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A New *Calcarobiotus* (Tardigrada: Macrobiotidae) from the Imperial Palace of Japan

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ABSTRACT—A new semiterrestrial tardigrade species of the genus *Calcarobiotus* is described from the Imperial Palace of Japan as the third species of the genus which has been previously known only from South Africa. The new species, *Calcarobiotus imperialis*, is closely related to *C. occultus* Dastych, but can be distinguished from the latter mainly by the absence of a spine on the tip of each egg process, and by having larger pores on egg surface, a narrower buccal tube, and larger claws.

INTRODUCTION

Since 1996, the National Science Museum, Tokyo, has been conducting biological investigations of the Imperial Palace of Japan, which is centrally located in Metropolitan Tokyo. It occupies a vast area with various environments, not only well-kept artificial and natural forests but also ponds, streams, and orchards. This project has been planned under the auspices of His Majesty the Emperor of Japan to obtain up-to-date knowledge of the fauna and flora, and also to monitor their changes in the Imperial Palace, which is surrounded by paved roads and concrete buildings. The first results will be published in this year, 2000.

During the investigation, we collected many samples of mosses, lichens, algae, and leaf litter from various habitats to obtain the tardigrades. Among the tardigrades extracted from mosses were some specimens in the genus *Calcarobiotus* (family Macrobiotidae), which was recently erected by Dastych (1993) based on two new species from South Africa. After close comparison with the type material of the two known species, the specimens from the Imperial Palace of Japan were determined to be a new species and are described here.

MATERIALS AND METHODS

Moss samples collected from artificially-mounded soil were soaked in water in a Petri dish and left overnight. Living tardigrades were picked up under a dissecting microscope at the highest magnification (X50) and then placed in a small amount of water on a glass slide and gradually heated (about 5 sec.) with a lighter from underside. Although this heat-killing technique has been commonly used in the fixation of free-living or plant-parasitizing nematodes to avoid the subsequent shrinkage of specimens, it is also effective with semiterrestrial tardigrades. Each specimen was mounted on a glass slide in a sufficient amount of Hoyer's medium; polyvinyl-lactophenol was used as a supplementary mounting medium; cover slips were sealed with Murrayite after the mounting media dried.

The specimens were closely examined using a phase contrast and Nomarski differential interference microscope (Zeiss Axiophot) and illustrated with the aid of camera lucida; specimens were measured using an eyepiece micrometer. The *pt* index, which is the percentage ratio between the length of any given structure and the length of the buccal tube (Pilato, 1981), was also calculated.

In order to get a clear image of the egg shell, a computer-enhancing technique was utilized. A series of egg shell images at different focal points was first captured on a UNIX Workstation with a CCD camera connected to a Zeiss Axiophot microscope. These images were then prepared on a Macintosh computer using the software Adobe Photoshop, and printed out using an Epson inkjet printer.

As comparative materials, the holotypes and paratypes of *Calcarobiotus filmeri* Dastych, 1993 and *C. occultus* Dastych, 1993 deposited in the Natal Museum, Pietermaritzburg, South Africa were examined.

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

DESCRIPTION

Genus *Calcarobiotus* Dastych, 1993
Calcarobiotus imperialis, **new species**
[Japanese name: Mikado-chômeimushi, new]
(Figs. 1, 2, Table 1)

Type series. HOLOTYPE: adult animal (NSMT Tg-45), sex undet.; side of Hasuikebori Pond near the Imperial Household, the Imperial Palace, Tokyo, central Japan (35°40'52"N, 139°45'30"E); ca. 10 m altitude; extracted from a moss, *Plagiomnium acutum* (Lindb.) Kop., on artificially-mounded soil; W. Abe; 14 Dec. 1998. PARATYPES: one adult animal (NSMT Tg-46), sex undet., same data as holotype; two adult animals (NSMT Tg-47, 48), one embryonate egg (NSMT Tg-49), and one egg (NSMT Tg-50), same locality as above, 12 Mar. 1999.

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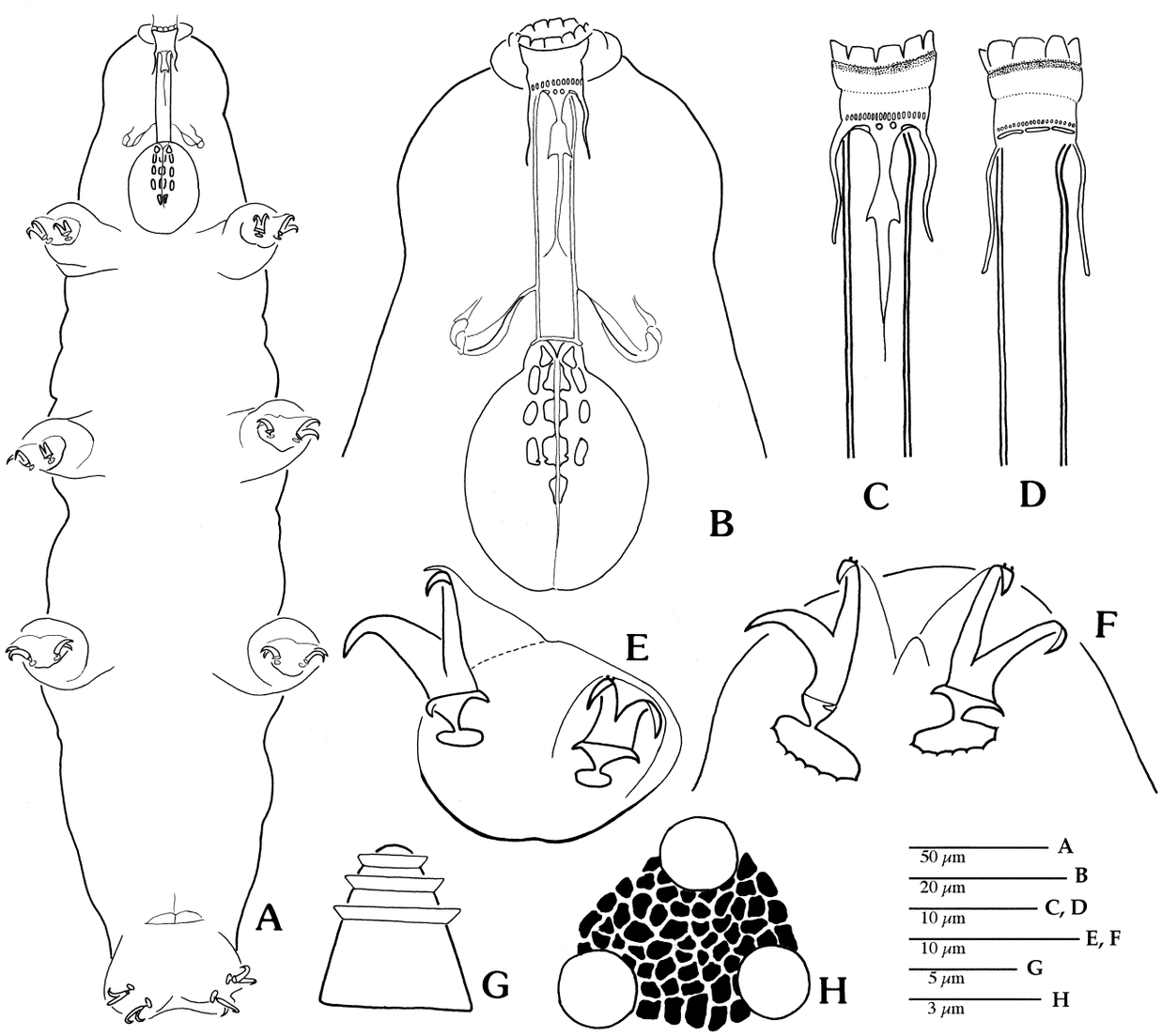

Fig. 1. *Calcarobiotus imperialis*, new species. A–D, F, Holotype (NSMT Tg-45); E, Paratype (NSMT Tg-46); G, H, Paratype (NSMT Tg-50). (A) Habitus, ventral view. (B) Buccopharyngeal apparatus, ventral view (pharynx artificially shifted posteriorly). (C) Buccal armature, ventral view. (D) Buccal armature, dorsal view. (E) Claws of leg II. (F) Claws of leg IV. (G) Egg process, lateral view. (H) Egg surface, dorsal view.

NSMT Tg-48 is mounted in polyvinyl-lactophenol, others in Hoyer's medium.

Holotype. Body elongate, length 353 μm excluding leg IV, width 81 μm; body width:body length, 1:4.3. Body whitish translucent in life, without pigmentation. Dorsal cuticle smooth, without pores. Eyespots absent.

Buccopharyngeal apparatus moderate in size. Mouth opening 9.8 μm in diameter, situated terminally, surrounded by 10 peribuccal lamellae. Circum-oral sensory field well-developed. Anterior band of buccal armature consists of minuscule and densely-distributed granules; posterior band consists of closely spaced teeth; each tooth rounded on the dorsal side and elongated on the ventral; dorsal crest well-marked, consists of three transverse bars; ventral crest consists of transverse structures, two lateral bars and two median rounded granules. Buccal tube rigid, 32.4 μm long, 5.1 μm in external diameter. Distance to stylet support insertion from posterior end of buccal cavity 26.0 μm, 80.3% of the length of the buccal tube; furca developed normally, similar to that of *Macrobiotus* species in size and shape.

Pharynx elongate spherical, 34.3 μm long, 22.6 μm wide; pharynx length:width, 1:0.66. Apophysis in pharynx well-developed; macroplacoid I as long as macroplacoid III; macroplacoid II shortest; macroplacoid III with conspicuous incision posteriorly; macroplacoids I–III 3.9, 3.1, and 3.9 μm long, respectively; microplacoid well-developed, 3.8 μm long.

Legs normally developed; cuticle of leg IV covered with densely-distributed granules that are fine and barely discernible; other legs without perceptible granules. Cuticular ring (="cuticular bar" in Dastych, 1993) in legs I–III well-developed;

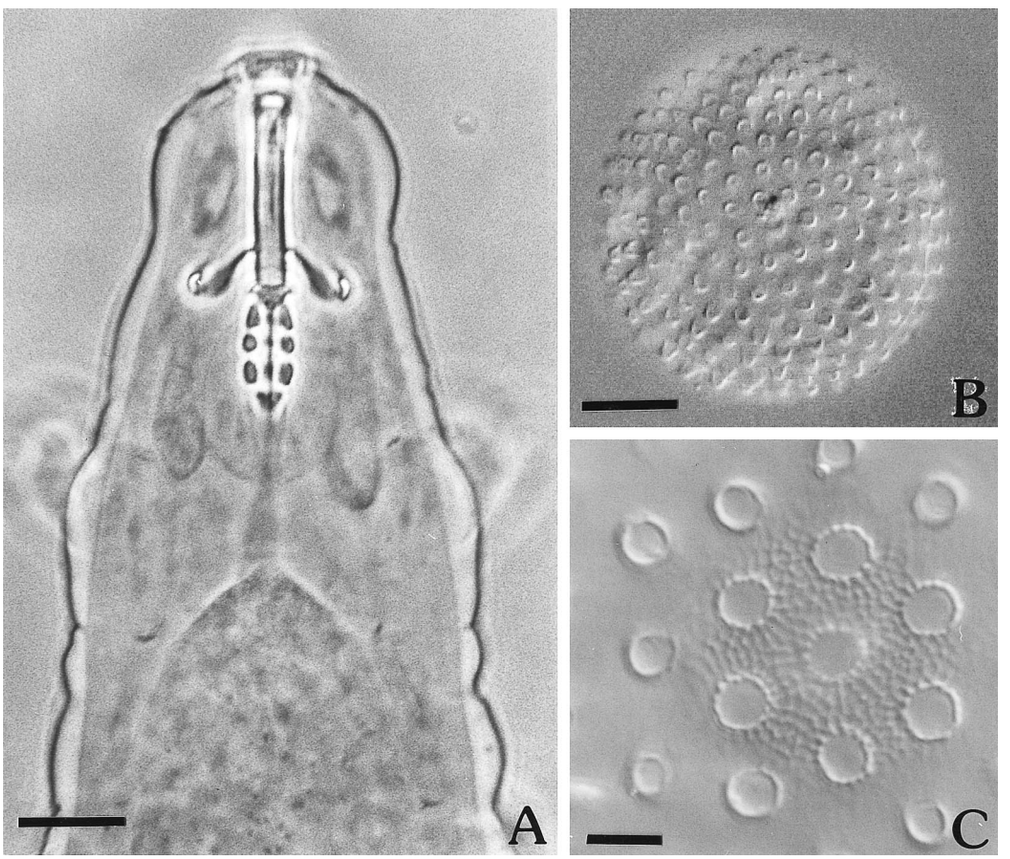

Fig. 2. *Calcarobiotus imperialis*, new species. A, Holotype (NSMT Tg-45); B, C, Paratype (NSMT Tg-50). (A) Anterior body. (B) Egg sculpture (computer-enhanced). (C) Detail of egg shell surface. Scales: A, B = 20 μm; C = 5 μm.

cuticular ring not restricted to ventral side of leg, but continued to its dorsal side, being more clearly observed on ventral side compared with dorsal side; on dorsal side of ring, thin cuticular sheet, attached to primary branch of each claw, forms distinct accessory point; leg IV without cuticular ring, but with cuticular sheet and accessory point.

Each claw with distinct, subtriangular base that is armed with a pair of laterally-protruded spurs. Internal and external claws similar to each other in shape, but external claw clearly larger than internal claw on legs I–III; claw IV larger than claws I–III; internal claws I–III, 8.2–9.0 μm long (including accessory point and lunula), external claws I–III, 10.5–11.7 μm long, anterior and posterior claws IV, 11.7 and 12.1 μm long, respectively. Each claw provided with well-developed lunula; width of lunula 2.7–3.1 μm on claws I–III, 5.9 μm on claw IV; edge of lunula smooth on claws I–III, dentate on claw IV.

Cloaca situated near base of leg IV, similar to that of *Macrobiotus* species in shape and size.

Paratype animals. Body 258–314 μm long; eyespots always absent. Embryo with posterior incision at macroplacoid III.

Paratype eggs. Whitish, spherical, 69 and 71 μm in diameter excluding processes; surface covered with small processes which are bell-shaped, uniform in size and shape, ca. 3.2 μm high, ca. 3.5 μm in diameter at base, and ringed concentrically with three well-marked cuticular ridges; tip of each process smooth, without any structure; ca. 36 processes on circumference of egg and ca. 170 processes per hemisphere. Surface covered with a net of irregularly shaped pores throughout; pores 0.3–0.8 μm in diameter.

Remarks. In the class Eutardigrada, especially in the family Macrobiotidae, the morphology of the egg shell is considered to be an important taxonomic character (Bertolani & Rebecchi, 1993). *Calcarobiotus imperialis* is readily distinguished from *C. filmeri* by the shape of egg processes, which are conical with strongly elongated and sometimes bi-trifurcate tips in the latter species. *Calcarobiotus imperialis* is very similar morphologically to *C. occultus*, both in the adult animal and egg, but can be distinguished from the latter by the following 1) the spiniform structure at the tip of each process on the egg shell is always absent, 2) the cuticular ridges on each process of the egg shell are well-marked, 3) the pores in the network on the egg shell surface are larger in size and irregular in shape, 4) the buccal tube is narrower (*pt* = 14.1–15.9), and 5) the claws are larger relative to the length of the buccal tube.

Table 1. Measurements (μm) and *pt* indices of the types of three *Calcarobiotus* species

	Calcarobiotus imperialis, new species					*C. occultus*					*C. filmeri*	
	Holotype	Paratype 1	Paratype 2	Paratype 3	Paratype 4	Holotype	Paratype 1	Paratype 2	Paratype 3	Paratype 4	Holotype	Paratype
Body length	353.0	314.0	258.0		embryo	207.5	315.0	180.0	190.0	embryo	457.5	embryo
Buccal tube length	32.4	28.9	33.2	34.3	27.2	40.0	39.0	33.6	35.2	26.8	46.8	34.0
Buccal tube diameter	5.1	4.3	4.7	5.5	3.9	8.5	8.0	6.8	7.6	4.8	8.4	5.6
pt	15.7	14.9	14.1	15.9	14.3	21.3	20.5	20.2	21.6	17.9	17.9	16.5
Mouth opening width	9.8	8.6	9.4	9.4		16.0	15.0	12.8	14.4		14.4	
Stylet support insertion level	26.0	23.0	26.2	26.9		32.0	30.0	25.8	27.0	20.8	38.8	26.6
pt	80.3	79.7	79.0	78.4		80.0	76.9	76.8	76.7	77.6	82.9	78.2
Macroplacoid I	3.9	3.7	3.9	3.9	3.2	6.0	5.5	4.8	5.6	3.2	8.0	3.6
pt	12.0	13.0	11.8	11.4	11.8	15.0	14.1	14.3	15.9	11.9	17.1	10.6
Macroplacoid II	3.1	3.0	3.0	3.1	2.4	4.0	4.0	3.6	4.4	2.2	6.4	2.6
pt	9.6	10.3	8.9	9.1	8.8	10.0	10.3	10.7	12.5	8.2	13.7	7.6
Macroplacoid III	3.9	3.7	4.1	4.0	3.0	6.2	5.6	5.2	6.0	3.0	6.4	3.6
pt	12.0	13.0	12.2	11.5	11.0	15.5	14.4	15.5	17.0	11.2	13.7	10.6
Microplacoid	3.8	3.5	3.9	4.1	2.4	4.0	4.0	3.2		2.8	5.4	3.6
pt	11.7	12.2	11.8	11.8	8.8	10.0	10.3	9.5		10.4	11.5	10.6
Pharynx length	34.3	29.3	31.2	29.6		42.0		33.2	39.2		58.0	
Pharynx width	22.6	22.2	25.0	27.3		38.0	36.4	30.0	37.6		47.0	
Internal claw I	9.0	7.8	7.8	9.4		9.0	8.6	7.2			13.2	
pt	27.7	27.0	23.5	27.3		22.5	22.1	21.4			28.2	
External claw I	10.9	9.4	9.4	10.3		9.2	9.2	8.4			14.4	
pt	33.7	32.4	28.2	30.0		23.0	23.6	25.0			30.8	
Internal claw II	8.7	7.8	8.6	9.0		8.8	8.6	7.2	7.6		14.0	
pt	27.0	27.0	25.9	26.4		22.0	22.1	21.4	21.6		29.9	
External claw II	11.7	10.9	10.9	11.7		9.2	8.8	8.4	8.4		12.4	
pt	36.1	37.8	32.9	34.1		23.0	22.6	25.0	23.9		26.5	
Internal claw III	8.2	7.4	8.6	9.6				6.8	8.2		12.4	
pt	25.3	25.7	25.9	28.0				20.2	23.3		26.5	
External claw III	10.5	10.5	11.7	11.8		9.2		8.8	8.8		15.2	
pt	32.5	36.5	35.3	34.4		23.0		26.2	25.0		32.5	
Anterior claw IV	11.7	9.4	10.1	10.1		10.8	10.7	9.4			14.0	
pt	36.1	32.4	30.6	29.6		27.0	27.4	28.0			29.9	
Posterior claw IV	12.1	10.9	11.3			10.5	11.0	9.2	10.0		16.8	
pt	37.3	37.8	34.1			26.3	28.2	27.4	28.4		35.9	

DISCUSSION

Dastych (1993) considered the cuticular ring (= "cuticular bar" in Dastych, 1993) on legs I–III as one of the distinctive characters of the genus *Calcarobiotus*. We confirmed that the new species from Japan also has a well-marked cuticular ring compared with the other genera of the Macrobiotidae. Based on our observations, the cuticular ring is located at the cuticular fold on the distalmost segment of the leg.

The genus *Calcarobiotus* was established by Dastych (1993) to accommodate two new species from South Africa. Our report from Japan suggests that *Calcarobiotus* is not an endemic genus to South Africa, but a taxon with a broad distribution. We collected *C. imperialis* from a moss growing directly on soil. Dastych (1993) also collected the two species mainly from soil or moss growing on soil. Furthermore, *Calcarobiotus* sp. was collected recently from a soil sample in a field of the National Institute of Agro-Environmental Sciences, Tsukuba, central Japan (M. Ito, pers. comm.). These collections suggest that *Calcarobiotus* species prefer (although not exclusively) to live in soil or moss growing on soil. As mentioned by Bertolani and Biserov (1996), the soil environment has been considered as a habitat of tardigrades rather recently, and thus the geographical distribution of the species living in soil is poorly understood. It is highly probable that many additional localities will be found by further investigations of soil environments.

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