uropods and telson, dorsal view. Scale bars: 1 cm in A, 200 μm in B–G.

generic characters of *Spherillo* except for the schisma of pereonite 1: the inner lobe is almost equal in length to the outer lobe (Kwon and Taiti, 1993). *Spherillo dorsalis* as redefined in this study appears to be very closely related to *S. obscurus* but is distinguished by 1) the noduli laterales on pereonites 1–6 are inserted at more or less the same distance from the lateral margin, 2) the setae on the inner margin of the carpus of pereiopod 1 is narrower than those of *S. obscurus*, 3) the merus of pereiopod 1 usually bears more than 3 long setae on the inner margin, and 4) the distal part on pleopod 2 exopodite is thicker than that of *S. obscurus*.

DNA sequences

The COI, 12S rRNA, 16S rRNA, 18S rRNA and 28S rRNA sequences were 615, 269–356, 386–391, 651 and 528–629 bases in length, respectively. The K2P distances between *S. obscurus* and *S. dorsalis* for the COI, 12S rRNA, 16S rRNA and 28S rRNA were 0.158, 0.075, 0.077 and 0.012, respectively, but there was no difference in the 18S rRNA region between these two species.

Discussions

Validity of '*dorsalis*' sensu Nunomura (1990, 2003)

We collected '*dorsalis*' sensu Nunomura (1990, 2003)

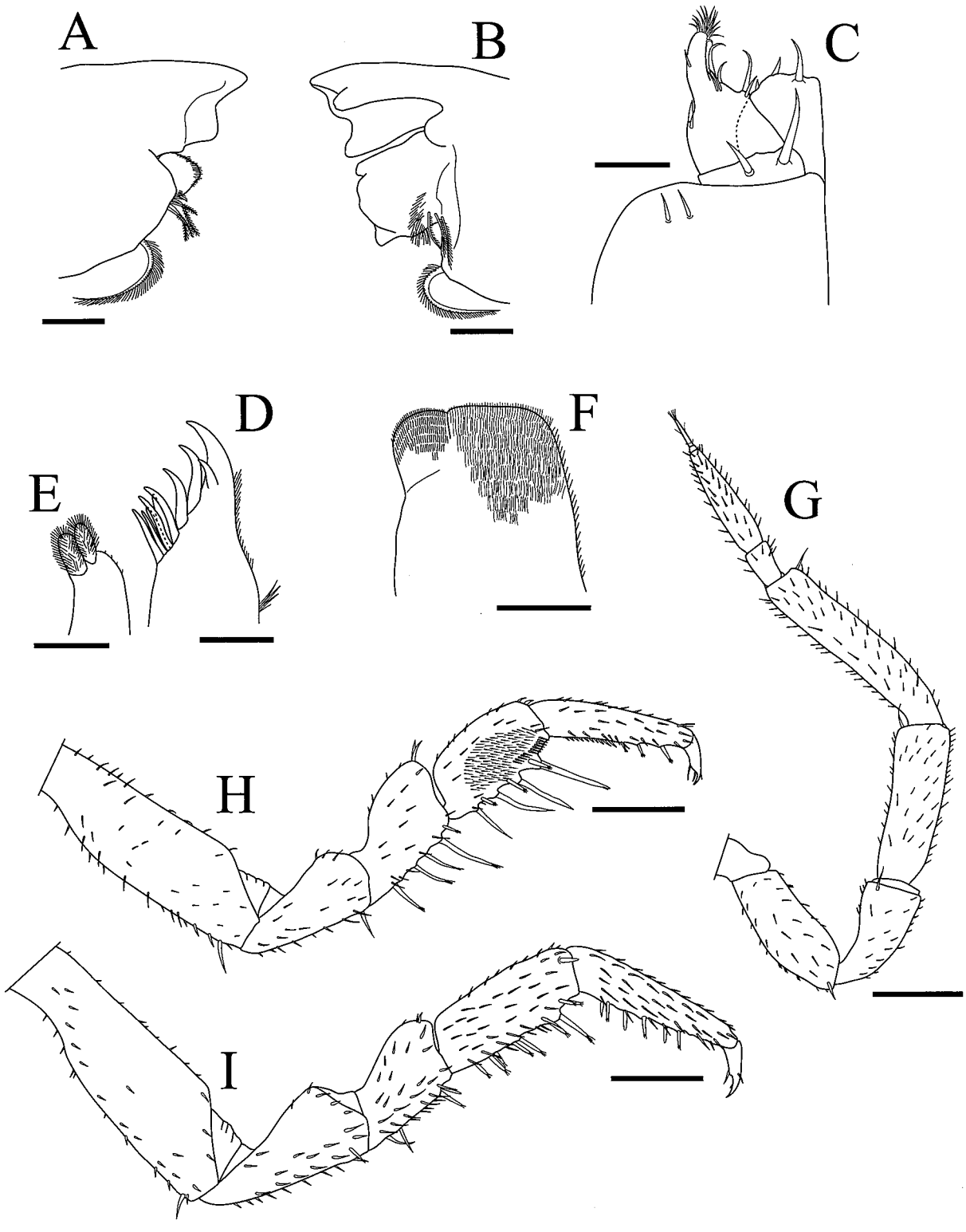

Fig. 9. *Spherillo dorsalis*, male, collected fromYokohama National University. A: Right mandible, KMNH-IvR 500696; B: left mandible, KMNH-IvR 500696; C: maxilliped, KMNH-IvR 500697; D: outer endite of first maxilla, KMNH-IvR 500697; E: inner endite of first maxilla, KMNH-IvR 500697; F: second maxilla, KMNH-IvR 500697; G: second antenna, KMNH-IvR 500697; H: pereiopod 1, frontal view, KMNH-IvR 500697; I pereiopod 7, frontal view, KMNH-IvR 500697. Scale bars: 100 μm in A–F, 300 μm in G–I.

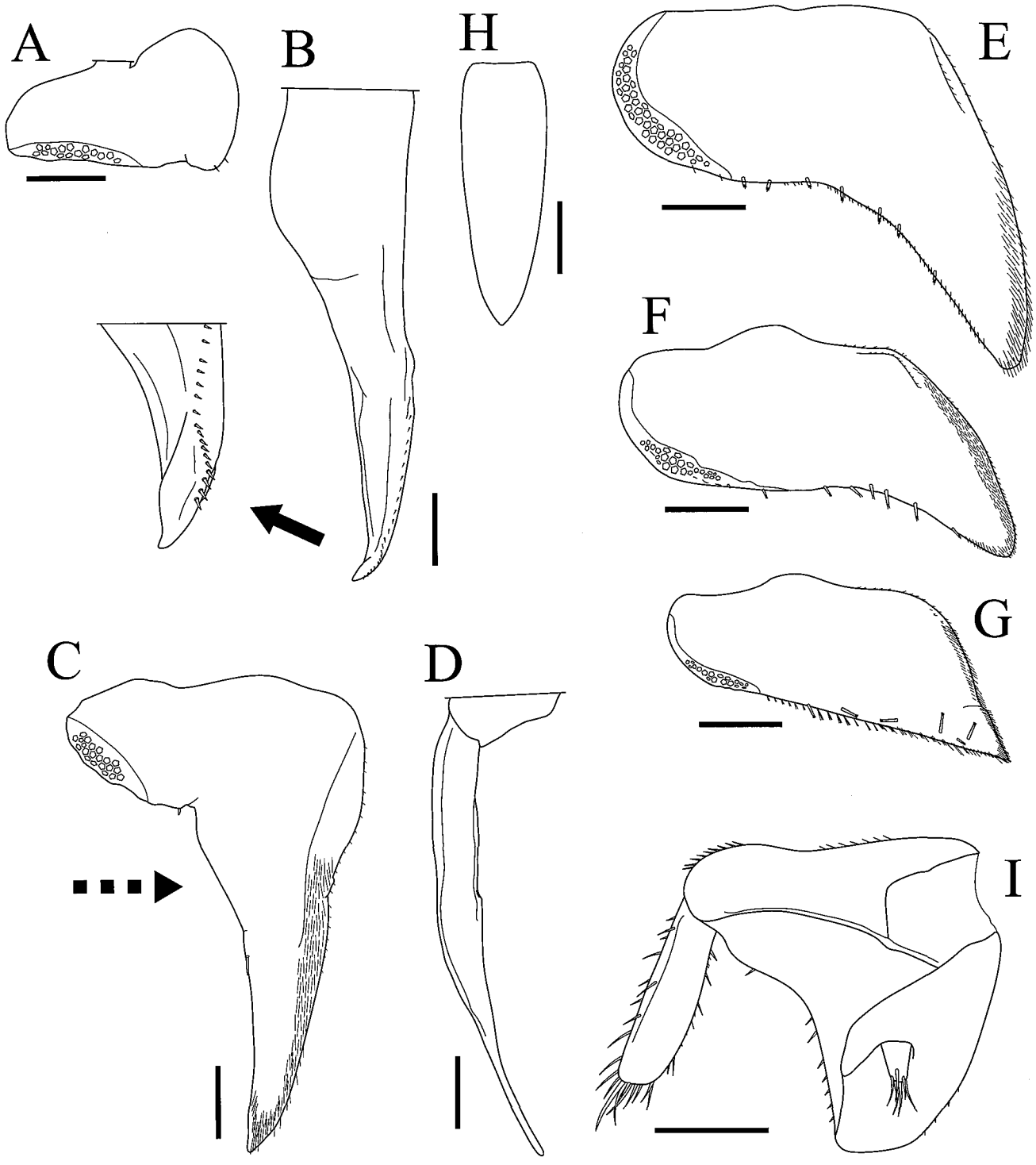

Fig. 10. *Spherillo dorsalis*, male, collected from Yokohama National University. A: Pleopod 1 exopodite, KMNH-IvR 500696; B: pleopod 1 endopodite, KMNH-IvR 500697; C: pleopod 2 exopodite, arrow indicates distal part, KMNH-IvR 500696; D: pleopod 2 endopodite, KMNH-IvR 500697; E: pleopod 3 exopodite, KMNH-IvR 500696; F: pleopod 4 exopodite, KMNH-IvR 500697; G: pleopod 5 exopodite, KMNH-IvR 500697; H: genital papilla (ventral shield), KMNH-IvR 500697; I: uropod, dorsal view, KMNH-IvR 500697. Scale bars: 200 μm.

from Sankeien Garden at Yokohama, where this species is known to occur in Kanto (N. Nunomura, person. comm.). We observed the following morphological characters: carpus of pereiopod 1 bears more than 10 long setae on inner margin; pleopod 1 exopodite is slender and low triangular; color of body sides is not pale or yellowish (Figs. 2, 12). These morphological characters are completely congruent with those of '*dorsalis*' sensu Nunomura (1990, 2003).

Nunomura (1990) stated that the pleopods of the specimens collected from Obama were morphologically similar to the figures of *A. dorsalis* by Iwamoto (1943). However, Nunomura's figures of the pleopod 1 exopodite (Fig. 143I in Nunomura, 1990; Fig. 2Q in Nunomura 2003) and the exopodite of specimens collected from Sankeien Garden (Fig. 12B) are obviously more slender than those in Iwamoto's figures (Fig. 11; Figs. 24D, E in Iwamoto, 1943). Moreover, Iwamoto (1943) described the lateral body of *A. dorsalis* as yellowish-brown, but the specimens collected from Sankeien Garden were almost black. Given the discordance of these morphological characters, we disagree with Nunomura's definition of '*dorsalis*'.

In contrast, the armadillid species collected from Yokohama National University had morphological characters (described above) that were completely consistent with the specific characters of *A. dorsalis* described by Iwamoto (1943). Thus, the species collected from Yokohama National University should be assigned to *A. dorsalis* sensu Iwamoto (1943) rather than to Nunomura's definition of '*dorsalis*', the scientific name of which remains unknown.

Generic status of the species obscurus and dorsalis

The taxonomy of Armadillidae is very confused (Taiti *et al.*, 1998), and the generic statuses of armadillid species in Japan have changed many times. First, both *obscurus* and *dorsalis* were described as members of the genus *Armadillo* (Budde-Lund, 1885; Iwamoto, 1943). Budde-Lund (1904) later transferred *A. obscurus* to the genus *Spherillo*. In 1990, *Spherillo obscurus* and *A. dorsalis* were assigned to the genus *Sphaerillo* by Nunomura (1990), and then Nunomura (1999a) transferred them to the genus *Venezillo*. More recently, these species were considered members of the genus *Spherillo* (Nunomura, 2011). *Spherillo* was first described by Dana (1852), but the name was not available because he did not designate a type species. Latter, Lehtinen *et al.* (1998) designated *Spherillo vitiensis* Dana, 1853 as type species of the genus *Spherillo*, making the genus available. The generic characters of *Spherillo* were described by Kwon and Taiti (1993); for examples, the frontal lamina of the cephalon does not or only slightly protrudes over the vertex; the schisma of pereonite 1 is deep and the inner lobe is shorter than the outer one; and pereonite 2 bears an oblique tooth-like lobe on ventral surface. The morphological characters of both *obscurus* and *dorsalis* as redefined here are congruent with the generic definition.

Species identification

We have not reexamined all of the specimens that have been reported as '*obscurus*' and '*dorsalis*', so we cannot make synonym lists for these species. Kwon (1995) regarded an armadillid species collected from Cheju Island, Korea, as *Venezillo obscurus*, but this species is obviously different from *S. obscurus* as redefined here in the positions of the noduli laterales and the morphology of pleopod 2 exopodite.

Spherillo obscurus and *S. dorsalis* are very similar to each other, but they can be distinguished by the position of the noduli laterales. Moreover, some morphological characters may be useful to identify these species: the long setae on the inner margin of pereiopod 1 of *S. obscurus* are thicker than those of *S. dorsalis*; the number and position of the setae on the inner margin of pereiopod 1 merus are different between these species; the distal part of pleopod 2 exopodite is more slender on *S. obscurus* than on *S. dorsalis*; the lateral body color change is more distinctive in *S. obscurus* than in *S. dorsalis*. Some morphological characters of these species, however, have been known to show intraspecific variation (Fig. 13), so precise identification of these species requires the careful observation of morphology and the use of DNA markers, e.g., the mitochondrial COI, 12S rRNA and 16S rRNA regions and the nuclear 28S rRNA region.

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We thank Dr. Charles Oliver Coleman (Museum für Naturkunde, Germany) for allowing S. Karasawa to examine the syntype of *A. obscurus*; Mr. Noboru Nunomura (Kanazawa University, Institute of Nature and Environmental Technology, Japan) for his helpful comments about terrestrial isopods; Mr. Hideki Kojima (Youkendo Co. Ltd, Japan) for permission to trace and reprint Iwamoto's figures; and Miss Juri Kato (Ibaraki, Japan), Mr. Kouki Yoshino (Chiba University, Japan), and Miss Atori Kurita (Kanagawa, Japan) for collecting specimens. This work was partly supported by Grants-in-Aid (20248015, 25281053, 25292082) from the Japan Society for the Promotion of Science.

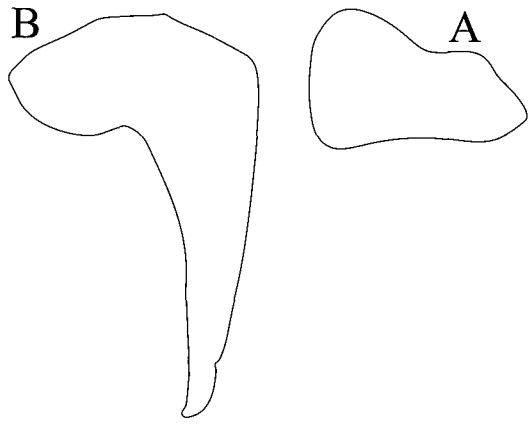

Fig. 11. Traces of original figures of pleopods 1 (A) and 2 (B) exopodites of *Armadillo dorsalis* described by Iwamoto (1943; Figs. 24D, E). Traced and printed by courtesy of Yokendo Co. Ltd, Japan.

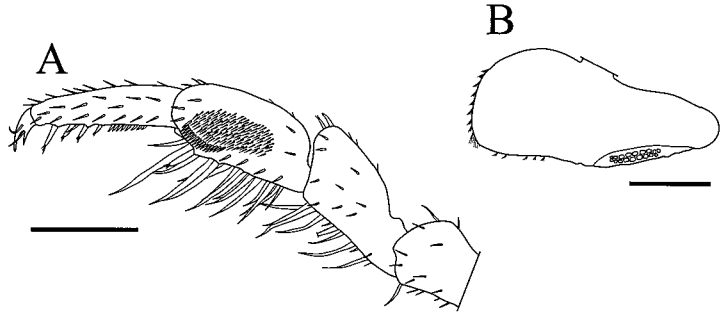

Fig. 12. *Spherillo* sp. (*S. dorsalis* sensu Nunomura), male, collected from Sankeien Garden. A: Pereiopod 1, frontal view, KMNH-IvR 500702; B: pleopod 1 exopodite, SK ve-3337. Scale bars: 300 μm in A, 200 μm in B.

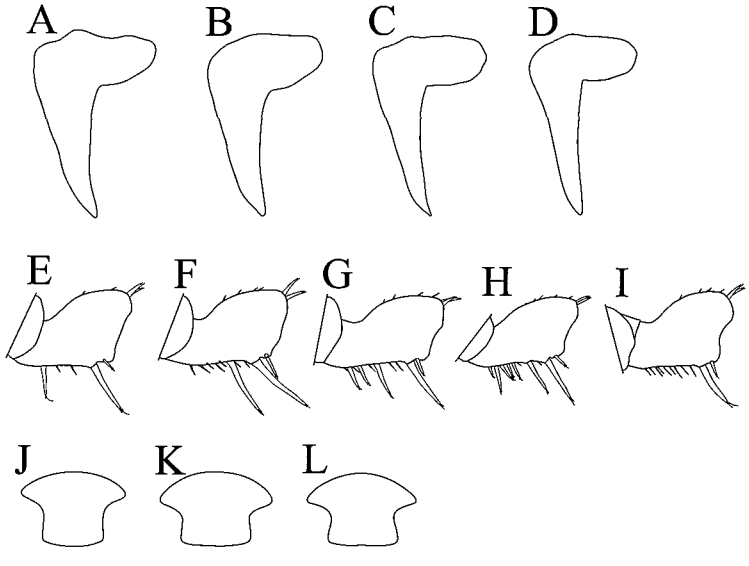

Fig. 13. Scheme of morphological variations in *S. obscurus* and *S. dorsalis*. A, B: Pleopod 2 exopodites, *S. dorsalis*, male, Yokohama National University; C, D: pleopod 2 exopodites, *S. obscurus*, male, The University of Tokyo; E–H: merus of pereiopod 1, *S. dorsalis*, male, Yokohama National University; I: merus of pereiopod 1, *S. obscurus*, male, The University of Tokyo; J–L: telsons, *S. dorsalis*, male, Yokohama National University.

摘 要

唐沢重考[1]・金澤泰人[2]・久保田耕平[2]（[1]福岡教育大学 〒811-4192 福岡県宗像市赤間文教町1-1・[2]東京大学 〒113-8657 東京都文京区弥生1-1-1）：*Spherillo obscurus* (Budde-Lund, 1885) と *S. dorsalis* (Iwamoto, 1943) の再定義，および，種同定のためのDNAマーカーの開発．

シンタイプおよび東京大学本郷キャンパスの広葉樹林内で得られた標本に基づき *Spherillo obscurus* (Budde-Lund, 1885) の再定義を行った．また，横浜国立大学の広葉樹林内で得られた標本に基づき *S. dorsalis* (Iwamoto, 1943) についての再定義も行った．さらに，種同定用のDNAマーカーを開発するため，ミトコンドリアDNAのCOI, 12S rRNA, 16S rRNA領域，および，核DNAの18S rRNA, 28S rRNA領域の一部の塩基配列を決定した．

キーワード：*Armadillo*, ミトコンドリアDNA, 核DNA, *Sphaerillo*, *Venezillo*

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