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SHORT COMMUNICATION

First data on the freshwater Tardigrada in India: a find of *Pseudobiotus kathmanae* in a small Himalayan lake

Первые данные по фауне пресноводных тихоходок Индии: обнаружение *Pseudobiotus kathmanae* в небольшом озере в Гималаях

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Abstract. A brief report on the tardigrade species *Pseudobiotus kathmanae* discovered in a small lake in Indian Himalayas is given, provided with morphometric data and photographs. Current state of knowledge of Indian fauna of Tardigrada is discussed, together with the discussion of the distribution of the freshwater genus *Pseudobiotus*.

Резюме. Кратко сообщается об обнаружении пресноводной тихоходки *Pseudobiotus kathmanae* в небольшом озере в Индийских Гималаях, с дополнением морфометрическими данными и фотографиями. Обсуждается текущее состояние изученности фауны тихоходок Индии и распространение видов рода *Pseudobiotus*.

Key words: zoogeography, distribution, Indian Hymalayas, freshwater, Tardigrada, *Pseudobiotus kathmanae*, new record

Ключевые слова: зоогеография, распространение, Индийские Гималаи, пресноводная фауна, тихоходки, *Pseudobiotus kathmanae*, новое указание

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Introduction

Little is known about the tardigrade fauna of India. Until now, only eight published papers are present in the literature, containing data on the Indian tardigrades. The main ones are the works of Murray (1907) and Iharos (1969), based on the analysis of the series of moss samples from continental India. Both of these papers should be considered obsolete compared with the current level of morphological data for this taxon. Investigations of last decades shows that a concept of cosmopolitan distribution of polymorphous species within Tardigrada should be replaced with a concept of the presence of similar, but distinct local species (Pilato et al., 2000; Tumanov, 2006; Michalczyk et al., 2012, Guidetti et al., 2016; Kaczmarek & Michalczyk, 2017; Kaczmarek et al., 2017). Within this approach, most species records that are traditionally considered in the above mentioned papers as “widely distributed” or “cosmopolitan” should be considered doubtful and need to be checked in new investigations.

Another work based on the analysis of several moss samples from the territory of the Republic of India is the paper of Maucci & Durante Pasa (1980), devoted to the Andaman Islands, the territory situated far from the mainland India. All

other works (Maucci, 1979; Kristensen, 1987; Abe & Takeda, 2000; Tumanov, 2006; Jørgensen et al., 2007) contains data on single species, obtained mostly from occasionally collected solitary samples. All the above mentioned papers are based on the investigation of terrestrial moss samples; data on the freshwater tardigrade fauna of India are completely absent.

Material and methods

The author received a small plastic vial containing formaldehyde-fixed specimens and exuviae with eggs of tardigrades in glycerol. Material was collected during the Third Western Himalayan Expedition of the St Petersburg Association of Scientists and Scholars in 2015 (Borkin & Gannibal, 2016) by S. Litvinchuk on 8 June 2015 in Lake Nako, Himachal Pradesh, Republic of India, 31.884607N, 78.627782E, 3592 masl, at a depth of c. 5 cm near the shore. Additional environmental data are as follows: substrate – silted soil, pH – 9.8, water temperature – 20.8 °C, conductivity – 0.16. mS, salinity – 0,08 ppt.

To identify the species, all specimens were mounted on microscope slides in Hoyer's medium and examined under a Leica DM 2500 microscope using Phase Contrast (PCM) and Differential Interference Contrast microscopy (DIC). Photographs were taken using a Nikon DS-Fi1 camera. All measurements are given in micrometers (μm) and were performed under PCM using NIS-Elements Software (Nikon Corporation, Japan). Body length was measured from the anterior to the posterior end of the body, excluding the hind legs. Elements of the buccal apparatus were measured according to Pilato et al. (1982). Claws were measured according to Beasley et al. (2008). The *pt* index used is the percentage ratio between the length of a structure and the length of the buccal tube (Pilato, 1981).

Results

Six adult specimens and three exuviae with eggs examined were attributed to the species *Pseudobiotus kathmanae* Nelson, Marley et Bertolani, 1999. The species differs from five other real species of the genus [excluding *Pseudobiotus longiunguis* (Iharos, 1968) in accordance with Pilato et al. (2010)] in the smooth cuticle, three macroplacoids, papillae on legs I–III, and claws on all legs similar in size and form, having widened bases of the outer claws. It should be noted, that such character as the presence or absence of the accessory points, used by Chang et al. (2007) to distinguish *P. kathmanae* from *P. matici* (Pilato, 1971) could not be used here, because the accessory points in *P. kathmanae* are extremely small and invisible in light microscopy (but their presence is proved by scanning electron microscopy), whereas *P. matici* was investigated by light microscopy only, so the presence of the minute accessory spines in this species could not be excluded.

In the original description of *Pseudobiotus kathmanae*, only SEM photographs and line drawings were given. Here I provide an additional set of light microscopy photographs (Fig. 1). The morphometry of the material examined (Table 1) is fully consistent with the description of *P. kathmanae* (Nelson et al., 1999), with the exception of the lengths of the claw bases. This discrepancy is most likely the result of different methods of measuring claws (D. Nelson, pers. comm.). A comparison of the published images of the claws of *P. kathmanae* (Nelson et al., 1999; Schuster et al., 1980) with the material examined shows the complete identity of the proportions of the claw parts.

Discussion

Since the initial descriptions of the genera *Pseudobiotus* Nelson, 1980 (Schuster et al., 1980) and *Thulinia* Bertolani, 1982 [later substituted with the name *Thulinius* (Bertolani, 2003)], the taxonomic position of species attributed to these genera was unclear. Only after the redescription of both genera based on the reinvestigation of the type material (Bertolani et al., 1999 and Nelson et al., 1999), these taxa became clearly delineated.

At present, the genus *Pseudobiotus* includes six exclusively freshwater species. One of them, *P. megalonyx* (Thulin, 1928), was often reported from various regions (McInnes, 1994) but due to previous taxonomic confusion between this species, *P. kathmanae* and *Thulinius augusti* (Murray, 1907), earlier reports in the literature should be considered dubious and need to be checked. Other species has restricted distribution: *P. hirsutellus* Pilato, Lisi et Binda, 2010 is known only

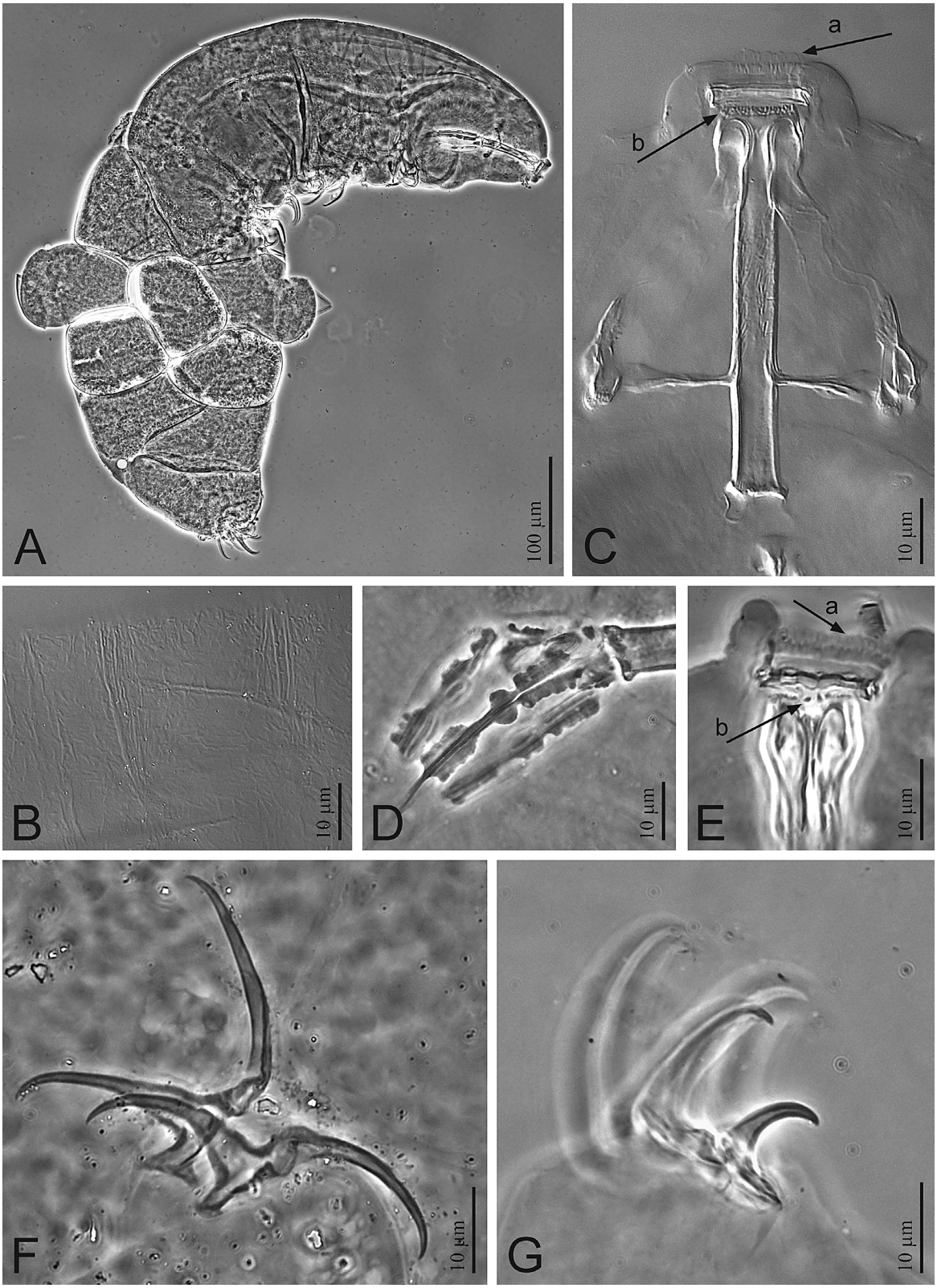

Fig. 1. *Pseudobiotus kathmanae* Nelson, Marley et Bertolani, 1999, details of morphology. **A**, total view of female bearing exuviae with eggs, attached to hind legs; **B**, structure of cuticle surface; **C**, buccal tube; **D**, placoids; **E**, lateral view of buccal armature; **F**, claws of legs III; **G**, modificated claws of legs IV of egg-bearing female; a – peribuccal lamellae, b – teeth of buccal armature. (A, D–G – phase contrast, B, C – DIC).

Table 1. Summary of morphometric data for six specimens of *Pseudobiotus kathmanae*. All measurements are given in μm, *pt* indices (in %) are given in parentheses.

Character / Specimen	1	2	3	4	5	6
Body length	454	491	–	591	–	474
Buccal tube length	50.3	57.0	55.8	–	59.5	58.1
Stylet support insertion point (Ss)	35.2 (*70.0*)	40.4 (*70.8*)	38.5 (*69.0*)	–	42.8 (*71.8*)	40.5 (*69.7*)
Buccal tube external width	5.5 (*11.0*)	7.5 (*13.1*)	7.7 (*13.7*)	–	7.5 (*12.6*)	6.5 (*11.2*)
Buccal tube internal width	3.4 (*6.8*)	5.6 (*9.9*)	6.1 (*10.9*)	–	6.0 (*10.0*)	4.5 (*7.8*)
1st macroplacoid length	8.7 (*17.3*)	10.8 (*19.0*)	12.2 (*21.9*)	–	11.7 (*19.7*)	11.1 (*19.2*)
2nd macroplacoid length	4.6 (*9.1*)	4.9 (*8.6*)	6.2 (*11.0*)	–	6.1 (*10.3*)	5.8 (*10.0*)
3rd macroplacoid length	9.4 (*18.6*)	10.5 (*18.5*)	12.6 (*22.6*)	–	11.8 (*19.8*)	12.1 (*20.8*)
Macroplacoid row length	26.3 (*52.3*)	32.1 (*56.3*)	34.5 (*61.8*)	–	35.2 (*59.2*)	34.0 (*58.5*)
Claw I lengths						
External base	–	–	–	–	12.4 (*20.8*)	–
External primary branch	–	–	–	–	33.1 (*55.6*)	–
External secondary branch	–	–	–	–	22.5 (*37.8*)	–
Claw II lengths						
External base	10.6 (*21.1*)	12.1 (*21.2*)	–	14.6 (*?*)	14.2 (*23.9*)	–
External primary branch	29.6 (*58.9*)	34.4 (*60.4*)	–	34.4 (*?*)	31.8 (*53.3*)	–
External secondary branch	23.7 (*47.1*)	27.9 (*48.8*)	–	26.6 (*?*)	27.1 (*45.6*)	–
Internal base	–	11.9 (*20.9*)	–	–	–	–
Internal primary branch	–	30.4 (*53.3*)	–	–	–	–
Internal secondary branch	–	26.1 (*45.8*)	–	–	–	–
Claw III lengths						
External base	11.0 (*22.0*)	–	–	12.1 (*?*)	14.6 (*24.5*)	–
External primary branch	29.2 (*58.2*)	–	–	31.0 (*?*)	–	–
External secondary branch	23.5 (*46.7*)	–	–	26.0 (*?*)	27.9 (*46.8*)	–
Internal base	10.1 (*20.0*)	–	–	11.4 (*?*)	–	11.5 (*19.8*)
Internal primary branch	23.1 (*46.0*)	–	–	–	–	–
Internal secondary branch	20.8 (*41.4*)	–	–	22.9 (*?*)	–	27.6 (*47.5*)
Claw IV lengths						
Anterior base	9.6 (*19.1*)	–	–	–	–	9.9 (*16.9*)
Anterior primary branch	–	–	–	–	–	–
Anterior secondary branch	21.1 (*42.0*)	–	–	–	–	16.2 (*27.8*)
Posterior base	10.5 (*20.8*)	–	–	–	–	18 (*20.3*)
Posterior primary branch	–	–	–	–	–	31.7 (*54.5*)
Posterior secondary branch	24.1 (*47.9*)	–	–	–	–	23.8 (*41.0*)

from Israel (Pilato et al., 2010), *P. matici* (Pilato, 1971) from Italy, *P. spinifer* Chang, Kaczmarek, Lee et Michalczyk, 2007 from South Korea (Chang et al., 2007), and *Pseudobiotus vladimiri* Biserov, Dudichev et Biserova, 2001 from Japan (Biserov et al., 2001). Being described on the base of material from United States and Italy simultaneously, *P. kathmanae* is the only species of the genus with confirmed wide distribution. The findings presented here support the presumable Holarctic distribution of this species (Nelson et al., 1999). The presence of the species with such a wide range seems to contradict the modern paradigm of the tardigrade zoogeography, and undoubtedly should be verified in the future using genetic analysis.

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References

Abe W. & Takeda M. 2000. A new record of *Cornechiniscus madagascariensis* Maucci, 1993 (Tardigrada: Echiniscidae) from India. *Proceedings of the Biological Society of Washington*, **113**: 480–485.

Beasley C.W., Kaczmarek L. & Michalczyk L. 2008. *Doryphoribius mexicanus*, a new species of Tardigrada (Eutardigrada, Hypsibiidae) from Mexico (North America). *Proceedings of the Biological Society of Washington*, **121**: 34–40. https://doi.org/10.2988/07-30.1

Bertolani R. 2003. *Thulinius*, new generic name substituting for *Thulinia* Bertolani, 1981 (Tardigrada, Eutardigrada). *Zootaxa*, **314**: 1–4. https://doi.org/10.11646/zootraxa.314.1.1

Bertolani R., Marley N.J. & Nelson D.R. 1999. Re-description of the genus *Thulinia* (Eutardigrada, Hypsibiidae) and of *Thulinia augusti* (Murray, 1907) comb. n. *Zoologischer Anzeiger*, **238**: 139–145.

Biserov V.I., Dudichev A.L. & Biserova N.M. 2001. Preliminary data on tardigrades of Lake Biwa (Japan). *Arthropoda Selecta*, **10**: 307–310.

Borkin L.J. & Gannibal B.K. 2016. Tret'ya Zapadno-Gimalayskaya ekspeditsiya Sankt-Peterburgskogo soyuza uchenykh (2015) [The Third Western Himalayan Expediton of the St Petersburg Association of Scientists & Scholars (2015)]. *Istoriko-biologicheskie Issledovanija*, **8**: 145–152. (In Russian).

Chang C.Y., Kaczmarek L., Lee J.M. & Michalczyk L. 2007. *Pseudobiotus spinifer,* a new Tardigrade species (Eutardigrada: Hypsibiidae) from Nakdong River, South Korea, with a redescription of *P. vladimiri* Biserov, Dudichev & Biserova. *Zoological Science*, **24**: 623–629. https://doi.org/10.2108/zsj.24.623

Guidetti R., Rebecchi L., Bertolani R., Jönsson K.I., Kristensen R.M. & Cesari M. 2016. Morphological and molecular analyses on *Richtersius* (Eutardigrada) diversity reveal its new systematic position and lead to the establishment of a new genus and a new family within Macrobiotoidea. *Zoological Journal of the Linnean Society*, **178**: 834–845. https://doi.org/10.1111/zoj.12428

Iharos G. 1969. Beiträge zur Kenntnis der Tardigraden Indiens. *Opuscula Zoologica, Budapest*, **9**: 107–113.

Jørgensen A., Møbjerg N. & Kristensen R.M. 2007. A molecular study of the tardigrade *Echiniscus testudo* (Echiniscidae) reveals low DNA sequence diversity over a large geographical area. *Journal of Limnology*, **66**: 77–83. https://doi.org/10.4081/jlimnol.2007.s1.77

Kaczmarek Ł., Gawlak M., Bartels P.J., Nelson D.R. & Roszkowska M. 2017. Revision of the genus *Paramacrobiotus* Guidetti et al., 2009 with the description of a new species, re-descriptions and a key. *Annales Zoologici*, **67**: 627–656. https://doi.org/10.3161/00034541ANZ2017.67.4.001

Kaczmarek Ł. & Michalczyk Ł. 2017. The *Macrobiotus hufelandi* group (Tardigrada) revisited. *Zootaxa*, **4363**: 101–123. https://doi.org/10.11646/zootaxa.4363.1.4

Kristensen R.M. 1987. Generic revision of the Echiniscidae (Heterotardigrada), with a discussion of the origin of the family. *In:* **Bertolani R.** (Ed.). *Biology of Tardigrada. Selected Symposia and Monographs, U.Z.I.*: 261–335. Modena, Italy: Mucchi.

Maucci W. 1979. I *Pseudechiniscus* del gruppo *cornutus* con descrizione di una nuova specie (Tardigrada, Echiniscidae). *Zeszyty Naukowe Uniwersytetu Jagiellońskiego, Prace Zoologiczne*, **25**: 107–124.

Maucci W. & Durante Pasa M.V. 1980. Tardigradi muscicoli delle Isole Andamane. *Bollettino Museo Civico di Storia Naturale di Verona*, **7**: 281–291.

McInnes S.J. 1994. Zoogeographic distribution of terrestrial/freshwater tardigrades from current lit-

erature. *Journal of Natural History*, **28**: 257–352. https://doi.org/10.1080/00222939400770131

Michalczyk Ł., Wełnicz W., Frohme M. & Kaczmarek Ł. 2012. Redescriptions of three *Milnesium* Doyère, 1840 taxa (Tardigrada: Eutardigrada: Milnesiidae), including the nominal species for the genus. *Zootaxa*, **3154**: 1–20. https://doi.org/10.11646/zootaxa.3154.1.1

Murray J. 1907. VI. – Some Tardigrada of the Sikkim Himalaya. *Journal of the Royal Microscopical Society*, **1907**: 269–273. https://doi.org/10.1111/j.1365-2818.1907.tb01654.x

Nelson D.R., Marley N.J. & Bertolani R. 1999. Re-description of the genus *Pseudobiotus* (Eutardigrada, Hypsibiidae) and of the new type species *Pseudobiotus kathmanae* sp. n. *Zoologischer Anzeiger*, **238**: 311–317.

Pilato G. 1971. Tardigradi delle acque dolci siciliane. Nota prima. *Bollettino delle Sedute della Accademia Gioenia di Scienze Naturali in Catania, Serie 4*, **11**: 126–134.

Pilato G. 1981. Analisi di nuovi caratteri nello studio degli Eutardigradi. *Animalia*, **8**: 51–57.

Pilato G., Bertolani R. & Binda M.G. 1982. Studio degli *Isohypslbius* del gruppo *elegans* (Eutardigrada, Hypsibiidae) con descrizione di due nuove specie. *Animalia*, **9**: 185–198.

Pilato G., Binda M.G., Napolitano A. & Moncada E. 2000. The specific value of *Macrobiotus coronatus* DeBarros 1942, and description of two new species of the *harmsworthi* group (Eutardigrada). *Bollettino delle Sedute della Accademia Gioenia di Scienze Naturali in Catania*, **33**: 103–120.

Pilato G., Lisi O. & Binda M.G. 2010. Tardigrades of Israel with description of four new species. *Zootaxa*, **2665**: 1–28.

Schuster R.O., Nelson D.R., Grigarick A.A. & Christenberry D. 1980. Systematic criteria of Eutardigrada. *Transactions of the American Microscopical Society*, **99**: 284–303. https://doi.org/10.2307/3226004

Tumanov D.V. 2006. Five new species of the genus *Milnesium* (Tardigrada, Eutardigrada, Milnesiidae). *Zootaxa*, **1122**: 1–23. https://doi.org/10.11646/zootaxa.1122.1.1

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