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Hypsibius thaleri sp. nov., a new species of a glacier-dwelling tardigrade from the Himalayas, Nepal (Tardigrada)

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ABSTRACT. – A new tardigrade species, *Hypsibius thaleri* sp. nov., from the Nare Glacier at Mt Ama Dablam in the Himalayas [NE Nepal, the Chomolungma (= Mt Everest) region], is described. *Hypsibius thaleri* belongs to the "*Hypsibius klebelsbergi*"-group, a complex of obligate glacier-dwelling tardigrades inhabiting cryoconite holes in the glacier ablation zone. The group represents a distinct supra-specific taxon, of new sub- or generic rank. A key for identification of the cryobiontic tardigrades is provided.

KEYWORDS: Tardigrada, taxonomy, *Hypsibius thaleri* sp. nov., glacier, cryoconite holes, the Himalayas, Nepal

Introduction

Tardigrades (or water-bears) of the Himalayas and the neighbouring Karakoram are very poorly known as the relevant literature is limited to a few publications (EHRENBERG 1859, MURRAY 1907, RAMAZZOTTI 1968, DASTYCH 1973, 1975, 1976, 1986, 2004, JANETSCHEK 1990, DASTYCH & KRISTENSEN 1995, DASTYCH 2004). Three papers (RAMAZZOTTI l.c., JANETSCHEK l.c., DASTYCH 2004) deal with or mention tardigrades inhabiting Himalayan cryoconite holes, a peculiar but neglected and still poorly understood glacial ecosystem found in the polar and high-mountain regions worldwide. Such cryoconite holes represent aquatic microcaverns which occur on the ice surface in a glaciers' ablation zone and which come into being by the actions of solar radiation on dark, fine particles of inorganic and organic debris that melt into the ice surface (*e.g.*, STEINBÖCK 1936, AN DER LAN 1963, WHARTON *et al.* 1981).

The flora and fauna of such cryoconite holes is poorly known and includes mostly bacteria, algae, protozoans, rotifers and tardigrades (*e.g.*, WITTROCK 1855, STEINBÖCK 1936, 1957, MIHELČIČ 1963, GRØNGAARD et al. 1999, WHARTON *et al.* 1981, DE SMET & VAN ROMPU 1994, SÄWSTRÖM et al. 2002, MARGESIN 2003). The tardigrades, when they occur in large numbers, play an important role within these independent, short-living ecosystems (*e.g.*, STEINBÖCK 1957, KRAUS 1977, DE SMET & VAN ROMPU 1994, DASTYCH *et al.* 2003). Among facultative inhabitants of the cryoconite holes, which include the majority of the tardigrade species reported from that habitat, there are also some water-bears which represent obligate ice-dwellers. To such true cryobiontic tardigrades belong two intensely dark pigmented species (*Hypsibius klebelsbergi* MIHELČIČ, 1959 and *H. janetscheki* RAMAZZOTTI, 1968), for which redescriptions have recently been provided (DASTYCH *et al.* 2003, DASTYCH 2004). The former has been reported from cryoconite holes on several glaciers in the Central Alps in Austria, the latter only from one glacial locality (and collection) in the Himalayas, in the vicinity of Chomolungma (= Mt Everest).

While redescribing *H. janetscheki* (see DASTYCH 2004), several individuals of a diferrent tardigrade species were observed mounted together with the type-series and topotypes. This species [originally identified by RAMAZZOTTI (1968) as the widely distributed *Hypsibius convergens* (URBANOWICZ, 1925)] was found to represent an undescribed taxon. The present paper describes this new species and provides a key for the identification of cryobiontic tardigrades.

Material and methods

The material was collected by the Austrian zoological expedition to the Nepalese Himalayas in 1961 (for details see JANETSCHEK 1990). The tardigrade sample (code "82": *l.c.*, p. 24) originated from the Nare Glacier (= Amai Dablang Glacier, *auct.*: 5620 m a.s.l.) "at the valley head of Mingbo, a snowless surface of the glacier, 5600 ± m, mixed sample of cryoconite, pipetted mud from various cryoconite holes, 4.6.1961..." (*l.c.*, p. 48, 49: from German).

The type-series of the new species, recorded in sympatry with *H. janetscheki* (see RAMAZZOTTI 1968, JANETSCHEK 1990), includes 49 specimens mounted by the former author on six microslides, five in PVL and one in FAURE'S medium. RAMAZZOTTI observed 186 specimens of this taxon (reported as *H. convergens*: l.c. 1968: p. 2), but the whereabouts of the other 137 individuals is unknown. All information about the new species (and *H. janetscheki*) is based exclusively on old, partly deteriorated material. Comparative material of *H. klebelsbergi* (Figs 3, 5) came from the Rotmoosferner glacier in the Ötztal Alps (for details see DASTYCH *et al.* 2003).

Photomicrographs were taken with a ZEISS "Photomikroskop III". The diameter of the buccal tube was taken just above the level of the insertion of stylet supports, the "stylet support attachments" characterizing the distance between the upper (dorsal) edge of the stylet sheaths and the anterior edge of stylet support. "External claw IV" means the most posteriorly located (i.e., the hind) claw on leg IV, homologous to external claws I-III. "Internal claw IV" denotes the most anteriorly located (i.e., the fore) claw on leg IV, homologous to the internal claws I-III. A slash (/) shows the separation of lines on each microslide label.

Morphometric data are presented by several indices: "*PT* index" describes the ratio between the length of the buccal tube and that of other structures taken into consideration (PILATO 1981). The macroplacoids' index (*MPLI*) characterizes the size ratio between the second and first macroplacoids (= *mpl*2 x 100 / *mpl*1: see DASTYCH *et al.* 2003). Four other indices are based on the morphometry of claws (see also DASTYCH 2004): (1) the external claws' index (*ECI*), (2) the internal claws' index (*ICI*), (3) the claws' main (= primary) branch index (*MBI*) and (4) the hind (= posterior IV) claw base index (*HCBI*).

The *ECI* index describes the length ratio between the external claw I and the hind claw (= external claw I length x 100 / hind (= posterior IV) claw length), the second, the *ICI* index, is the ratio between internal claw I and the fore claw (= internal claw I length x 100 / fore (= anterior IV) claw length). The *MBI* index, characterizes the size ratio between the claws' main branches (i.e., the external claw I branch length x 100 / hind claw main branch length). The *HCBI* index defines the size ratio between the hind claw base and its main branch (= hind claw base height x 100 / hind claw main branch length).

The coefficient of variability (*V*) is defined as the standard deviation divided by (arithmetic) mean (i.e., $SD / \bar{x}$). The coefficient of determination (r^2, *r* squared) is the square of the PEARSON'S product-moment correlation coefficient, *r* (*e.g.*, SOKAL & ROHLF 1981). It describes the proportion of variance in one variable explained by variation in the other variable, i.e., presenting degree of correlation of examined variables (the values of *r* squared range between 0.0 and 1.0). Here *r* squared involves the mouth tube length and that of considered structure (*PT* indices) or the variables characterizing *MPLI, ECI, ICI, MBI* and *HCBI* indices as defined above. All indices and coefficients are presented in %.

Abbreviations used are: *DIC*- differential interference contrast, *ECI* - external claws' index, *HCBI* - hind claw base index , *ICI* - internal claws' index, *LM* - light microscope, *n* - sample size, *MBI* - claws' main branch index, *min-max* - minimum-maximum range, *mpl* - macroplacoid, *MPLI* -macroplacoids' index, *PHC* - phase contrast, *PVL* - polyvinyl-lactophenol, r^2 - coefficient of determination, *SD* - standard deviation, *ss* - stylet support, *V* - coefficient of variability, $\bar{x}$ - (arithmetic) mean.

Description of species

Hypsibius thaleri sp. nov.
(Figs 1-39)

Hypsibius convergens, – RAMAZZOTTI 1968: 2; 1972 (in part); RAMAZZOTTI & MAUCCI 1983 (in part); JANETSCHEK 1990; MCINNES 1994 (in part).
Hypsibius janetscheki, – DASTYCH 1993: "active form".

H o l o t y p e (Figs 1, 22, 30, 34). – 445 µm long, sex unknown: mounted together with six paratypes on microslide labelled "XI-22" (see below, "**D**"). Deposited in the RAMAZZOTTI'S Collection lodged at the Museo Civico di Storia Naturale, Verona.

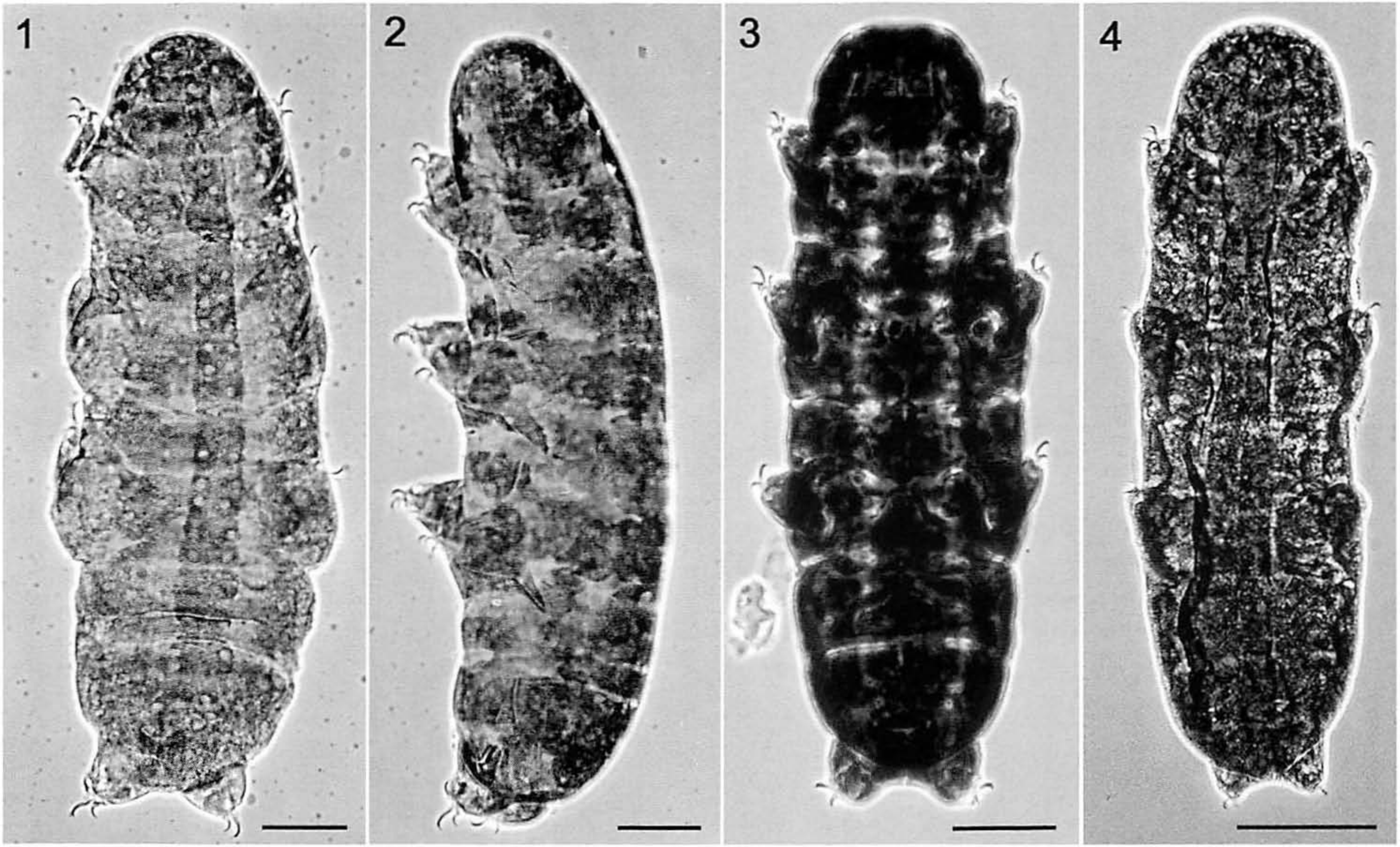
T y p e l o c a l i t y. – NE Nepal, Saragmatha National Park, Khumbu Himal, sediment from cryoconite holes of various size, within an area of *c.* 20 m² on the Nare Glacier in the Mingbo Valley, 5570 m a.s.l.; 4 June 1961, collected by H. JANETSCHEK (altitude data from JANETSCHEK 1990: p. 21, 48, 49 and RAMAZZOTTI 1968).

P a r a t y p e s (48 individuals) on six microslides labelled: (**A**), "Tipo 178 / 10 *H.* (*H.*) / *janetscheki* /Ramazzotti (Polyvi)" and "Pozzetto / glaciale m 5600 / Himalaya, Ghiac / ciaio Amar Dablam / Tipi 2" (seven paratypes); (**B**), "Tipo 180 / 14 *Hyps.* (*H.*) / *janetscheki* / Ramazzotti" and " Pozzetti / glaciali m 5600 / Himalaya / 4.VI.61 / Faure" (seven paratypes); (**C**), "XI-14 / 5 *Hypsibius* /Ramazzotti (Poliv.)" and "Pozzetto glaciale 5600 m / Himalaya - Ghiac- / ciaio Amar / Dablam" (five paratypes); (**D**), "XI-22 / 8 *H.* (*H.*) / Ramazzotti / (Poliv.)" and "Pozzetti glaciali Himalaya / Ghiacciaio Amar / Dablam 5600 m" (six paratypes + holotype); (**E**), " XI-26 / 11 *H.* / Ramazzotti (Poliv)" and "Pozzetto / glaciale m 5600 / Hymalaia - ghiac= / ciaio Amar Dablam" (11 paratypes); (**F**), "XI-27 / 17 *H.* (*H.*) /Ramazzotti" and "Pozzetto glaciale m 5600 / Himalaya (Poliv.) (12 paratypes)". Locality data and depository as for the holotype; one microslide with 12 paratypes (slide No. XI-27) in the Zoologisches Museum Hamburg (ZMH Acc. No. A20/04).

The slides "Tipo 178" and "Tipo 180" also bear type specimens of *H. janetscheki* (see redescription of the latter taxon in DASTYCH 2004). The abbreviation "*H.* (*H.*)" stands for "*Hypsibius* (*Hypsibius*)" [sp.] and is written, together with the locality data and the type of mounting medium, in black Indian ink; the name "janetscheki" and "Tipi 2" in blackish-violet ballpoint ink.

Note: The incorrectly spelled name "Amar Dablam" on the microslide labels refers to the impressive summit at the Nare Glacier, i.e., the Mt Ama Dablam (= Amai Dablang, *auct.*, 6856 m a.s.l.) in the region of Chomolungma (= Mt Everest).

E t y m o l o g y. – In honour of my colleague and arachnologist, Univ. Doz. Dr. KONRAD THALER (Innsbruck), in recognition of his interests and contribution to the knowledge about high mountain invertebrates.



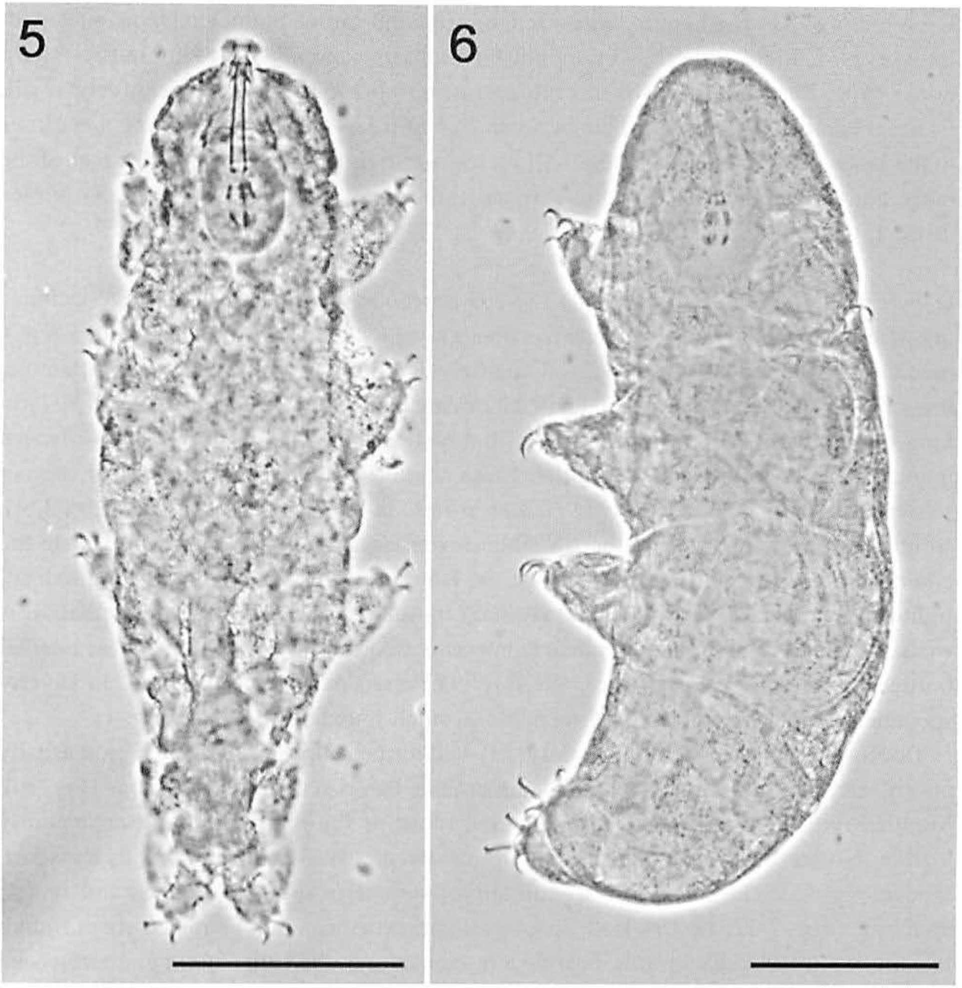
Figs 1-4. Adults of: **1-2**, *Hypsibius thaleri* sp. nov, dorsal and lateral view, respectively; **3**, *Hypsibius klebelsbergi* MIHELČIČ; **4**, *Hypsibius janetscheki* RAMAZZOTTI (all *PHC*. Fig. 1: holotype. Fig 3: FAURE'S medium; others: *PVL*. Scale bar: 50 µm.).

D i a g n o s i s. – Medium to large sized, mostly light-brown pigmented hypsibiids with large eyes. Cuticle smooth. The mouth tube anterior apophyses resemble the "sharp hook"-type. Pharynx with two macroplacoids, no microplacoid. Legs relatively small. Claws medium sized, intermediate between *Isohypsibius*- and *Hypsibius*-type, but closer to the latter. External claws on legs I-III increasing slightly in size towards the rear of the body, thus being smaller than the external (hind) claws on leg IV. Accessory spines distinct, wide.

D e s c r i p t i o n. – Length of body 157-504 µm (360-500 µm: Ramazzotti 1968), cuticle in *LM* smooth. Colour in life unknown, animals mounted on microslides in *PVL* or Faure's medium light-brownish, one specimen intensely dark-brown. Most specimens with large, brownish-black eye-dots (*e.g.*, 8.0 and 12.0 µm in diameter in a specimen 487 and 504 µm long, respectively). The pigmentation of the body is produced by tiny blackish-brown granules located in the epidermal cells. These granules, except in one individual, are not so densely accumulated as those in *H. klebelsbergi* or *H. janetscheki* (Figs 1, 2 vs 3, 4), so internal organs are usually more visible. Juveniles markedly less pigmented than the adults (Fig. 6). On the dorsal surface of the latter are often traces of two longitudinal, lighter pigmented strips along the two main muscle cords, where the pigmentation is weaker (Fig. 1). Such weaker pigmentation occurs frequently also at the pseudosegmental borders, producing an indistinct, variably checkered pattern (Figs 1, 2). In several specimens epidermal nuclei are discernible as small roundish dots (*e.g.*, Fig. 1).

Bucco-pharyngeal apparatus (Figs 14-19) well formed, large. Peribuccal region usually poorly visible, a few animals only with discernible, barely retracted mouth cone (Fig. 16). Mouth cavity median sized, its details and those of the mouth opening area poorly visible. No teeth visible within the mouth cavity in *LM*. Mouth tube relatively short, moderately wide (Figs 14-19) and with almost symmetrical anterior dorsal and ventral apophyse (Figs 7-12, 14-19). Both apophyses terminated caudally with a distinct, spine-like process directed backwards, bearing a resemblance to the barb (flue) of an arrowhead (*e.g.*, Figs 8, 14, arrowhead). The external edge of both apophyses, when observed in lateral view, distinctly concave in its median part (e.g., Figs 9-11). Terminal posterior apophyses of the mouth tube tiny, barely visible (Figs 16, 18, arrowhead). Stylets relatively large, their furcas "typically" formed (Fig. 13). Stylet supports well developed and located more or less in anterior position (their *PT* values are between 60-69 %).

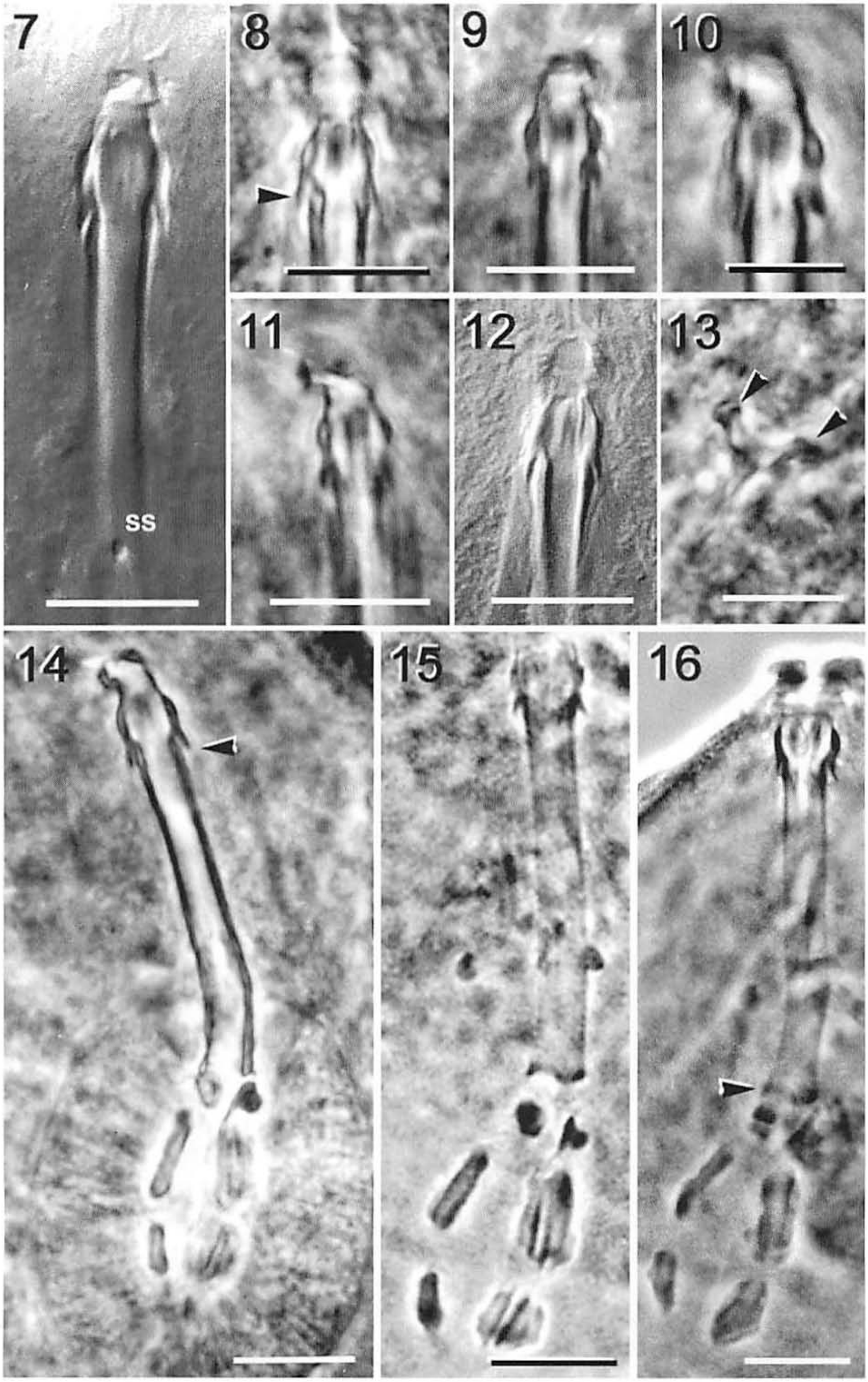
Pharynx relatively large, sub-spherical (Figs 6, 20), with distinct pharyngeal apophyses and two macroplacoids (Figs 14-19). No microplacoid. Pharyngeal apophyses well separated from the first macroplacoid (Figs 18-20). Macroplacoids rod-shaped, moderately long and broad, the second macroplacoid 50-85 % of the length of the first one (Figs 14-25). Both macroplacoids distinctly separated from each other, usually without or, rarely (mainly in juveniles), only with poorly marked constrictions. When present, the constriction occurs in the middle of the first placoid and at the caudal end of the second. Juveniles with markedly short macroplacoids (Fig. 6) and the constriction occurs in the middle of the second macroplacoid. Several adults with obscure external thickening at the caudal part of the second macroplacoid.



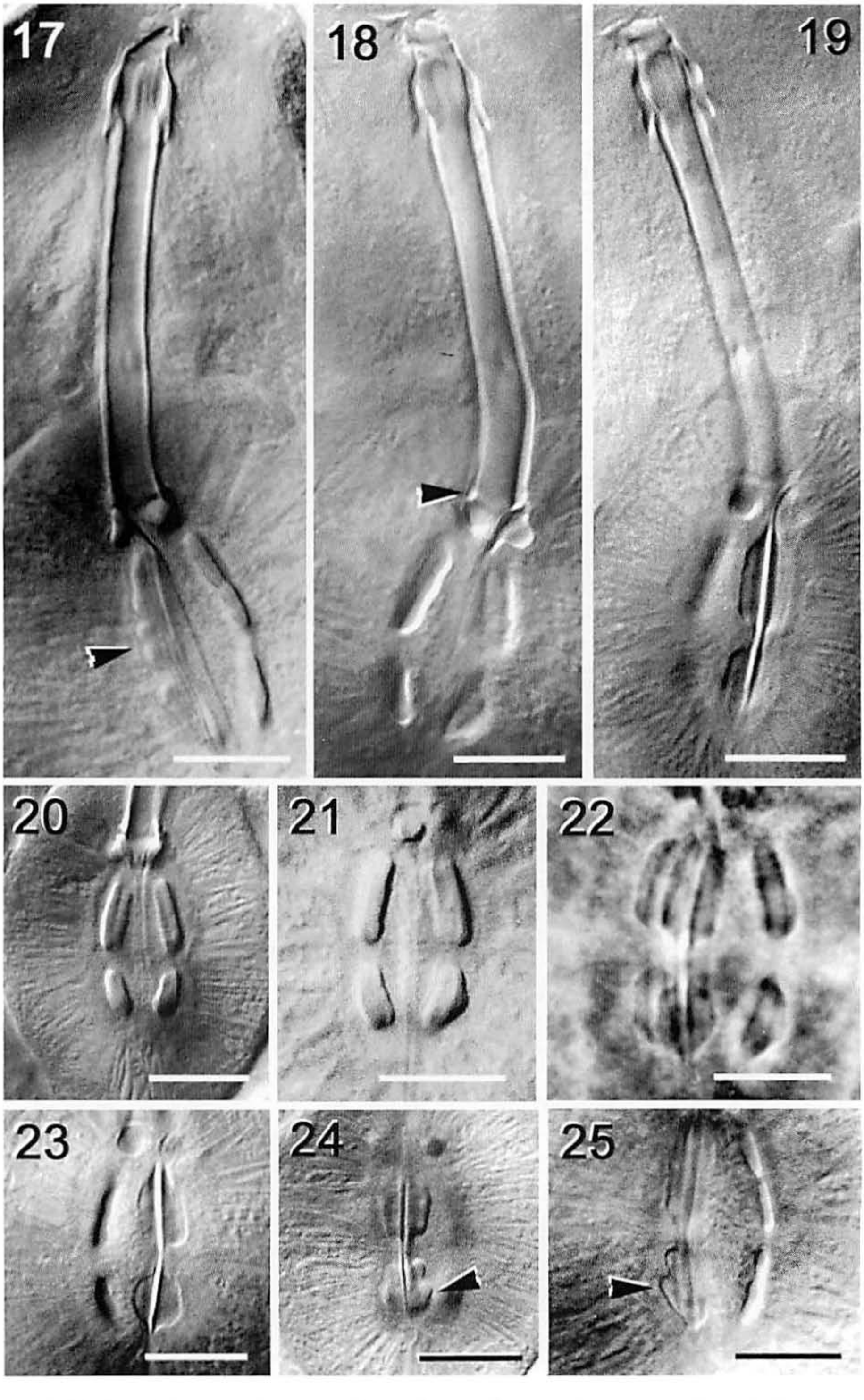
Figs 5-6. Juveniles of: **5**, *Hypsibius klebelsbergi* MIHELČIČ; **6**, *Hypsibius thaleri* sp. nov. (*PHC*. Fig. 5: Faure's medium; Fig. 6: *PVL*. Scale bar = 50 µm.).

Legs relatively small, claws medium sized and similar in shape to those of *H. klebelsbergi*, though distinctly larger and more slender (Figs 26-39). The claws of shape between *Isohypsibius*- and *Hypsibius*-type, but resembling more the latter type. Main branches slender, relatively long (*e.g.*, Figs 26, 27, 37), accessory spines well developed and wide (Figs 30, 33, 39). Lunules absent, no cuticular bars between the claws and their bases. The size of claws (and their main branches) increases towards the body posterior, so that the claws on the first pair of legs are distinctly smaller that those on the fourth legs (Figs 1, 2). Accordingly, the values of the indices *ECI, ICI* and *MBI* are markedly lower than 100 % (see morphometrics below).

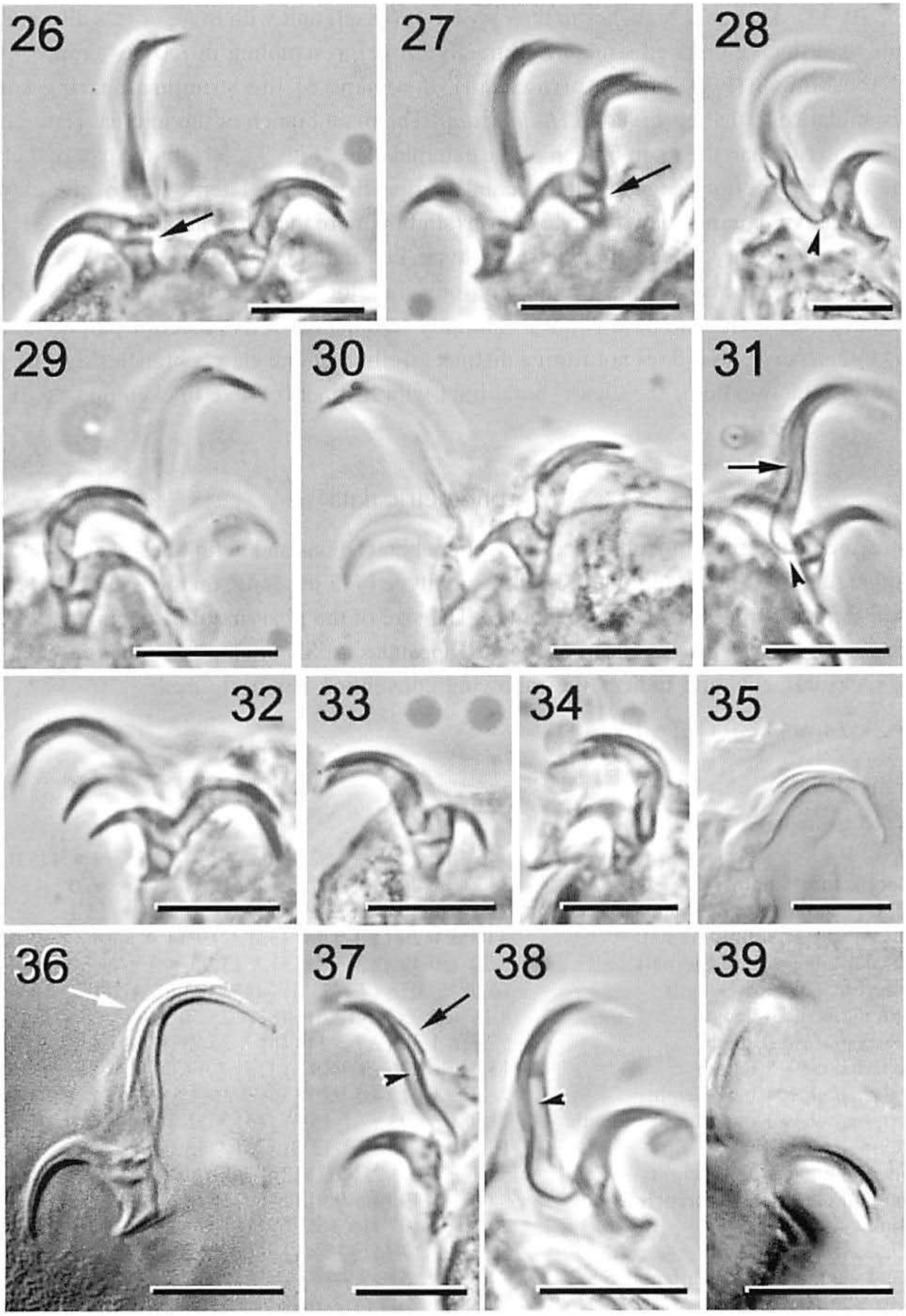
Main branches of all external claws narrow, elongated and slightly "S"-shaped, when observed in profile (Figs 28, 31, 37, 38), i.e., with a distinct curvature in its middle (Fig. 31, arrow) and the (short) base of the branch strongly bent at its connection to the claw's basal tract (Figs 28, 31, arrowhead). The secondary branches relatively long (Figs



Figs 7-16. *Hypsibius thaleri* sp. nov.: **7-12**, the anterior mouth apophyses; **13**, stylet's furca; **14-16**, bucco-pharyngeal apparatus (Figs 7, 12: *DIC*; others: *PHC*. Paratypes. Scale bar: 10 μm.).



Figs 17-25. *Hypsibius thaleri* sp. nov.: **17-19**, bucco-pharyngeal apparatus, lateral view; **20-25**, variability of macroplacoids [Fig. 22 (holotype): *PHC*; others (paratypes): *DIC*. Scale bar: 10 μm].



Figs 26-39. *Hypsibius thaleri* sp. nov.: **26**, claws of leg IV; **27**, claws of leg II; **28**, external claw of leg III; **29**, claws of leg III; **30**, claws of leg IV; **31**, external claw of leg III; **32**, claws of leg III; **33**, internal (fore) claw of leg IV; **34**, internal claw of leg I; **35**, main branch of external claw II; **36**, external claw of leg I; **37**, external (hind) claw of leg IV; **38**, external claw of leg II; **39**, external (hind) claw of leg IV (explanations in text; Figs 35, 36, 39: *DIC*; others: *PHC*. Figs 30, 34: holotype; others: paratypes. Scale bar: 10 μm).

27, 30, 32). The main branches in their posterior (basal) unit with more or less discernible sub-superficial elongated structure (refractive zone), resembling in *LM* an "empty" part of the branch (Figs 37, 38, arrowhead), the shape of the structure bearing some resemblance to that observed in *H. dujardini*. The main branch of the internal claw often more curved than the main branch of the external claw (Figs 32, 34). In the external claw the upper (anterior) edges of the secondary branch and the lateral side of the claw's basal tract of external claw form an obtuse (slightly more than 90°) angle (Figs 28, 31, 37, 38). The accessory spines of *klebelsbergi*-type, i.e., flattened and wide (Figs 33, 36, 39). The base of accessory spines relatively long, i.e., located over the largest part of the main branch arch. The base is not as abruptly terminated in its posterior part as that in *H. klebelsbergi*, so it does not form a distinct swelling on the claw's branch (Figs 36, 37, arrow). The middle of the claws' basal tract with distinct bar-like thickening (*e.g.*, Figs 26, 27, arrow).

Morphometric data

Measurements are in µm, all indices in %. For abbreviations and definitions see § "Material and methods". The morphometrics of the holotype (445 µm long, on microslide "XI-22") is separated from other data by a dot (•). The size of the pharynx of a specimen 308 µm long is 31.5 x 21.6 µm, its bucco-pharyngeal apparatus is 58.5 µm long. For the presentation of measurements and indices the following convention has been used:

$\bar{x} \pm SD$ (*min-max*) [*n*] * V / r^2

Individuals

A) Measurements (µm)

Body length	346.30 ± 88.63 (157.0-504.0) [33] * 25.59 • 445.0
Buccal tube length	34.74 ± 4.66 (23.4-43.2) [45] * 13,41 • 36.0
Stylet supports attachments	23.27 ± 2.85 (16.2-27.9) [28] * 12.24 • 23.4
Buccal tube width (external)	4.08 ± 0.79 (2.3-5.04) [39] * 19.44 • 5.0
Buccal tube width (internal)	2.70 ± 0.49 (2.3-3.8) [8] * 17.95 • 3.8
Macroplacoid row length	16.07 ± 3.76 (7.2-23.4) [48] * 22.85 • 18
Macroplacoid 1 length	8.18 ± 2.9 (3.2-12.6) [48] * 25.59 • 9.9
Macroplacoid 2 length	5.37 ± 1.38 (2.7-8.1) [48] * 25.77 • 5.9
External claw 1 length	15.43 ± 2.26 (9.9-19.8) [25] * 14.65 • 18.0
External claw 1 base height	7.30 ± 1.41 (4.5-9.0) [36] * 19.35 • 8.6
External claw 1 main branch length	11.98 ± 1.93 (7.7-15.8) [45] * 16.12 • 13.5
Internal claw 1 length	10.67 ± 1.45 (7.2-12.6) [23] * 13.62 • 11.25
Internal claw 1 base height	5.61 ± 1.21 (2.7-7.2) [21] * 21.60 • 6.3
Internal claw 1 main branch length	8.00 ± 1.49 (4.5-9.9) [14] * 18.61 • 9.0
Hind claw length	20.10 ± 1.97 (15.3-23.8) [18] * 9.83 • 21.6
Hind claw base height	8.94 ± 2.17 (4.5-14.4) [23] * 24.3 • 10.4
Hind claw main branch length	14.63 ± 2.57 (9.9-18.0) [35] * 17.57 • 15.3
Fore claw length	11.58 ± 2.02 (7.7-16.0) [17] * 17.49 • 13.5
Fore claw base height	5.57 ± 1.39 (3.1-9.9) [24] * 24.97 • 6.0
Fore claw main branch length	7.91 ± 1.32 (5.4-9.9) [24] * 16.79 • 9.5

B) Indices

PT stylet supports	66.24 ± 2.02 (60.3-68.9) [28] * 3.06 / 94.44 • 65.14
PT buccal tube width (ext.)	11.60 ± 1.36 (7.6-14.7) [38] * 11.69 / 74.34 • 13.9
PT buccal tube width (int.)	7.85 ± 1.32 (6.4-10.6) [8] * 16.93 / 20.76 • 10.6

PT macroplacoid row length	45.31 ± 6.26 (26.7-57.8) [45] * 13.81 / 83.71 • 50.0
PT macroplacoid 1 length	23.11 ± 3.92 (11.7-33.3) [45] * 16.97 / 78.15 • 27.5
PT macroplacoid 2 length	15.19 ± 2.54 (9.09-20.0) [45] * 16.74 / 79.12 • 16.3
PT claw 1 (ext.) length	45.17 ± 4.28 (39.5-56.4) [24] * 9.47 / 60.57 • 50.0
PT claw 1 (ext.) base height	20.87 ± 2.83 (14.8-28.6) [35] * 13.57 / 63.99 • 23.9
PT claw 1 (ext.) main branch length	34.49 ± 3.39 (27.8-43.2) [43] * 9.82 / 63.41 • 37.8
PT claw 1 (int.) length	30.49 ± 1.80 (26.3-34.9) [22] * 5.90 / 82.79 • 31.3
PT claw 1 (int.) base height	15.94 ± 2.43 (11.1-20.5) [20] * 15.29 / 66.97 • 17.5
PT claw 1 (int.) main branch length	22.67 ± 3.46 (17.8-27.5) [13] * 15.25 / 36.73 • 25.0
PT hind claw length	55.87 ± 4.14 (47.7-62.5) [17] * 7.40 / 40.58 • 60.0
PT hind claw base height	25.07 ± 4.27 (16.7-35.3) [22] * 17.05 / 66.57 • 28.9
PT hind claw main branch length	41.78 ± 4.15 (33.3-47.5) [34] * 9.94 / 70.67 • 42.5
PT fore claw length	32.68 ± 4.05 (25.0-43.4) [17] * 12.40 / 51.85 • 37.5
PT fore claw base height	15.65 ± 3.13 (9.7-26.19) [23] * 20.03 / 43.11 • 16.7
PT fore claw main branch length	22.41 ± 3.17 (14.3-27.5) [23] * 14.16 / 33.17 • 26.4
Macroplacoid index (*MPLI*)	66.11 ± 7.83 (50.0-85.7) [48] * 11.84 / 81.57 • 59.1
External claws index (*ECI*)	82.72 ± 8.37 (70.0-95.2) [9] * 10.11 / 43.77 • 83.3
Internal claws index (*ICI*)	88.38 ± 7.95 (70.6-94.1) [8] * 8.99 / 69.55 • 83.3
Claw main branch index (*MBI*)	82.70 ± 5.87 (71.8-93.4) [34] * 7.10 / 79.77 • 82.2
Hind claw base index (*HCBI*)	60.33 ± 10.35 (45.5-94.1) [22] * 17.15 / 55.05 • 68.0

Eggs: not known.

V a r i a b i l i t y. – Specimens of *H. thaleri* sp. nov. show little intra-specific variability, compared to those of *H. klebelsbergi*. The variability is mainly found in the shape of the anterior mouth tube apophyses (particularly the length of their caudal processes), the curvature of the mouth tube, the shape and length of macroplacoids and in the curvature of distal units of the main claw branches. Furthermore, several individuals have aberrantly developed macroplacoids (*e.g.*, Figs 17, 24, 25, arrowhead).

D i f f e r e n t i a l   d i a g n o s i s. – Three tardigrade species, *H. klebelsbergi*, *H. janetscheki*, and *H. thaleri* sp. nov., hitherto reported exclusively from the glacier habitats, are characterized by several features, combinations of which clearly separate these ice-dwellers from other water-bears. These features are the peculiar shape of the anterior mouth tube apophyses (resembling the "sharp-hook"-type described recently for the genus *Acutuncus*: see PILATO & BINDA 1997), the intermediate shape of claws between *Isohypsibius*- and *Hypsibius*-types (see DASTYCH *et al.* 2003), the large eyes and distinctly pigmented epidermal layer. These characters also indicate a separate supra-specific taxonomic status for the species-complex (= "*H. klebelsbergi*-group") within the family Hypsibiidae.

Hypsibius klebelsbergi and *H. janetscheki* are characterized by intense brown-blackish or dark-brown body pigmentation, a feature which easily separates the taxa from the distinctly paler *H. thaleri* (However, one specimen of the new species – a *simplex*-state? – was also intensely dark pigmented). *H. thaleri* also clearly differs from these two species by: 1). markedly larger and more slender claws (*e.g.*, Figs 1, 2, 26-39 vs 3, 4; comp. also DASTYCH *et al.* 2003: Figs 46-54 and DASTYCH 2004: Figs 11-23); 2). the shape of the anterior mouth tube apophyses, with more concave profile and their caudal processes which are on average longer and more spine-like as those of *H. janetscheki* (or *H. klebelsbergi*); 3). the first macroplacoid in *H. thaleri* is distinctly less (if at all) incised in its middle and the second macroplacoid is usually without a caudal external incision, compared to those of *H. klebelsbergi* and *H. janetscheki* (see DASTYCH *et al.* 2003 and DASTYCH 2004 for differences between two latter taxa).

Differences in the claw size trend divides the new species (with *H. klebelsbergi*) from *H. janetscheki*. Claw size, particularly that of the external claws, increases from anterior to posterior in *H. thaleri* and *H. klebelsbergi* (i.e., they are the largest on legs IV), while decreasing in size for *H. janetscheki* (i.e., the largest on leg I: comp Figs 1, 2 and 3 vs 4). This contrasting trend is corroborated by lower average values of the claw indices in *H. thaleri* sp. nov. (and *H. klebelsbergi*) and markedly higher in *H. janetscheki*. They are (data for *H. klebelsbergi* in parenthesis): *ECI* = 82.72 (89.70) vs 132.60, *ICI* = 88.38 vs 104.60 and *MBI* = 82.70 (90.75) vs 140.00 %, respectively). These differences are already easily recognised in the claw size of juvenile forms (comp. Figs 5 and 6; see DASTYCH 2004: Fig 3 for a juvenile form of *H. janetscheki*).

The less intense (lighter) body pigmentation, larger claws, different shape of the anterior mouth apophyses and normally less incised placoids in the new species differentiate it from *H. klebelsbergi* (for other differences compare also the identification key and morphometrics in DASTYCH *et al.* 2003 and the present paper).

Hypsibius thaleri differs from frequently dark pigmented *H. dujardini* DOYÈRE, 1840 (the type species for the genus *Hypsibius* EHRENBERG, 1848) by: 1). the absence of microplacoids, 2). a relatively broader mouth tube, 3). more symmetric shape of the anterior mouth tube apophyses, 4). divergent shape of claws, 5). absence of a small cuticular bar that occurs at the base of external and internal claws IV in *H. dujardini* (see *e.g.*, BERTOLANI 1982: Fig 47 E).

The new species differs from *H. convergens*, for which it was originally misidentified by RAMAZZOTTI (1968), mainly by: 1). the presence of epidermal pigment, 2). the concave profile of the anterior mouth tube apophyses and their longer, spine-like caudal processes (in *H. convergens* the epidermis is transparent, the profile is convex and the processes shorter: see *e.g.*, BINDA & PILATO 1986: Fig. 1D), 3). differently shaped claws. The accessory spines in *H. thaleri* are of the *klebelsbergi*-type, compared to *H. convergens*, which are less robust (i.e., thinner), more spine-like and their posterior unit (the base) is much more continuously elongated and not abruptly interrupted. (However, it should be borne in mind that the status of *H. convergens* represents a serious taxonomic problem and this species-group is in a need of urgent revision).

Biology and distribution. – The reproduction strategy of the new species is unknown. The only available information on the biology of *H. thaleri* sp. nov. and the sympatric *H. janetscheki* is that about their type locality at the Nare Glacier (RAMAZZOTTI 1968, JANETSCHEK 1990; see also this paper, § "Material and methods", "Description of species"). The taxa were then listed as "*Hypsibius (H.) janetscheki* nov. spec., 41 Exp., *Hypsibius* (*H.*) *convergens* (URBANOVITZ [*sic*], 1925) 186 Ex., Kosmopolit..." (JANETSCHEK l.c.: 49). As the sample represented a pooled collection from a number of cryoconite holes located on a glacier surface area about 20 m^2, it is not possible to hypothesize about the level of co-occurrence of these two species in individual cryoconite holes. The scanty information was supplemented by JANETSCHEK (l.c.) with a list of other cryoconite biota encountered in the sample. The author separated the sample fixative from the cryoconite and found in the condensed substrate (its volume 4.8 cm^3) "tardigrades 240, bdelloid rotifers 72, small crustaceans, immat. 27, testate amoebae, at least 2 ssp, n. n., diatoms, n. n...." (from German: l.c.: 49). It was suggested in this reference that this was the first such quantitative data from the cryoconital substrate; it should also be noted that the locality at the Nare Glacier (5570 m) represents one of the highest altitudinal reports for tardigrades.

An individual of *H. convergens* (together with *Diphascon scoticum* MURRAY, 1905) was also reported from a warmer stream in the vicinity of Pangpoche, 3900 m a.s.l. (JANETSCHEK, l.c.). However, as there was no mention of the animal being pigmented, so it can be assumed that it probably did represent a species from the confusing *Hypsibius convergens*-group, not *H. thaleri*.

R e m a r k s. – Tardigrades collected from cryoconite holes on the Nare Glacier in the Himalayas (RAMAZZOTTI 1968) represent two distinct morphotypes, i.e., intensely dark pigmented form with small claws and light pigmented form with longer, medium-sized claws. The first morphotype has been described as *H. janetscheki*, the second one refered to by RAMAZZOTTI (l.c.: see also JANETSCHEK 1990) as *H. convergens*. The latter morphotype is described in the present paper as a new species. Curiously, none of the labelled microslides bears the name *"H. convergens"*, so consequently there are two slides labelled "*H. janetscheki*" with both the type material of the darkly pigmented *H. janetscheki* ("Tipo 178" and "Tipo 180") and the light morphotype of a second species (see § "Description of species"). Such labelling is misleading and suggested that only *H. janetscheki* was present in the material, thus exaggerating the actual number of individuals on the microslides. One slide label ("Tipo 178") also had "Tipi 2" as an additional inscription (in ballpen ink, poorly visible) which presumably should indicate the presence of (two?) dark pigmented individuals mounted alongside light pigmented individuals of the new taxon. This would indicate which species Ramazzotti had recognized as *H. janetscheki* within two taxa had been mounted together on one slide. However, there are three dark pigmented individuals (*H. janetscheki*) and seven light pigmented (*H. thaleri* sp. nov.) specimens on this microslide.

The reasons behind the lack of information on the microslide labels by RAMAZZOTTI is not known. It is possible that this confusion exemplifies some of the general difficulties that have occurred in the past for those trying to distinguishing species within the *H. klebelsbergi*-group (see DASTYCH *et al.* 2003 for the perplexing taxonomic history of that species-complex). Moreover, it should be noted that these two Himalayan taxa were recently considered to be two cyclomorphic life forms of *H. janetscheki*, i.e., a dark pigmented dormant form with a short claws, and a light pigmented, active form with longer claws (DASTYCH 1993). However, in the light of newly available information on glacier tardigrades (DASTYCH *et al.* 2003, DASTYCH 2004), this hypothesis can no longer be supported (see l.c. 2004). Both morphotypes should be considered as two separate "good" species, i.e., *H. janetscheki* and *H. thaleri*.

Identification key for the species of the *Hypsibius klebelsbergi*-complex

The distinction between the three known (morpho)species of the *H. klebelsbergi*-complex can be problematic, particularly when only a limited number of individuals are available. This is due to the considerable morphological variability within these taxa, which produce an overlap of the morphometric ranges of several characters (comp. DASTYCH *et al.* 2003: 86-87, DASTYCH 2004: 189, present paper). Furthermore, the body pigmentation often obscures key features thus preventing the required measurements.

1. Hind claw smaller than the corresponding (external) claw on legs I. Body intensely dark brown. Distribution: the Himalayas . . . . *H. janetscheki*

– Hind claw larger than the corresponding claw on legs I. Body either dark pigmented or pale. Distribution: the Alps, the Himalayas 2

2. Body intensely dark brown, or blackish-brown, claws small and stumpy. *PT* values for hind claw on average *c.* 40 %, the *PT* values for its main branch *c.* 30 %. The second macroplacoid relatively long, the *MPL*-index values on average *c.* 78 %. The anterior mouth apophyses weakly indented (in profile slightly concave), their caudal processes short and stumpy. Distribution: the Alps . . . . *H. klebelsbergi*

– Body pale, partly transparent, light brown, claws large and slender. *PT* values for hind claw on average *c.* 56 %, the *PT* values for its main branch *c.* 42 %. The second macroplacoid short, the *MPL*-index values on average *c.* 65 %. The anterior mouth apophyses distinctly indented, their caudal processes longer, thin and spine-like. Distribution: the Himalayas . . . . *H. thaleri* sp. nov.

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