# Semiterrestrial Tardigrades from the Tokiwamatsu Imperial Villa, Tokyo, Japan

By

## Wataru Abe<sup>1)</sup> and Masatsune Takeda<sup>2)</sup>

阿部 渉1)・武田正倫2): 常盤松御用邸に生息する陸産緩歩動物(クマムシ類)

#### Introduction

The tardigrades, or water bears, are one of the lesser-known phyla of the protostome micrometazoans. They have a ubiquitous distribution, including marine, freshwater, and semiterrestrial environments. All tardigrade species require ambient water to be active, and semiterrestrial species live in moist habitats, such as soil and leaf litter or among mosses and lichens (Nelson, 2002).

In this paper, we report for the first time the semiterrestrial tardigrades from the Tokiwamatsu Imperial Villa, Shibuya Ward, central Tokyo  $(35^{\circ}39' 27'' N, 139^{\circ}42' 47'' E; ca. 30 m above the sea level). In the garden of the villa, which has an area of ca. 19 thousand square meters, not only various tree and shrub species are planted, but also a pond and Japanese rock garden are arranged harmoniously, forming a good green tract in the central part of metropolitan Tokyo.$ 

### Materials and Methods

Investigations were conducted in 2002 and 2003 under the auspices of His Imperial Highness Prince Masahito. In total, 58 cryptogam samples, including mosses and lichens, were examined for tardigrades. The cryptogam samples were removed from substrata, stored in paper envelopes, and brought back to the laboratory. Tardigrades were extracted from the cryptogam samples and processed for microslides following the method by Abe and Takeda (2000a). Tardigrade specimens were examined under differential interference contrast and phase contrast microscopes at the highest magnification  $1000 \times$ , photographed with a digital camera, and illustrated with a camera lucida. Terminology of the dorsal plates in Echiniscidae followed Abe *et al.* (1998). Specimens are deposited in the collection of the Department of Zoology, National Science Museum, Tokyo (NSMT).

<sup>1)</sup> Department of Biological Sciences, Graduate School of Science, Hokkaido University (Present address: Faculty of Horticulture, Chiba University) 北海道大学大学院理学研究科生物科学専攻(現所属:千葉大学園芸学部)

<sup>&</sup>lt;sup>2)</sup> Department of Zoology, National Science Museum, 3–23–1 Hyakunincho, Shinjuku-ku, Tokyo, 169–0073 Japan E-mail: takeda@kahaku.go.jp

国立科学博物館動物研究部 東京都新宿区百人町 3-23-1

#### Results

Following 5 species belonging 4 genera representing 3 families were identified.

Class Heterotardigrada Order Echiniscoidea Family Echiniscidae

## 1. Echiniscus cf. perviridis Ramazzotti, 1959 (Fig. 1)

Six females (NSMT-Tg 202–207), 1 immature (NSMT-Tg 208), 1 animal of undetermined sex (NSMT-Tg 209); extracted from moss growing on stone wall (collection no. 20021213-H10); collected 13 December 2002 by W. Abe.

Measurements. Body length 193-215 µm.

*Remarks.* This is a member of the *Echiniscus viridis*-group characterized by having a greenish dorsal cuticle and no body cirri other than that at position A (Ramazzotti & Maucci, 1983). The specimens from the Tokiwamatsu Imperial Villa agree well with the diagnosis of *E. cf. perviridis* from the Imperial Palace, Tokyo (Abe & Takeda, 2000b). As wrote in Abe and Takeda (2000b), we already confirmed that *E. cf. perviridis* sensu Abe & Takeda and *E. japonicus* sensu Utsugi (1994, 1996) reported from various localities in Japan are conspecific. Taxonomic identity of the Japanese species, however, is still uncertain, and, as pointed by Abe and Takeda (2000b), direct comparison of type specimens of the above two taxa is necessary to dispel the taxonomic confusion. Although reports of the *E. viridis*-group from the world are not many, this species is one of the commonest tardigrades inhabiting xerophilous mosses (especially the cosmopolitan moss *Bryum argenteum* Hedw.) in urban areas of central and southern Japan (Utsugi, 1994, 1996).

Class Eutardigrada Order Parachela Family Hypsibiidae

## 2. Hypsibius dujardini (Doyère, 1840) (Fig. 2)

Two animals of undetermined sex (NSMT-Tg 210, 211); extracted from moss growing on stone wall (collection no. 20021213-H10); collected 13 December 2002 by W. Abe.

Measurements. Body length 255 and  $263 \,\mu m$ .

*Remarks*. These specimens agree well with the diagnosis of *Hypsibius dujardini* by Abe and Takeda (2000b), which is based on the specimens from the Imperial Palace, Tokyo. This species is a cosmopolitan (McInnes, 1994) and, in Japan, reported from the Imperial Palace, Tokyo and several other localities (Abe & Takeda, 2000b).

#### Family Macrobiotidae

## 3. Macrobiotus mauccii Pilato, 1974

(Fig. 3)

One ovigerous female (NSMT-Tg 212), 1 animal of undetermined sex (NSMT-Tg 213), 1 animal of



Fig. 1. Echiniscus cf. perviridis from the Tokiwamatsu Imperial Villa, Tokyo. A, habitus, dorsal view; B, dorsal sculpture; C, leg 3, ventral view; D, E, dentate collar on leg 4, dorsal view. A–C, E, NSMT-Tg 203; D, NSMT-Tg 202. Arrowhead, spur; mp1, median plate 1; mp2, median plate 2; pp2, segmental paired plate 2; pp3, segmental paired plate 3; sp, scapular plate; tp, terminal plate. Differential interference photomicrographs.

undetermined sex in molting simplex stage (NSMT-Tg 214); extracted from moss growing on concrete (collection no. 20021213-H02); collected 13 December 2002 by W. Abe. One animal of undetermined sex in molting simplex stage (NSMT-Tg 215); extracted from moss growing on trunk of *Prunus armeniaca* L. var. *ansu* Maxim. (collection no. 20021213-H04); collected 13 December 2002 by W. Abe. Nine animals of undetermined sex (NSMT-Tg 216–224), 1 ovigerous female in molting simplex stage (NSMT-Tg 225), 1



Fig. 2. *Hypsibius dujardini* from the Tokiwamatsu Imperial Villa, Tokyo. A, habitus, ventral view; B, buccopharyngeal apparatus, ventral view; C, leg 4. A–C, NSMT-Tg 210. Differential interference photomicrographs.

animal of undetermined sex in molting simplex stage (NSMT-Tg 226), 1 embryonated egg (NSMT-Tg 227), 8 eggs (NSMT-Tg 228–235); extracted from moss growing on stone wall (collection no. 20021213-H 10); collected 13 December 2002 by W. Abe.

*Diagnosis*. Body 340–580  $\mu$ m long. Dorsum smooth, without cuticular pores. Eyespots present. Buccal tube fairly wide, its internal diameter ca. 18% of its length; all three macroplacoids rod-shaped; macroplacoids 1 and 3 subequal in length; macroplacoid 2 the shortest; microplacoid well-developed, subconical, located near to macroplacoid 3. Claw normal in size for the genus; lunule subcircular, its margin entire, without dentation. Egg 58–75  $\mu$ m in diameter, excluding process. Process large, bell-shaped, with taper and elongate tip, reticulated with fine and circular mesh.

*Remarks*. This species is described originally from Guangzhou (Canton), southeastern China (Pilato, 1974) and reported subsequently from the Andaman Islands, India (Maucci & Durante Pasa, 1980), Hokkaido, northern Japan (Utsugi, 1988), and the Imperial Palace, Tokyo, central Japan (Abe & Takeda, 2000b). Specimens from the Tokiwamatsu Imperial Villa agree well with the original description of this species both in animals and eggs, except for having eyespots, which are absent from the type specimens. This species belongs to the *Macrobiotus harmsworthi*-group characterized mainly by having three rod-



Fig. 3. Macrobiotus mauccii from the Tokiwamatsu Imperial Villa, Tokyo. A, habitus, ventral view; B, buccopharyngeal apparatus, ventral view; C, ventral side of buccal armature; D, claws on leg 1; E, egg; F, egg process, lateral view. A–D, NSMT-Tg 217; E, NSMT-Tg 227; F, NSMT-Tg 229. Differential interference photomicrographs.

shaped macroplacoids, a large microplacoid placed near to the macroplacoid 3, and a dorsal cuticle devoid of cuticular pores (Ramazzotti & Maucci, 1983). Hitherto, following two species of the *M. harmsworthi*-group are reported from Japan: *M. harmsworthi* Murray, 1907 (Mathews, 1937a, b; Watanabe *et al.*, 1961; Utsugi, 1994, 1996) and *M. orcadensis* Murray, 1907 (Ito, 1997). In the *M. harmsworthi*-group, morphology of animals is fairly similar among species, and identification to the species level is virtually reliable only if there is egg, which has species-specific ornamentation and processes on its shell. In this study, we were able to collect the eggs, and they allowed us to make an accurate identification of the speciements to the species level.

#### 4. Macrobiotus cf. hufelandi Schultze, 1833

One animal of undetermined sex (NSMT Tg-236); extracted from moss growing on bark of tree (collection no. 20020523-H05); collected 23 May 2002 by M. Takeda. One animal of undetermined sex (NSMT-Tg 237); extracted from moss growing on stone wall (collection no. 20021213-H10); collected 13 December 2002 by W. Abe.

*Diagnosis*. Dorsum smooth; eyespots absent. Buccal tube internal diameter ca. 13% of its length; apophysis in pharyngeal bulb typical for the genus in size and shape; macroplacoids 1 and 2 rod-shaped, the former approximately two times longer than the latter, both without conspicuous constriction; microplacoid fairly small, subconical, placed immediately posterior to macroplacoid 2. All claws provided with lunules, which are subcircular and with smooth edge.

*Remarks.* This species may belong to the *Macrobiotus hufelandi*-group characterized mainly by having two rod-shaped macroplacoids, a microplacoid, and the egg with reticulated shell and inverted goblet egg processes (Bertolani & Rebecchi, 1993). Identification of the specimen from the Tokiwamatsu Imperial Villa to species level was impossible because of the absence of the egg.

#### 5. Minibiotus cf. intermedius (Plate, 1889)

(Fig. 4)

One animal in molting simplex stage (NSMT-Tg 238); extracted from moss growing on stone lantern (collection no. 20021213-H01); collected 13 December 2002 by W. Abe. Two animals of undetermined sex (NSMT-Tg 239, 240); extracted from moss growing on stone lantern (collection no. 20030205-H01); collected 5 February 2002 by M. Owada.

*Diagnosis*. Body small, 100–185  $\mu$ m long, translucent. Cuticle smooth, without granulation or pores. Eyespots complex. Buccal tube narrow. Apophysis in pharyngeal bulb subtriangular and large, similar to macroplacoids in size; macroplacoids 1–3 round, subequal in length from one another; microplacoid minute, situated immediately posterior to macroplacoid 3. Edge of claw lunule smooth, without dentation.

*Remarks*. Using taxonomic key of Claxton (1998), the specimens from the Tokiwamatsu Imperial Villa can be identified as *Minibiotus intermedius* (Plate, 1889). We, however, postpone the final taxonomic decision of the specimens, because of the absence of the egg, which has a species-specific ornamentation on



Fig. 4. *Minibiotus* cf. *intermedius* from the Tokiwamatsu Imperial Villa. Buccopharyngeal apparatus, lateral view. NSMT-Tg 239. bt, buccal tube; ma, macroplacoids; mi, microplacoid; mo, mouth. Differential interference photomicrograph.

its shell, and thus is virtually inevitable material to make an accurate identification of Minibiotus species.

#### Acknowledgments

We are grateful to Prince Masahito and Dr. M. Owada (NSMT) for their help and collecting mosses, and Dr. K. Okabe (Forestry and Forest Products Research Institute, Tsukuba) for providing us with facilities in the laboratory. This study was partly supported by the Japan Society for the Promotion of Science Research Fellowship for Young Scientists to W.A. (no. 13010556).

#### 摘 要

2002 年および 2003 年に、東京都渋谷区内に位置する常盤松御用邸において陸産緩歩動物(クマムシ類)の調査を行った。樹幹や石の上などに着生する蘚類から分離抽出されたクマムシ類を微分干 渉顕微鏡および位相差顕微鏡を用いて観察し、分類学的な検討を加えた。同定された 2 綱 2 目 3 科 4 属 5 種のクマムシ類の分類学的所属は以下のとおりであり、このうち Echiniscus cf. perviridis, Hypsibius dujardini, Macrobiotus mauccii の 3 種は近隣の皇居からも報告されているものである。

```
Phylum Tardigrada 緩歩動物門
```

```
Class Heterotardigrada 異クマムシ綱
 Order Echiniscoidea トゲクマムシ目
   Family Echiniscidae ヨロイトゲクマムシ科
     Genus Echiniscus トゲクマムシ属
       1. Echiniscus cf. perviridis
Class Eutardigrada 真クマムシ綱
 Order Parachela ヨリヅメ目
   Family Hypsibiidae ヤマクマムシ科
     Genus Hypsibius ヤマクマムシ属
       2. Hypsibius dujardini ドゥジャルダンヤマクマムシ
   Family Macrobiotidae チョウメイムシ科
     Genus Macrobiotus チョウメイムシ属
       3. Macrobiotus mauccii マウッチチョウメイムシ
       4. Macrobiotus cf. hufelandi ナガチョウメイムシ類似種
     Genus Minibiotus コガタチョウメイムシ属
       5. Minibiotus cf. intermedius チョウメイムシ類似種
```

#### References

- Abe, W. & M. Takeda, 2000a. A new Calcarobiotus (Tardigrada: Macrobiotidae) from the Imperial Palace of Japan. Zool. Sci., 17: 259–263.
- Abe, W. & M. Takeda, 2000b. Tardigrades from the Imperial Palace, Tokyo. Mem. natn. Sci. Mus., Tokyo, (35): 165–177.
- Abe, W., K. Utsugi & M. Takeda, 1998. Pseudechiniscus asper, a new Tardigrada (Heterotardigrada: Echiniscidae) from Hokkaido, northern Japan. Proc. biol. Soc. Wash., 111: 843–848.
- Bertolani, R. & L. Rebecchi, 1993. A revision of the Macrobiotus hufelandi group (Tardigrada, Macrobiotidae), with some observations on the taxonomic characters of eutardigrades. Zoologica Scr., 22: 127–152.
- Claxton, S. K., 1998. A revision of the genus *Minibiotus* (Tardigrada: Macrobiotidae) with descriptions of eleven new species from Australia. *Rec. Aust. Mus.*, 50: 125–160.

Ito, M., 1997. Taxonomic study on the Eutardigrada from the northern slope of Mt. Fuji, central Japan. III. Families Macrobiotidae and Milnesiidae. Species Diversity, 2: 167–178.

Mathews, G. B., 1937a. The Tardigrada or water-bears. China J., 26: 97-105.

Mathews, G. B., 1937b. Tardigrada from Japan. Peking nat. Hist. Bull., 11: 411-412.

- Maucci, W. & M. V. Durante Pasa, 1980. Tardigradi muscicoli delle isole Andamane. Boll. Mus. civ. Stor. nat. Verona, 7: 281–291.
- McInnes, S. J., 1994. Zoogeographic distribution of terrestrial/freshwater tardigrades from current literature. J. nat. Hist., 28: 257–352.
- Nelson, D. R., 2002. Current status of the Tardigrada: Evolution and ecology. Integ. comp. Biol., 42: 652-659.

Pilato, G., 1974. Tre nuove specie di tardigradi muscicoli di Cina. Animalia, 1: 59-68.

- Ramazzotti, G. & W. Maucci, 1983. Il philum Tardigrada: III edizione riveduta e aggiornata. Memorie Ist. ital. Idrobiol., 41: 1–1012.
- Utsugi, K., 1988. Tardigrades in Hokkaido area. Zool. Sci., 5: 1335.
- Utsugi, K., 1994. Study on terrestrial tardigrades in Japan. I. Urban areas in Kanto region. *Nat. env. Sci. Res.*, 7: 29–34. (In Japanese, with English summary.)
- Utsugi, K., 1996. Study on terrestrial tardigrades in Japan. II. Summary of urban tardigrades in Japan. *Nat. env. Sci. Res.*, **9**: 33-46. (In Japanese, with English summary.)
- Watanabe, Y., K. Sasaki & K. Taira, 1961. Preliminary survey of tardigrades on dead bamboo-leaves from Chiba Prefecture. Sci. Rep. Yokosuka City Mus., (6): 93–96.