Preliminary data on tardigrades of Lake Biwa (Japan)

Предварительные данные о тихоходках (Tardigrada) озера Бива (Япония)

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КЛЮЧЕВЫЕ СЛОВА: тихоходки, фауна, систематика, Бива.

ABSTRACT: Fourteen species of freshwater tardigrades were found in Lake Biwa (Japan). One of them, *Pseudobiotus vladimiri* is new for science. It differs from the other species of the genus the sculptured cuticle and the emerging accessory points in the main branch of the claws. Apart *P. megalonyx*, it differs from the other *Pseudobiotus* also in the shape of the placoids.

РЕЗЮМЕ: Четырнадцать видов пресноводных тихоходок были найдены в озере Бива (Япония). Один из них, *Pseudobiotus vladimiri*, описан как новый для науки. Он отличается от других видов этого рода скульптурированной кутикулой и выступающими дополнительными шипиками на главных ветвях коготков. Кроме *P. megalonyx*, он отличается от всех других *Pseudobiotus* формой плакоидов.

Introduction

Little is known on freshwater tardigrades in the world, on which most studies were carried out in Italy [Ramazotti, 1945; Pilato, 1971, 1973, 1974; Binda, 1978; Bertolani, 1982; Pilato et al., 1989]. A few were carried out in Japan, including a very interesting paper on Thermozodium (the unique Mesotardigrada known up to date) by Rahm [1937], a paper by Sudzuki [1975] two papers by Okano [1991] slightly concerning tardigrades of Lake Biwa, and the last publication on Japanese freshwater material by Ito and Tagani [1993]. A list of papers concerning freshwater and terrestrial tardigrades of Japan was reported by McInnes [1994: 348]. In regard to tardigrades of Lake Biwa, in our opinion (as H. Noda's opinion, in lettr.) the unique species reported and illustrated by Okano does not belong to Macrobiotus (now Minibiotus) intermedius (Plate, 1889), as referred, because it does not have claws of the hufelandi type.

Due to the kindness of Dr. O. A. Timoshkin (Limnological Institute, RAS, Irkutsk), some freshwater samples from Lake Biwa were available for a study of tardigrades. This material is the basis for the present contribution.

Material and methods

The examined material consisted of specimens extracted from 11 sediment samples from interstitial, littoral and sublittoral zone of Lake Biwa and 3 mounted slides with waterbears. Samples containing tardigrades were collected from the following areas: North Biwa, off Kita-Komatzu, two interstitial, 4 littoral and one sublittoral samples; a small stream off Kita-Komatsu, near its mouth, one sample; Station 4, off Wani River mouth, one sample with one specimen; South Biwa, one sample. A sample from Shin-asahi-cho contained no tardigrades. Samples were collected in 1996–1997 except for the sample from station 4 that was collected in 1994.

The specimens were fixed in 2% acetic acid, 70% ethanol or 4% formaldehyde. Then they were stained with acetic carmine or KOH and then mounted in Faure's fluid and examined using phase contrast microscopy.

Results

Fourteen species of tardigrades (Table 1) were found one of them new to science. For many of them (*Diphascon sp.*, *Dactylobiotus sp.*, *Macrobiotus* group*harmsworthi*, *M.* group *hufelandi* and *M.* aff. *occidentalis*) a definitive diagnosis was impossible, due to the material conditions (*Diphascon*) and to the absence of eggs (*Dactylobiotus sp.* and *Macrobiotus*).

Pseudobiotus vladimiri **sp.n.** Fig. 1.

Material examined: three specimens on 3 slides furnished by Dr. O.A. Timoshkin and the fourth specimen in *simplex stadium* was extracted from the sample.

Type locality. Japan, northern part of Lake Biwa, Kita-Komatzu, stones at a depth of one meter (one specimen): sand at a depth of three meters (3 specimens), July-August 1996.

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Holotype. Adult specimen. Slide number 2484(2), collected by O.A. Timoshkin, August 1996, determined by V. I. Biserov. Deposited in the Lake Biwa Museum, Japan.
Paratypes. Slides number 2484(1), 2484(3). Deposited in the

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DESCRIPTION. Body long, slender, broadest in region of legs III. No eyes. Cuticle wrinkled and with sculpture,

Table 1. Complete list of tardigrades of Lake Biwa. Таблица 1. Полный список видов тардиград оз. Бива.

Species	Sampling locality	World distribution
	Class Eutardigrada	
Order Parachela		
Family Hypsibiidae		
Subfamily Hypsibiinae		
Genus <i>Hypsibius</i> Ehrenberg, 1848		
H. convergens Urbanowicz, 1925	off Kita-Komatzu, stones at a depth of 2 m.	Cosmopolitan (according to McInnes, 1994)
	a small stream off Kita-Komatsu, near by its mouth, sand and leaves	
H. dujardini (Doyere, 1840).	a small stream off Kita-Komatsu, near by its mouth, sand and leaves	Cosmopolitan (according to McInnes, 1994)
Genus Pseudobiotus Nelson, 1980		
P. vladimiri sp.n.	off Kita-Komatzu, sand at a depth of 3 m.	Lake Biwa
Genus Isohypsibius Thulin, 1928		
I. aff. granulifer	off Kita-Komatzu, stones and sand.	
	off Kita-Komatzu, sand and stones at a depth of 1–1.2 m.	
I. aff. saltursus	a small stream off Kita-Komatsu, near by its mouth, sand and leaves	
I. prosostomus Thulin, 1928	a small stream off Kita-Komatsu, near by its mouth, sand and leaves	Cosmopolitan (according to McInnes, 1994)
I. tetradactyloides (Richters, 1907)	off Kita-Komatzu, stones at a depth of 1 m.	Subcosmopolitan (according to McInnes, 1994)
	a small stream off Kita-Komatsu, near by its mouth, sand and leaves	
	Subfamily Itaquasconinae	
	Genus Diphascon Plate, 1889	
Diphascon sp.	a small stream off Kita-Komatsu, near by its mouth, sand and leaves	
Family Macrobiotidae		
Genus Dactylobiotus Schuster, 1980		
D. dispar (Murray, 1907)	South Biwa, sand at a depth of 1.5 m.	Subcosmopolitan (according to McInnes, 1994)
	off Kita-Komatzu, interstitial, sand.	
	off Kita-Komatzu, stones and sand at a depth of 1–1.2 m.	
Dactylobiotus sp.	off Kita-Komatzu, interstitial.	
	off Kita-Komatzu, stones at a depth of 2 m.	
	off Kita-Komatzu, stones at a depth of 1 m.	
	off Kita-Komatzu, depth 5 m.	
	off Kita-Komatzu, stones and sand.	
Genus Macrobiotus C. A. S. Schultze, 1834		
M. gr. harmsworthi	a small stream off Kita-Komatsu, near by its mouth, sand and leaves	
M. gr. hufelandi	a small stream off Kita-Komatsu, near by its mouth, sand and leaves	
M. aff. occidentalis	a small stream off Kita-Komatsu, near by its mouth, sand and leaves	
Order Apochela		
Family Milnesiidae		
Genus <i>Milnesium</i> Doyere, 1840		
<i>M. tardigradum</i> Doyere, 1840	a small stream off Kita-Komatsu, near by its mouth, sand and leaves	Cosmopolitan (according to McInnes, 1994)

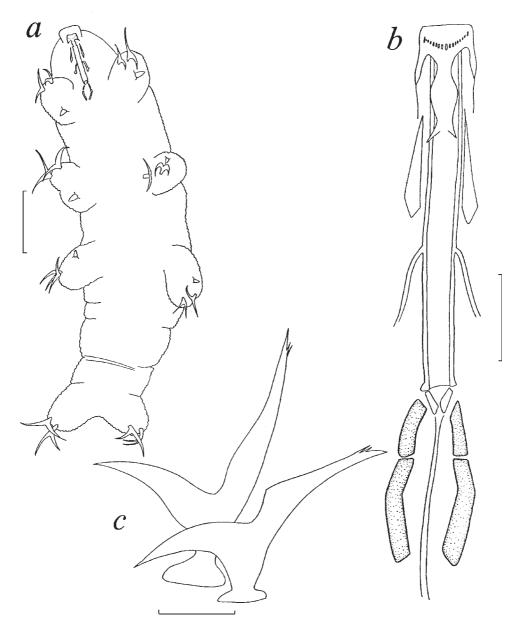


Fig. 1. *Pseudobiotus vladimirii* sp.n.: a — habitus; b — bucco-pharyngeal apparatus; c — claws on the hind legs. Puc.1. *Pseudobiotus vladimirii* sp.n.: a — общий вид; б — вооружение глотки; в — коготки на задних ножках.

reaching legs I dorsally and laterally; ventral sculpture present only in the caudal portion of the body approximately up to legs III. Sculpture represented by rare distributed small conical processes, 1.5–2.5 mm in length. Peribuccal lamellae not revealed. Buccal armature represented by well developed row of teeth. Buccal tube with two well developed crests for insertion of stylet musculature. Stylets curved, with well developed furcae. Pharyngeal bulb slightly oval with small apophyses, two macroplacoids, the first one shorter than the second; microplacoid absent. The shape of the macroplacoids is very similar to that of *Pseudobiotus megalonyx* (Thulin, 1928).

Legs long and relatively thin, with big papillae (similar to that of *P. megalonyx*) on the legs I–III. Claws of *Isohypsibius*-type. Outer claws with long primary branches with short accessory points. Inner claws with small accessory points on

primary branches. Base of the claws in legs IV enlarged. Lunules absent. No cuticular bars on the legs.

Eggs unknown.

REMARKS. This species is similar to *Pseudobiotus megalonyx* (arrangement of the buccal armature and macroplacoids, long claws, papillae on the legs I–III) and in our opinion it can be attributed to the genus *Pseudobiotus* in spite of the impossibility to discern peribuccal lamellae. The small number of specimens and the absence of animals for SEM studies make impossible to verify the presence of these structures. *Pseudobiotus vladimiri* sp.n. can be distinguished from the other species of the genus for the presence of a sculptured cuticle, emerging accessory points in the claws and, apart *P. megalonyx*, also for the shape of the placoids. It differs from *P. megalonyx* also by having the stylet supports inserted on the buccal tube in a more cephalic position.

Specimens with characteristics, which overstep the limits of the known species description or with some divergences from them, are discovered.

Isohypsibius aff. granulifer

Material examined: one specimen jointly with *Dactylobiotus* sp. extracted from stones and sand off Kita-Komatzu and two specimens jointly with *Dactylobiotus dispar* on 2 slides from sand and stones at a depth of 1–1.2 m off Kita-Komatzu.

The specimens are distinguished from other descriptions of the species (e.g. that in Bertolani, 1982) because of the absence of granules on the ventral surface of the body and the absence of cuticular bars near the inner claws on legs I–III.

Isohypsibius aff. *saltursus*

Material examined: one specimen jointly with *Hypsibius* convergens, H. dujardini, I. prosostomus, I. tetradactyloides, *Diphascon* sp., M. gr. harmsworthi, M. gr. hufelandi, M. aff. occidentalis, M. tardigradum.

The specimen differs from *I. saltursus* in the presence of lunules and cuticular bars at the bases of inner claws. It is similar to *Thulinia ruffoi* Bertolani, 1981, but it lacks buccal lamellae.

Dactylobiotus dispar (Murray, 1907).

Adult animals agree well with Bertolani's [1982] and Binda & Pilato's [1999] description, except for almost invisible accessory points on claws in some individuals. Eggs with smaller diameter (75 mm against typical 90 mm) than Bertolani indicated may belong to this species. Unfortunately, eggs with embryos were not found.

Discussion

Cosmopolitism and endemism of the tardigrades are very difficult to understand. McInnes [1994] lists 22 cosmopolitan species of tardigrades. On the other hand we know that many other species were found only once or are known only for the type locality. On this topic we can make two considerations. 1. Many of the supposed cosmopolitan species can hardly be considered as true widespread species, due to the discovery of numerous sibling species among them, often differing in the pattern of distribution (e.g. the "hufelandi group", see Biserov, 1990a, 1990b; Bertolani, Rebecchi, 1993). 2. It is premature to believe that numerous endemic species are found only in the type locality. For example, for a long time Isohypsibius baldii was known only from Lake Tovel, Italy [Ramazzotti, 1945; Bertolani, Balsamo, 1989]. In 1996 V.I. Biserov found this species in the Novaya Zemlya.

The new species has several characters intermediate between the genera *Pseudobiotus* and *Isohypsibius*. It is premature to consider the endemicity of the Tardigrada of Lake Biwa, partly because of the limited material at hand and partly because of the poorly investigated situation of fresh-water Tardigrada in general, and in particular in Japan and in other ancient lakes.

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