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# A New Species of the Genus *Hypsibius* (Tardigrada: Parachela: Hypsibiidae) from Sakhalin Island, Far East Russia

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**ABSTRACT**—A new species of semiterrestrial eutardigrade, *Hypsibius stilliferus*, is described from Sakhalin Island, Far East Russia. The new species is distinguished from its congeners by having an irregular, polygonal or subtriangular dorsal sculpture, which increases in size posteriorly, two granular macroplacoids, and a cuticular bar near the base of posterior claw 4, and by lacking a microplacoid and septulum. It is currently known from its type locality and several other localities in northern and southern Sakhalin Island. This is the first report concerning tardigrades from Sakhalin Island.

**Key words:** taxonomy, water bear, Eutardigrada, Hypsibiidae, Russian Far East

## INTRODUCTION

*Hypsibius* Ehrenberg, 1848, the type genus of the family Hypsibiidae, is one of the oldest genera in the phylum Tardigrada. This genus is widely distributed from tropical to Arctic and Antarctic regions (McInnes, 1994) and inhabits both semiterrestrial (e.g., mosses, lichens, or surface soil) and freshwater environments. Although numerous taxonomic problems surround the genus (e.g., Binda and Pilato, 1987; Nelson and McGlothlin, 1993), more than 30 species are currently considered to be valid.

Knowledge of the tardigrade fauna of Russia is still incomplete, and most papers deal with the fauna of European or central Russia (e.g., Bozhko, 1936; Biserov, 1988, 1989, 1991, 1996a, 1998a, 1999). In Far East Russia, Tchusunov and Mokievsky (1995) and Biserov (1996b) described a new marine and a semiterrestrial species, respectively, from islands in the Sea of Japan off Vladivostok; Biserov (1998b) reported 42 semiterrestrial species from the Commander Islands in the Bering Sea; and Biserov (1998c) reported a marine species from the Sea of Japan.

In summer of 2001, I had the opportunity to investigate the semiterrestrial tardigrades of Sakhalin Island, Far East Russia as a member of the International Sakhalin Island Project (ISIP), which is a collaboration among American, Japanese, and Russian scientists. Although Sakhalin is the largest island in Far East Russia, more than 900 km long

from north to south, none of its tardigrade fauna has previously been reported. As a result of investigations with ISIP, an undescribed *Hypsibius* species was found from lichen samples collected in four localities and is described here as new. Other tardigrade species collected with ISIP will be published elsewhere.

## MATERIALS AND METHODS

Collections were made both on southern Sakhalin Island, mainly around Yuzhno-Sakhalinsk, and in the northern part of the island, mainly on the Schmidt Peninsula (Fig. 1). Lichens and mosses growing on rocks, tree trunks, or surface soil were scraped off the substratum and stored in paper envelopes to avoid mold. The lichen and moss samples were soaked and stirred gently in tap water in petri dishes and left overnight. Living tardigrades released onto the bottom of the petri dish were picked up under a dissecting microscope at 30 × magnification, killed by heating, and prepared for slides by the method described in Abe and Takeda (2000). Hoyer's medium was used as a mountant, and cover slips were ringed with Murrayite after the medium dried completely.

The specimens were examined under a phase contrast and Nomarski differential interference microscope at 1250 × magnification, measured using a calibrated ocular micrometer, illustrated with the aid of a camera lucida, and photographed with a digital camera attached to the microscope. The *pt* value, which is the percentage ratio between the measurement of any given structure and the length of the buccal tube (Pilato, 1981), was calculated; buccal tube length was measured from the posterior end of the buccal cavity to the distal base of the apophysis in the pharyngeal bulb. The body length was measured from the rostral tip of the body to the caudal-most point of the body, excluding leg 4.

The type specimens of the new species are deposited in the collection of the Department of Zoology, National Science Museum, Tokyo (NSMT).

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## TAXONOMIC ACCOUNT

### *Hypsibius stiliferus* sp. nov.

(Figs. 2, 3; Table 1)

#### Holotype

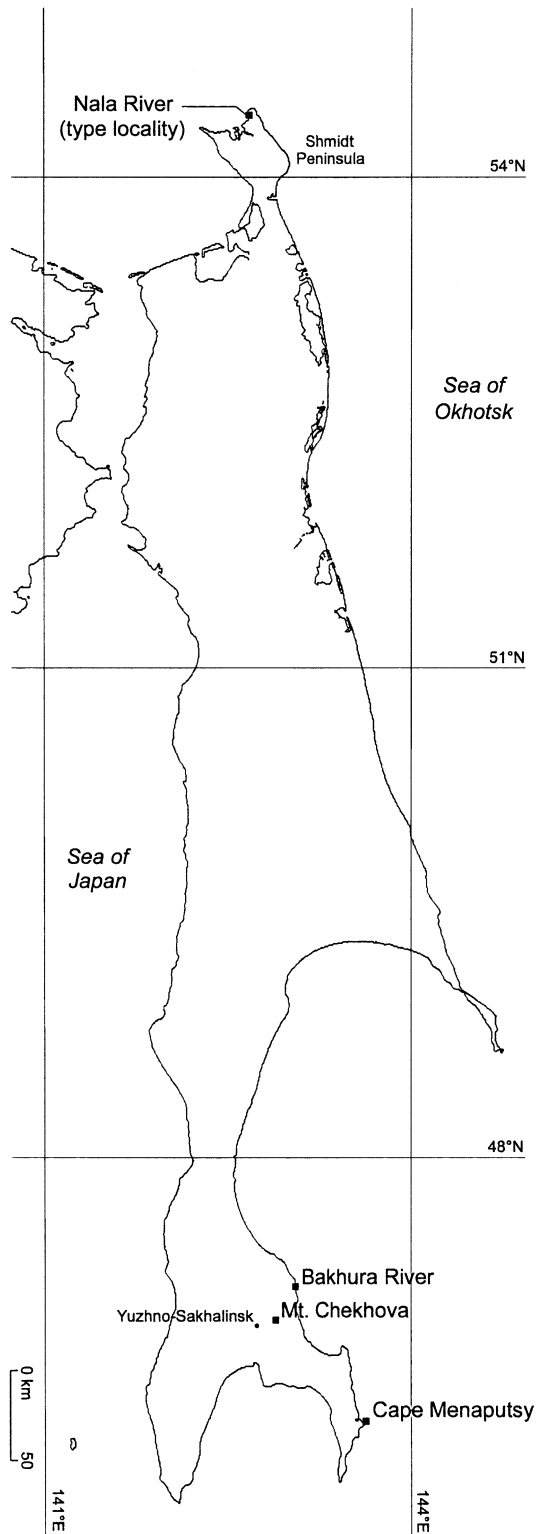
Animal of undetermined sex (NSMT-Tg 114); near mouth of the Nala River facing Severnyy Bay in the Sea of Okhotsk (ca. 6 km south-southwest of Cape Yelizavety), Shmidt Peninsula, northern Sakhalin Island, 54°22.5'N, 142°40.9'E, 50 m above sea level; extracted from a foliose lichen growing on rock (collection no. 20010807-01); collected 7 August 2001 by W. Abe.

#### Paratypes

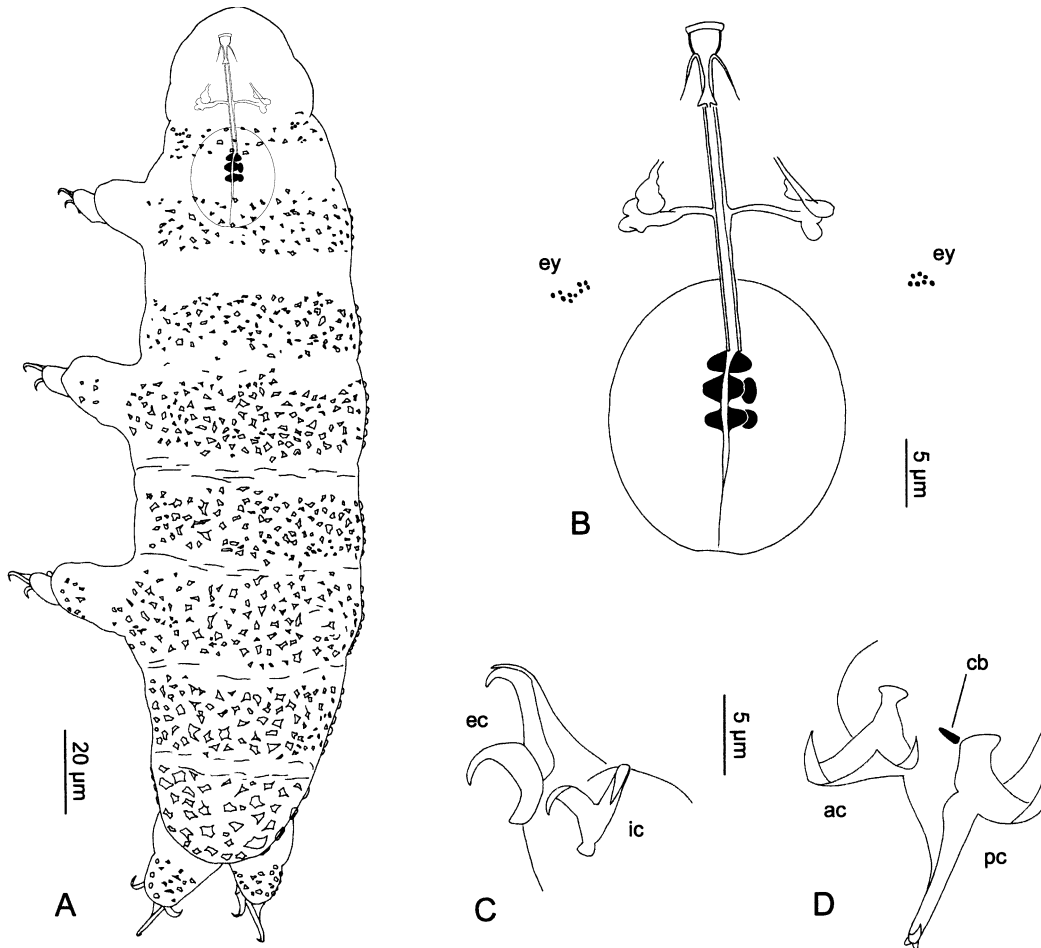
Twenty-six animals of undetermined sex (NSMT-Tg 115–140), 15 animals in molting simplex stage (NSMT-Tg 141–155), 1 exuvia with 2 eggs (NSMT-Tg 156); other collection information same as holotype. Twenty-six animals of undetermined sex (NSMT-Tg 157–182), 6 animals in molting simplex stage (NSMT-Tg 183–188), 1 exuvia (NSMT-Tg 189), 1 exuvia with 2 eggs (NSMT-Tg 190); ca. 2 km south of the peak of Mt. Chekhova (ca. 9 km east-northeast of Yuzhno-Sakhalinsk), southern Sakhalin Island, 46°59.4'N, 142°50.3'E, 740 m above sea level; extracted from a foliose lichen growing on rock (collection no. 20010722-04); collected 22 July 2001 by W. Abe. One animal of undetermined sex (NSMT-Tg 191); extracted from a foliose lichen growing on rock (collection no. 20010722-03); other collection information same as immediately preceding. Two animals of undetermined sex (NSMT-Tg 192, 193); near mouth of the Bakhura River facing the Sea of Okhotsk (ca. 20 km east of Sokol), southern Sakhalin Island, 47°13.2'N, 143°00.9'E, 5 m above sea level; extracted from a foliose lichen growing on trunk of *Alnus* sp. (collection no. 20010719-05); collected 19 July 2001 by W. Abe. Three animals of undetermined sex (NSMT-Tg 194–196), 5 animals of molting simplex stage (NSMT-Tg 197–201); near Cape Menaputsky facing the Sea of Okhotsk (ca. 94 km southeast of Yuzhno-Sakhalinsk), southern Sakhalin Island, 46°23.0'N, 143°36.8'E, 2 m above sea level; extracted from a lichen growing on rock (collection no. 20010821-02); collected 21 August 2001 by W. Abe.

#### Diagnosis

Eyespots present or absent, irregular if present; dorsum, at least caudally, covered with irregularly shaped, mostly polygonal or subtriangular sculpture, which increases in size posteriorly; dorsal sides of legs may also be tuberculate; buccal cavity simple, without tooth or ridge; buccal tube narrow; macroplacoids 1 and 2 granular; macroplacoid 1 with very shallow constriction dorsomedially; macroplacoid 2 without constriction; macroplacoid 1 longer than macroplacoid 2; microplacoid and septulum absent; primary branches of external claws 1–3 and posterior claw 4 distinctly longer and more slender than primary branches of internal claws 1–3 and anterior claw 4; cuticular bar developed near base of posterior claw 4; cuticular bar absent in the other legs.



**Fig. 1.** Localities (squares) where *Hypsibius stiliferus* sp. nov. was collected on Sakhalin Island, Far East Russia.



**Fig. 2.** *Hypsibius stilliferus* sp. nov. A, habitus, dorsal view; B, buccopharyngeal apparatus and eyespots, ventral view; C, leg 2, ventral view; D, leg 4, ventral view. A–E, holotype (NSMT-Tg 114). (ac, anterior claw; cb, cuticular bar; ec, external claw; ey, eyespots; ic, internal claw; pc, posterior claw).

### Description of holotype

Body fusiform (Figs. 2A, 3A; see Table 1 for measurements); color whitish translucent in life, almost transparent in preservative. Eyespots irregular, composed of several subcircular or oval granules, situated posterolaterally on head segment; adjacent granules not in contact, but well demarcated from one another (Fig. 2B).

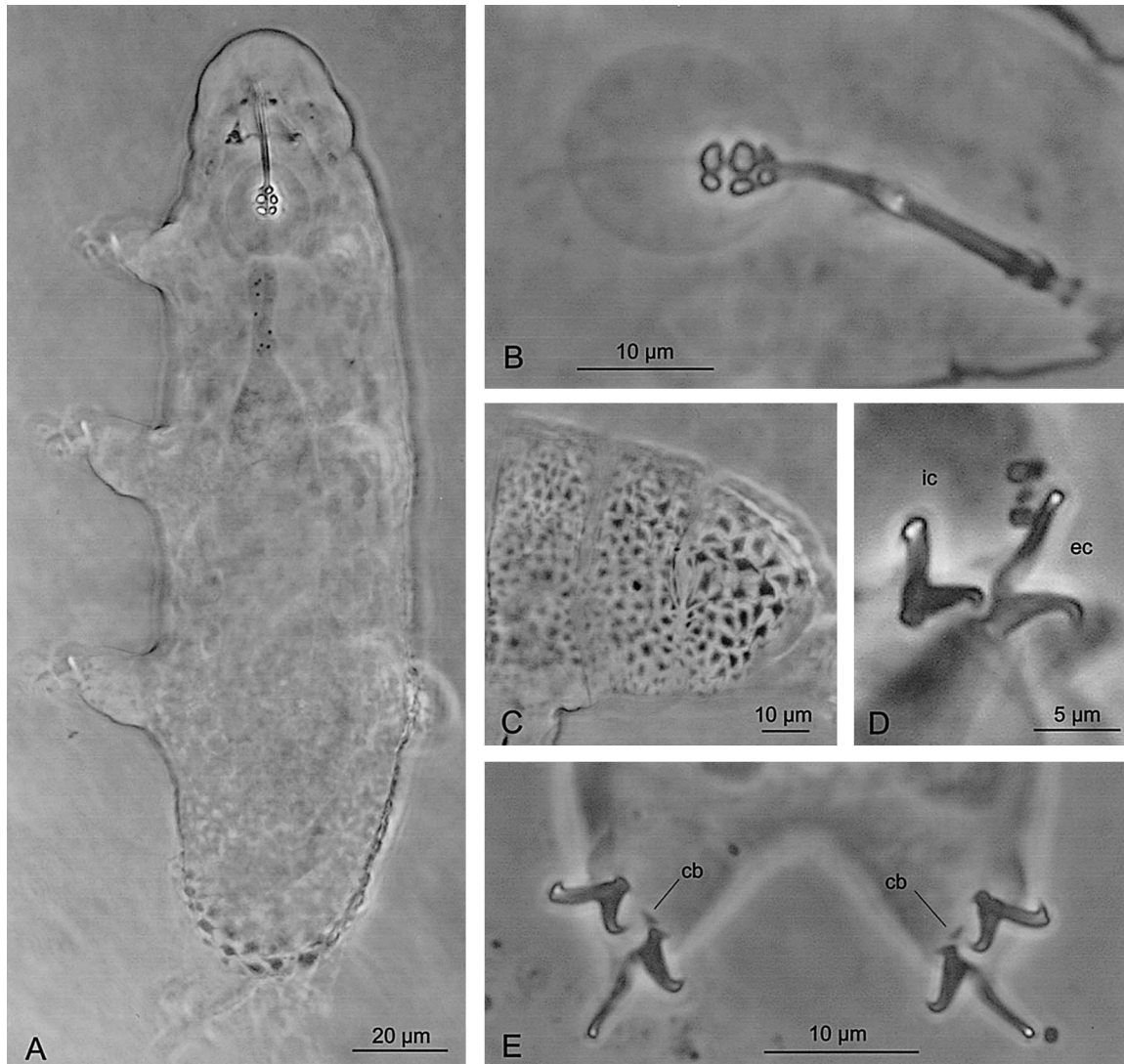
Dorsum and dorsal sides of legs 2–4 sculptured with irregularly shaped and distributed cuticular tubercles (Figs. 2A, 3A, C); tubercles mostly polygonal or subtriangular, increasing in size posteriorly, 0.8–4.6  $\mu\text{m}$  long; adjacent tubercles not in contact, but distinctly spaced; body tubercles developed as 8 transverse bands, which correspond with body segments; 2nd band developed on segment bearing leg 1, 4th on segment bearing leg 2, 6th on segment bearing leg 3, and 8th, or posteriormost, on segment bearing leg 4; cuticle between bands smooth, or with shallow, short, transverse cuticular furrows (Fig. 2A). Head, dorsal side of leg 1, venter, ventral sides of legs 1–4 smooth, without sculpture. Cuticle without pores.

Buccopharyngeal apparatus small, its total length 38.9

$\mu\text{m}$  from mouth opening to posterior end of pharyngeal bulb, 19.2% of body length (Figs. 2B, 3A, B). Mouth clearly situated subterminally, 2.7  $\mu\text{m}$  in diameter. Buccal cavity small and simple, without tooth and ridge, subcylindrical with tapered posterior end; buccal tube rigid, rather narrow, clearly curved ventrally near posterior end; stylet support inserted near midpoint of buccal tube. Furca typical for the genus in size and shape, with two processes posteriorly (Fig. 2B).

Pharynx subglobular, 19.5  $\mu\text{m}$  long, 18.2  $\mu\text{m}$  in diameter (Figs. 2B, 3A, B). Apophysis well developed, granular, similar in size and shape to macroplacoids. Macroplacoid 1 granular, with very shallow constriction dorsomedially, which is discernible only from lateral view; macroplacoid 2 granular, shorter than macroplacoid 1, without constriction; macroplacoids 1 and 2 well-demarcated from one another (Figs. 2B, 3A, B). Microplacoid and septulum absent.

Legs typical for the genus in size and shape (Figs. 2A, 3A). Claw 2-1-2-1 in sequence (Figs. 2C, D, 3D, E). Claw 4 larger than claws 1–3. External claws 1–3 and posterior claw 4 larger and longer than internal claws 1–3 and anterior



**Fig. 3.** *Hypsibius stilliferus* sp. nov. A, habitus, ventral view; B, buccopharyngeal apparatus, lateral view; C, dorsal sculpture, between legs 3 and 4; D, claw 2, ventral view; E, leg 4, ventral view. A, holotype (NSMT-Tg 114); B, paratype (NSMT-Tg 159); C, paratype (NSMT-Tg 115); D, E, paratype (NSMT-Tg 192). (cb, cuticular bar; ec, external claw; ic, internal claw). Phase contrast photomicrographs.

claw 4. Primary branches of external claws 1–3 and posterior claw 4 distinctly longer and more slender than primary branches of internal claws 1–3 and anterior claw 4; primary branches of external claws 1–3 and posterior claw 4 clearly swollen basally, substraight medially, curved distally, attached to secondary branch somewhat proximal to middle of latter. Secondary branches of all claws stout, clearly expanded basally, but without lunule; edge of claw base entire, without dentation or sinuation. Internal structure indiscernible in all claws. Accessory point evident, with acute tip, developed near tip of each primary branch (Fig. 2C, D). Cuticular bar positioned very near the base of, but not fused with, posterior claw 4, short, bluntly long-triangular, slightly tapered toward end opposite posterior claw base (Figs. 2D, 3E); its tip directed toward base of anterior claw 4; edge of cuticular bar entire, without dentation or sinuation; cuticular bar absent in the other claws.

#### Notes on paratypes

Of 83 specimens examined, 50 specimens provided with eyespots and 33 specimens devoid of eyespots. Dorsal sculpture, which is composed of cuticular tubercles, highly variable in extent; tubercles widely covering dorsum except for anteriormost part (similar to holotype) in 7 specimens (of 83 specimens), but distributed only posterior to segment bearing leg 3 in 16 individuals. Tubercles on legs present or absent; in 9 individuals (of 74 specimens), tubercles completely absent from all legs but, in 65 individuals, may occur on dorsal sides of legs 2–4. Macroplacoid 1 always with small and shallow constriction dorsomedially. Cuticular bar always present near the base of posterior claw 4. Egg laid in exuviae, oval or subglobular, 43.4–48.6 µm in diameter, its surface smooth, without ornamentation. See Table 1 for measurements.

**Table 1.** Morphometric data of *Hypsibius stiliferus* sp. nov.

	Holotype		Paratypes			
	Measurement	Pt value	Range	Pt value	S.E. of pt value	N
Body length	202.9		106.9–267.2			10
Buccal tube length	22.4		14.1–24.1			10
Buccal tube inside diameter	0.7	3.1	0.5–0.8	2.7–3.5	0.1	10
Stylet support insertion level	12	53.6	7.3–14.3	51.8–61.1	0.8	10
Macroplacoid 1 length	2	8.9	1.6–2.6	8.9–12.8	0.4	10
Macroplacoid 2 length	1.4	6.3	1.3–2.0	5.8–9.2	0.3	10
Placoid row length	4.6	20.5	3.3–5.2	18.3–23.4	0.8	10
Internal claw 1 height	5.9	26.3	4.6–5.9	22.5–26.3	0.4	9
External claw 1 height	8.1	36.2	5.2–9.8	29.5–40.7	1.5	8
Anterior claw 4 height	6.5	29	4.7–7.7	26.7–36.9	1.2	9
Posterior claw 4 height	13.7	61.2	7.2–14.3	44.3–65.6	2.1	10

Measurements are given in micrometers. Pt value is the percent measurement of a structure relative to the buccal tube length. N, number of specimens measured; S.E., standard error.

### Etymology

The specific name of the new species, *stiliferus*, is a masculine adjective in the nominative singular; *stilus* means bar or stick and *fer* means bearing, referring to the cuticular bar on leg 4.

### Range

The new species is known so far from semiterrestrial habitats (lichens growing on rocks or tree trunks) in the type locality (near Nala River) in northern Sakhalin Island and in three localities (near Bakhura River, Mt. Chekhova, and Cape Menaputsky) in southern Sakhalin Island (Fig. 1). This species seems to be widely distributed on Sakhalin Island.

### Differential diagnoses

The dorsal sculpture is one of the most readily observable characters in *Hypsibius stiliferus* sp. nov. It is composed of cuticular tubercles that increase in size posteriorly. Similar dorsal sculpture is also present in the following *Hypsibius* species: *H. camelopardalis* Ramazzotti and Maucci, 1983 from Portugal and Spain; *H. macrocalcaratus* Beasley, 1988 from the USA; *H. ragonesei* Binda and Pilato, 1986 from Italy; *H. roanensis* Nelson and McGlothlin, 1993 from the USA; and *H. scabropygus* Cuénot, 1929 from Europe, Africa, Japan, and USA. *Hypsibius stiliferus* sp. nov. is readily distinguished from *H. camelopardalis*, *H. macrocalcaratus*, *H. roanensis*, and *H. scabropygus* by having the cuticular bar near the base of posterior claw 4 and a dorsomedial constriction on macroplacoid 1. The new species is differentiated from *H. roanensis* by having the dorsal sculpture separated in several transverse bands (dorsal sculpture continuous in *H. roanensis*), and from *H. scabropygus* by having a more irregularly distributed dorsal sculpture (dorsal sculpture more regularly distributed in *H. scabropygus*). The new species differs from *H. ragonesei* by

having shorter and granular macroplacoids 1 and 2 (macroplacoids not granular, but elongated in *H. ragonesei*) and a very shallow dorsomedial constriction on macroplacoid 1 (dorsomedial constriction on macroplacoid 1 deeper and more conspicuous in *H. ragonesei*).

### DISCUSSION

Morphology of macroplacoids in the pharyngeal bulb is an important taxonomic character in the family Hypsibiidae (e.g., Ramazzotti and Maucci, 1983). The dorsal constriction on macroplacoid is, if present, rather deep and conspicuous in most species, but the constriction is fairly shallow and small in *Hypsibius stiliferus* sp. nov. Similar shallow constriction is rarely reported but known in some species in the family Hypsibiidae, e.g., *Ramazzottius szeptyckii* (Dastych, 1980). The type of sculpture on dorsum is considered to be a useful taxonomic character in the family Hypsibiidae. But, in the present new species, the sculpture on dorsum shows high variability. This variability, however, is continuous and related only to its extension, and the type of the sculpture is still the same in different individuals.

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