

Redescription and notes on the biology of the glacier tardigrade *Hypsibius klebelsbergi* MIHELČIČ, 1959 (Tardigrada), based on material from the Ötztal Alps, Austria

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ABSTRACT. – A redescription of a cryobiotic tardigrade, *Hypsibius klebelsbergi* MIHELČIČ, 1959, is presented, based on material from cryoconite holes on the glacier Rotmoosferner in the Ötztal Alps (Austria). Much of the basic morphometric data of *H. klebelsbergi* is provided here for the first time and the bulk of available biological and ecological information about the species and its distribution is evaluated and discussed. The combination of some characters of *H. klebelsbergi* (e.g., the shape of anterior apophyses of the mouth tube and of the claws) indicates its separate generic status. A bisexual (amphimictic) reproduction mode for *H. klebelsbergi* is implied. The latter and the taxonomic position of the species, including its possible synonymy with *H. janetscheki* Ramazzotti, 1968, known only from a Himalayan glacier, require further studies.

KEYWORDS: Tardigrada, *Hypsibius klebelsbergi*, redescription, SEM, taxonomy, glaciers, cryoconite holes, cryobiont, ecology, the Alps, Austria.

Introduction

Only a few invertebrate taxa dwell permanently on glaciers, where all available habitats are characterized by harsh environmental conditions. Cryoconite holes (= Kryokonitlöcher, Mittagslöcher), are aquatic microcaverns that occur on the ice surface (Fig. 1) in the glaciers' ablation zone. These holes are caused by dark particles of fine dark inorganic and organic debris warmed by solar radiation and sinking into the ice surface (black body effect). The term for the mainly wind transported debris, cryoconite or "ice dust" (*kryos* = ice, *konis* = dust, Gr.), was introduced by NORDENSKJÖLD in 1870 (see STEINBÖCK 1936). Cryoconite holes occur in polar regions and mountain glaciers worldwide and play an important role in the acceleration of the deglaciation processes (e.g., TAKEUCHI et al. 2001). Still, little is known about physical conditions and chemical components of cryoconite holes and even less about their flora and fauna. Some characteristics of cryoconite holes, including partly their biota, have been provided by, e.g., STEINBÖCK 1936, 1957, AN DER LAN 1963, MIHELČIČ 1963, KRAUS 1977, DE SMET & VAN ROMPU 1994, GRØNGAARD et al. 1999, TAKEUCHI et al. 2001, SÄWSTRÖM et al. 2002.



Fig. 1. Cryoconite holes on the Rotmoosferner glacier (the Ötztal Alps, July 2002: the largest holes are up to a dozen or so cm in diameter); inset: inhabitant of the holes, *Hypsibius klebelsbergi* MIHELČIČ, dorsal view (BOUIN'S fixed, scale bar: 75 μ m).

Protozoans, rotifers and tardigrades are the most frequently reported invertebrates from these small and cold waterbodies, their species spectrum and abundance varying with the particular geographic region. Tardigrades are an important component of these small insular ecosystems on the ice surface, from which at least 12 species have been reported, with two being found exclusive to this habitat (see list on p. 97). *Hypsibius klebelsbergi* MIHELČIČ, 1959, one of the species, was the first tardigrade to be described (and named) from cryoconite holes. While the identity of this animal is beyond doubts (STEINBÖCK 1957, MIHELČIČ 1959), the species still remains enigmatic due to the unsatisfactory original description, and the incomplete redescription that was based on a recently re-discovered small but badly preserved type-series (DASTYCH 1993). A comprehensive thesis by KRAUS (1977) had reminded unpublished.

The present study is based on an ample material of *H. klebelsbergi* from its *terra typica* (Ötztal Alps, Austria), which has added previously unknown information on its morphology, and provides some observations on the biology of this elusive, cold-loving tardigrade.

Material and methods

The material for this study originates from the Rotmoosferner glacier (46°52'N, 11°02'E) near Obergurgl in the Ötztal Alps (Nordtirol, the Austrian Central Alps). A short description of the periglacial zone and glacier foreland can be found in KAUFMANN (2001). In total, 62 qualitative samples were collected from the glacier surface, with a limited number from habitats at its margin. The following two localities were sampled:

(A). Orographically the right side of the glacier, at the base of the northerly exposed crest of Mt. Kirchenkogel. Ablation zone at 2715-2800 m a.s.l., 15 July 2002, coll. H. DASTYCH & K. THALER:

- 1) bottom sediment from cryoconite holes, with a few samples from small grooves on the ice surface (44 samples: more than 4500 tardigrades, including 15 eggs of *Hypsibius*, the latter found outside the exuviae),
- 2) a blackish sediment (cryoconite) from and with glacier's firn, near a lateral moraine (one sample, c. 100 ml: 257 tardigrades, three eggs of *Hypsibius* outside the exuviae),
- 3) similar sediment frozen in a piece of ice (one sample, c. 50 ml: 76 tardigrades),
- 4) a blackish, wet and strongly macerated tuft of a phanerogam on the surface of the ice (one sample, c. 150 ml: 28 tardigrades, two exuviae with nine and seven eggs of *Hypsibius*, respectively; 37 empty, ornamented and partly macerated egg shells of *Macrobotus*),
- 5) fine, wet gravel and sand on a slope of the lateral moraine, at the edge of ice (one sample, c. 50 ml: 14 tardigrades),
- 6) silt from lateral moraine, at the edge of ice (one sample, c. 50 ml: no tardigrades).

(B). In close vicinity to locality "A", but at slightly lower altitude. Wet cryoconite sediment from the ice surface, 2500 m a.s.l., 18 August 2001, coll. K. THALER (13 samples of cryoconite collected on filter papers: 28 specimens in two samples).

The sediment from cryoconite holes and grooves was taken up with a pipette (diameter 1 cm) into a measuring cylinder (of 4 cm diameter, 300 ml capacity) until full. The cryoconite holes were up to 15 cm in diameter and deep, but were more usually in the range of 2-5 cm. The contents of the cylinder was stirred and poured into three smaller cylinders of 100 ml capacity. After c. 20 minutes most of water from each cylinder was carefully decanted, the reminding water and sediment (c. 30 ml) was poured into small (30 ml) polyethylene tubes which were closed for transportation. Altogether 44 pooled samples were collected, each with material from several cryoconite holes or grooves.

Initial processing and fixation of material took place at the Alpine Forschungsstelle Obergurgl of the University of Innsbruck. With the exception of ten samples selected for drying, all the other samples from cryoconite holes were reduced with a small pipette in volume up to 2-3 ml. The discarded water was checked for the presence of tardigrades under stereo microscope. Before fixation, the sediment in each sample was briefly examined for the presence of living water bears. Gravel, silt and macerated plant remains were initially processed by the method described by DASTYCH (1985). All reduced samples were transferred with a pipette to smaller (10 ml) polyethylene tubes and fixed either in 70 % ethanol or hot (*c.* 60-70 °C) BOUIN'S medium (17 and 17 samples, respectively).

The contents of each cryoconite sediment sample selected for drying (ten samples, *c.* 30 ml) was stirred, poured onto a filter paper in a funnel and allowed to drain. The filter papers with sediment were placed onto a blotting paper to remove further water (the latter changed 2-3 times) and dried at room temperatures for several hours. Tardigrades were extracted from the dried cryoconite by a method described for bryophyte samples in DASTYCH (1985), and these specimens were used for most of the morphological and cytological observations in *LM*. Unfortunately, using this method caused that all the tardigrades extracted were dead, although several rotifers returned to active life. These tardigrades were mostly badly shrunken, with many strongly adhered to organic or mineral particles, whilst numerous others were so damaged that only a part of them was suitable for further examination. Moreover, markedly less specimens were observed in this dried material (739 individuals counted, including those qualified for preparation), compared to BOUIN'S or ethanol fixed samples, suggesting that the technique used to dry the samples was too fast and/or used too high a temperature, thus causing less than optimal results.

Selected specimens from the dried samples were fixed in a mixture of methanol and acetic acid (3:1) and stained with aceto-lactic orcein (e.g., BERTOLANI 1971). A squash technique was employed for detailed observations, as the unusual dark pigmentation of the tardigrades makes standard light microscope examination of internal organs virtually impossible (see e.g., Figs 1: inset, 4, 6). Claws were similarly affected by the extremely dark-pigmented layer of epidermis and were poorly visible, if at all, when viewed against such a background. Specimens fixed in BOUIN'S medium or ethanol were useless for squashed preparations. A part of the whole material was mounted permanently on microslides either in gum chloral media (FAURE'S, HOYER'S, SWAN'S) or polyvinyl-lactophenol. Some specimens were treated with 10% KOH, washed in distilled water and mounted in SWAN'S medium.

For SEM examination numerous specimens were transferred from BOUIN'S medium into 70% ethanol (or selected from those fixed in 70 % ethanol), subsequently dehydrated, critical-point-dried and carbon-coated. For histological observations several specimens were transferred from BOUIN'S medium into 70% ethanol and dehydrated in a graded ethanol series with propylenoxide as the intermedium. They were then embedded in Epon resin, semi-thin sectioned (longitudinal sections, 1.5 µm thick), stained in 2% Toluidine Blue (pH 12), the sections then mounted on slides in Entellan medium (MERCK). The tardigrades were examined with phase- or differential interference contrast microscopy and scanning electron microscopy. Photomicrographs were taken with ZEISS 'Photomikroskop III', SEM micrographs with LEO 1525.

For the presentation of morphometric data several indices have been employed: "*pt* index" describes the ratio between the length of the buccal tube and that of other structures taken into consideration (PILATO 1981); "*mpl* index", introduced here, characterizes the size ratio between the second and first macropiloids ($= \text{mpl}_2 \times 100 / \text{mpl}_1$). The coefficient of variability (*V*) is defined as 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^2 range between 0.0 and 1.0). All these indices are presented in %.

Permanent microslide preparations (33, with 296 individuals, 31 eggs) and two pooled samples with *H. klebelsbergi* have been deposited at the Zoologisches Museum Hamburg (ZMH Acc. No A8/03). The latter include 13 sub-samples from cryoconite holes preserved in 70 % ethanol (with the tardigrades only partly extracted and/or counted). The samples were either fixed in BOUIN'S medium (7) and transferred to ethanol or originally preserved in ethanol (6). Furthermore, microslides with several specimens each have been deposited in the Naturhistorisches Museum (Wien), the Università di Modena (BERTOLANI'S Collection), the Università di Catania (PILATO'S Collection) and in the Zoologisk Museum (Copenhagen).

The following abbreviations are used in text or illustrations: *alo* - aceto-lactic orcein, *ar* - artefact, *Bf* - BOUIN'S fixative, *cg* - lobe of cerebral ganglion, *DIC* - differential interference contrast, *ep* - epidermal pigment, *fu* - furca, *LM* - light microscope, *min-max* - minimum-maximum values, *mpl* - macroplicoid, *mo* - mouth opening, *mt* - mouth tube, *mu* - mucrones, *n* - sample size, *ph* - pharynx, *PHC* - phase contrast, *pt* - length ratio, *r²* - coefficient of determination, *SD* - standard deviation, *SEM* - scanning electron microscope, *ss* - stylet support, *TEM* - transmission electron microscope, *V* - coefficient of variation, \bar{x} - (arithmetic) mean.

Historical summary

The first information about tardigrades in Alpine cryoconite holes is provided by STEINBÖCK (1957), one of the pioneers of high-mountain and Arctic invertebrate zoology, who found them in large numbers on the glacier Sulztalerferner in the Stubai Alps, Austria. STEINBÖCK (1957) recorded that species also from the Ötztal Alps (the glaciers Niederjochferner and Marzellferner) and described the colour of the animals as "blackish". He introduced tardigrades in his paper with an example of a *Macrobotus* sp., with a figure cited from the paper by RICHTERS (1926) (see STEINBÖCK *l.c.*: Fig. 2), but did not specify which genus had been found on the Alpine glaciers.

MIHELČIĆ (1959), who received tardigrades from STEINBÖCK, described them as a new species, *Hypsibius klebelsbergi*. The original description is short, in some aspects not precise and even misleading. The author partly supplemented the description in a second paper (*l.c.* 1963) and also in a letter to RAMAZZOTTI, who published MIHELČIĆ'S remarks in his tardigrade monographs (*l.c.* 1972, RAMAZZOTTI & MAUCCI 1983).

RAMAZZOTTI (1968) described a new tardigrade from Himalayan cryoconite holes as *Hypsibius janetscheki*, and compared the species with *H. klebelsbergi*.

KRAUS (1977) devoted his Ph.D. thesis to the morphology and biology of *H. klebelsbergi*, producing a comprehensive and very important contribution to the knowledge on this species. Unfortunately, the work remained unpublished.

DASTYCH'S (1993) redescription of *H. klebelsbergi* was based on four greatly deteriorated type specimens. This was limited by the quantity and quality of the surviving material, and did not include the work by KRAUS (*l.c.*), as it was then unknown to the author.

GRØNGAARD et al. (1992) reported *H. klebelsbergi* from cryoconite holes at the Lyngmarksbraen glacier, Disco Island, Greenland. Later, R. M. KRISTENSEN informed us (pers. comm. to H.D., 1997) about the misidentification of individual(s?) from the *Hypsibius dujardini*-group as *H. klebelsbergi*. That misidentification was also mentioned in a subsequent paper by GRØNGAARD et al. (1999), and was cited as an unidentified *Hypsibius* species.

Complementary description

Hypsibius klebelsbergi MIHELČIĆ, 1959
(Figs 1-65)

Hypsibius (H.) klebelsbergi MIHELČIĆ, 1959: 259-261, Figs 4a-c; MIHELČIĆ 1962.

Hypsibius (Iso-) klebelsbergi; - MIHELČIĆ 1963

Hypsibius (I.) klebelsbergi; - MIHELČIĆ 1963

Hypsibius klebesbergi (sic). - RAMAZZOTTI 1967, 1978

Hypsibius (Hypsibius) klebelsbergi; - RAMAZZOTTI 1968, 1972; KRAUS 1977; RAMAZZOTTI & MAUCCI 1983

Hypsibius klebelsbergi; - GRØNGAARD et al. 1992, 1999; DASTYCH 1993; MCINNES 1994; THALER 1999

Type material. – Lectotype and two paralectotypes in the Tiroler Landesmuseum Ferdinandeum (Innsbruck), one paralectotype (examined here) in the Zoologisches Museum Hamburg (ZMH Acc. No. A32/92). The type specimens had not been designated in the original description. Original labels on microslide with type series read: “*H. klebelsbergi* sp. n.” and “Öztaler Alpen / leg. STEINBÖCK” (see DASTYCH 1993).

Type locality. – Originally described only as: “Kryokonitlöcher der Gletscher im Tirolergebirge”: MIHELČIČ 1959, p. 259 (= cryoconite holes on the glaciers in the mountains of Tirol). Later MIHELČIČ (1963: p. 46) restricted the type locality to “Öztaler Alpen”. As he received the specimens from STEINBÖCK (MIHELČIČ 1959: p. 254), the “Niederjoch- und Marzellferner”, i.e., two adjacent glaciers in the Ventertal (= Vent Valley), represent the exact type locality (see STEINBÖCK 1957: p. 67 and footnote, p. 68). [Prof. JANETSCHKE made a note in his reprint of the paper by MIHELČIČ 1959: “l. cl. Marzellferner zwischen 2650-2780 m, leg. STEINBÖCK, 10. Sept. 1956, aus Kryokonit” (the abbreviation “l. cl.” means “*locus classicus*”, for the type locality)].

Etymology. – Dr. FRANZ MIHELČIČ named the species after the Austrian geologist, Prof. RAIMUND VON KLEBELSBERG, on a request of the species collector, Prof. OTTO STEINBÖCK.

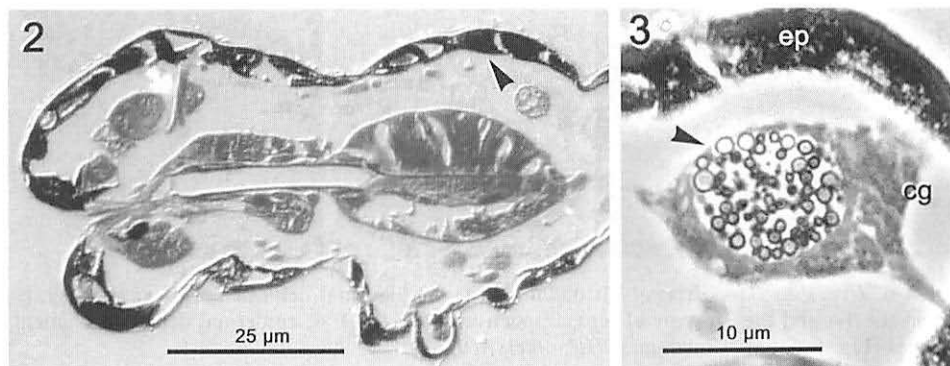
Diagnosis. – Small to large, highly pigmented, dark-brown or blackish hypsibiids. Eyes large, cuticle with tiny wrinkles. The mouth tube anterior apophyses resembling the “sharp hook”-type, pharynx with two macroplacoids, no microplacoid. Claws small, somewhat modified and intermediate between *Isohypsibius*- and *Hypsibius*-type, but more closer to the latter type. Smooth eggs found both within and outside the exuviae. A glacier dweller.

Material examined. – 658 specimens and 31 eggs from the glacier Rotmoosferner in the Öztal Alps (for details see ‘Material and methods’).

Description. – Small sized to large animals, 162-532 μm long (in the original description: “rund 600 μm ”, MIHELČIČ 1959; 313-640 μm in type-series ($n = 4$), DASTYCH 1993; 100-115 μm ($n = ?$), 140-560 μm ($n = 617$), KRAUS (*l.c.*).

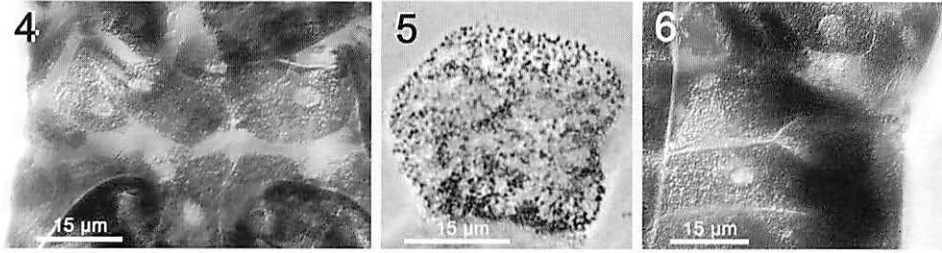
Body mostly elongated, usually with well marked head segment (Fig. 9, 10); the latter with usually well discernible prominent peribuccal ring on terminally located mouth cone (Figs 1: inset, 9, 13, 14). Elliptical organs on the head absent. Legs median sized, claws small. Hind legs usually directed down at right angle to long axis of the body or slightly tucked up. Dorsal side of hind legs markedly convex, with a distinct bulge (Fig. 12, arrowhead); lateral sides of the legs often with a distinct external curvature at their basal part (Figs 9, 20, arrows; 19, arrowhead). The dorsal bulging of the legs supposedly represents “a large hump” (“großer Buckel”), already noted by MIHELČIČ (1959: however, see comment in RAMAZZOTTI 1972). Cloacal opening an elongated, transverse slit (Fig. 21), rarely with a poorly marked triangular pattern below (Fig. 20). (Usually the latter is distinct in many other hypsibiid species).

Cuticle transparent, delicately wrinkled all over the body, but without a perceptible pattern (Figs 10, 18, 20) and seemingly smooth at moderate *LM* and *SEM* magnification. No pore-like structures on the cuticle have been observed at the muscles’ attachments to the body wall (Fig. 18).



Figs 2, 3. *Hypsibius klebelsbergi* MIHELČIČ. – 2, anterior part of the body with epidermal pigment; 3, eye dot (longitudinal sections, *DIC* and *PHC*, respectively; explanations in text).

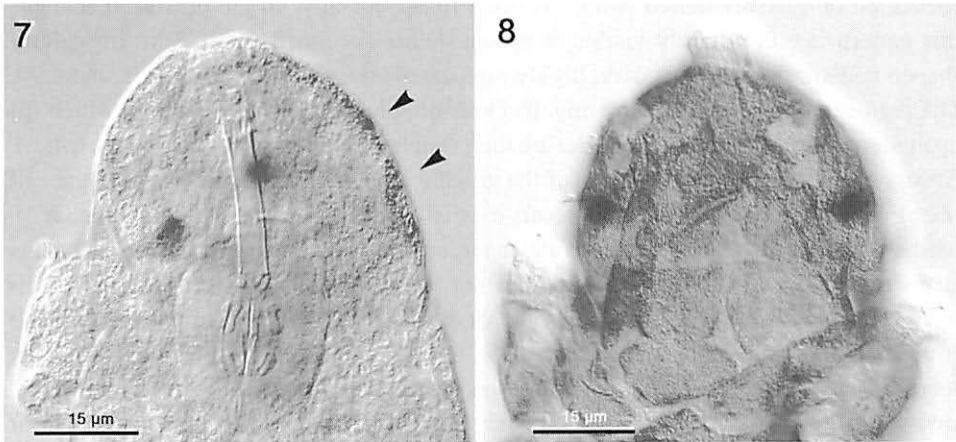
Newly hatched individuals almost hyaline and slightly whitish, its epidermal cells without pigment. Subsequent juveniles with increasing epidermal pigmentation, which appears at first in the head region (Fig. 7, arrowheads). Juveniles and adults highly pigmented, dark-brown, blackish-brown (Fig. 1: inset) or almost black and often with a slight metallic glimmer when lit directly. Internal organs are almost always hidden because of the pigmentation (Figs 1: inset, 4, 6), the latter with variable pattern. Some specimens dorsally with traces of two longitudinal, lighter pigmented stripes along the location of two main muscle cords. Similar, but transverse stripes occur frequently at the pseudosegmental borders, where pigmentation is weaker. As a result, a somewhat checkered or variably dotted pattern is often found dorsally. In the darkest specimens this pattern is either hardly visible or absent. Other specimens with light, irregularly shaped and sized spots on a dark, highly pigmented background (e.g., Figs 1: inset, 4). The pigmentation is produced by tiny, blackish-brown granules (of melanin?) which fill up epidermal cells (Fig. 5); the nuclei are then visible as lighter dots (Figs 1: inset, 4, 6). KRAUS provided *TEM* micrographs of the granules and their distribution within the cell (*l.c.*: Figs 15, 16, 18). The pigment occurs only in epidermal cells (Figs 2, arrowhead; 3), which form a relatively thick layer under the cuticle (Figs 2, 3). The function of this characteristic and strong pigmentation has not been studied. As in other glacier dwelling species, an enchytraeid (e.g., GOODMAN & PARRISH 1971, SHAIN et al. 2000) and collembolan (e.g., EISENBEIS & MEIER 1999), this intensive epidermal pigmentation may serve as protection against high radiation on the glacier surface. Storage cells in body cavity whitish, body fluid transparent. Midgut contents of many specimens grayish or grayish-yellow, with some light orange parts or similarly coloured small crystal-like structures. Eye-spots large, composed of numerous blackish-brown granules, which are distinctly larger than those of epidermal cells (Fig. 3, arrowhead). Eyes only easily recognizable in the first instars or very young animals (Figs 7, 8), having a 5 µm diameter in a specimen 220 µm long; in older individuals the eyes are mostly hidden by the epidermal pigmentation.



Figs 4-6. *Hypsibius klebelsbergi* MIHELČIĆ. – 4, 6, epidermal cells in dorsal view, slightly behind the 1st and the 3rd pair of legs, respectively (*Bf*, *DIC*); 5, epidermal cell with pigment granules (Fig. 5: SWAN'S medium, *PHC*; others: *DIC*).

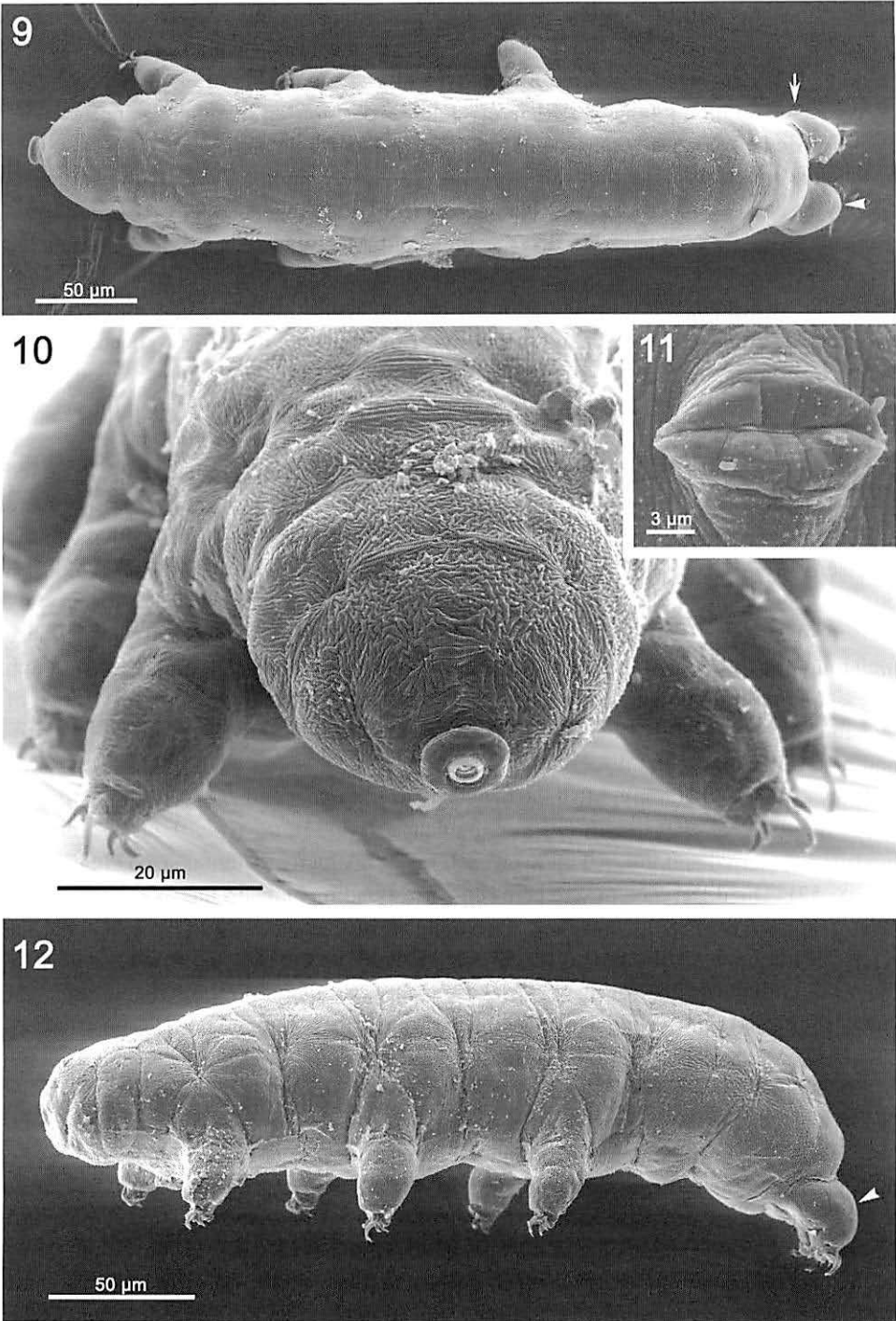
Bucco-pharyngeal apparatus well formed, large, *c.* 30-35 % of the body length in the smallest specimens, *c.* 20 % in adults. Mouth opening surrounded by a large peribuccal ring (Figs 9, 10, 15), located terminally on a distinct cone (Fig. 13). The ring is usually more or less smooth or, exceptionally, with (six) hardly discernible remnants of peribuccal lobes (Figs 13; 14, asterisk). The ring has a collapsed appearance in animals in the *simplex* state (Fig. 11). No other external peribuccal structures (e.g., peribuccal papillae) have been observed. Mouth cavity median sized, with a posterior ring of tiny teeth (= mucrones), discernible in *LM* only in some largest individuals. The teeth are well visible when examined with *SEM* (Fig. 16).

Mouth tube relatively short, wide and only slightly bent; in lateral view uniformly wide (Figs 22, 25, 32), but often slightly narrowed anteriorly when seen dorsally. The tube has distinct, almost symmetrical anterior apophyses (Figs 22, 24, 34, arrowheads).



Figs 7, 8. *Hypsibius klebelsbergi* MIHELČIĆ. – The body pigmentation in a very young (Fig. 7) and slightly older (Fig. 8) juvenile, respectively (*DIC*, *Bf*; SWAN'S medium, respectively).

The dorsal apophysis slightly larger and more rounded than the ventral; both terminated caudally with a tiny but distinct spine-like process. The processes are directed backwards and bear a resemblance to the barbs of an arrowhead (Fig. 34). The apophyses are distinctly flattened, slightly concave (Figs 23, 34) and in their anterior parts evenly fused with the cavity wall of the mouth. Behind the dorsal apophysis, for *c.* 1/3 of the length



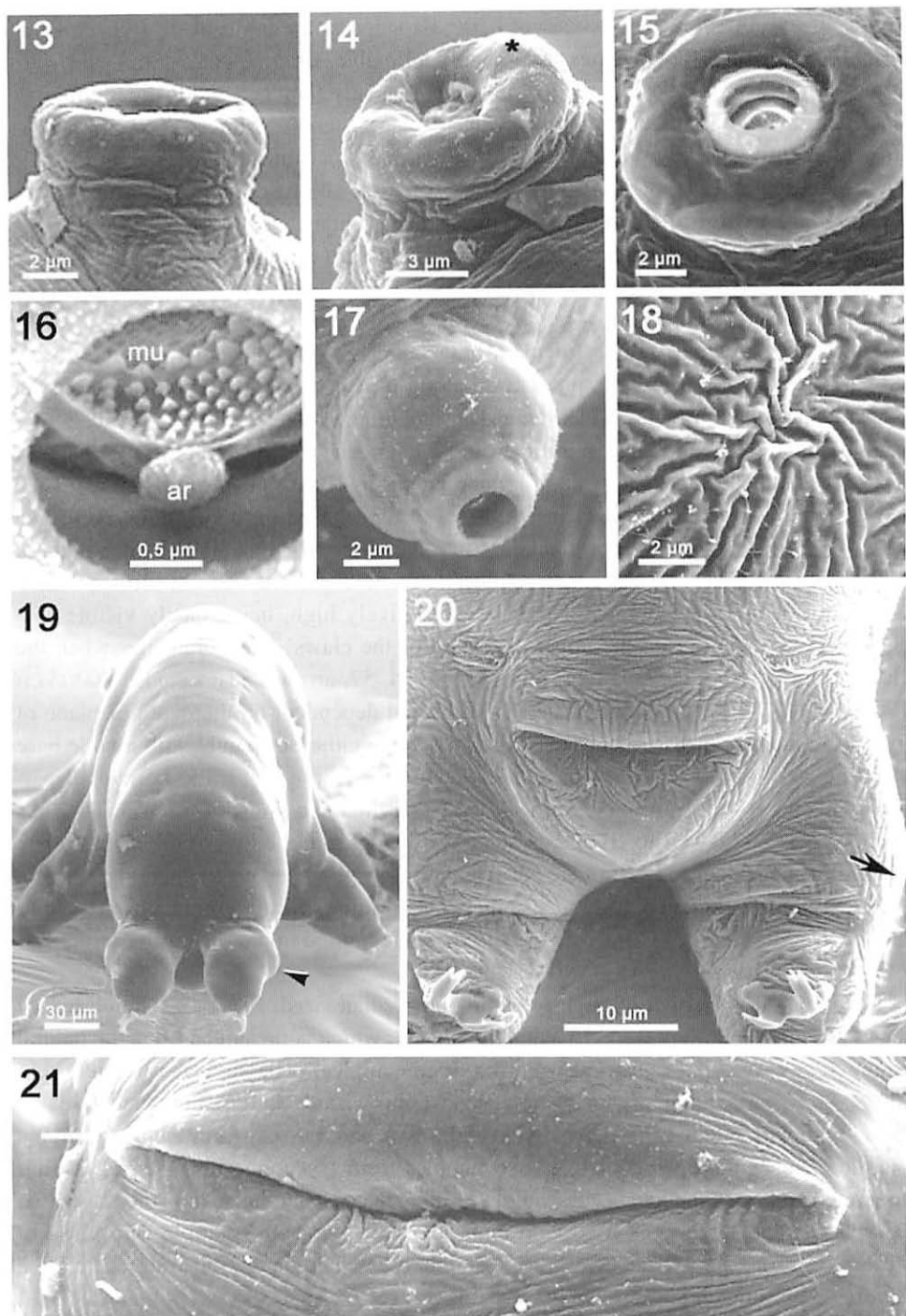
Figs 9-12. *Hypsibius klebelsbergi* MIHELČIČ. – Animal in dorsal (Fig. 9), lateral (Fig. 12), and frontal (Fig. 10) view; 11, closed (collapsed) mouth opening in a *simplex*-state (explanations in text).

of the tube, the wall of the tube is dorsally thicker than on the ventral side. Terminal posterior apophysis of the mouth tube absent, as the tube's wall is posteriorly unthickened. Stylets relatively large, furca "typically" formed (Fig. 28: *fu*). Stylets' supports located on mouth tube in a relatively anterior position, so that their pt index values ($= \frac{pt}{ss}$) are between 60-70 % (see morphometric data for details).

Pharynx relatively large, sub-spherical (Fig. 22), rarely slightly ovoid, with pharyngeal apophyses and two macroplacoids. No microplacoid. Pharyngeal apophyses small, roundish and distinctly remoted from first macroplacoid. Macroplacoids strikingly variable in shape and size (Figs 22, 25-33, 35-37), often within the same pair of placoids (e.g., Figs 30, 35, arrowheads). It is quite common for macroplacoids to differ even within the two halves (Fig. 26, arrowhead). This variability of the placoids has already been noted in the type-series (DASTYCH 1993: Fig. 8). Macroplacoids rod-shaped, mostly moderately long and broad (Figs 22, 25, 31), the first one mostly about 1/3 longer than the second. Often, however, they are thin and long (Figs 28, 33, 37), similar to those in the original illustration by MIHELČIČ (1959: Fig. 4a). Usually macroplacoids are markedly separated from each other (e.g., Figs 25, 31, 36). The first macroplacoid often greatly narrowed and pointed at both ends, while second is then pointed anteriorly, as already found in the type-series (DASTYCH 1993: Fig. 8). Frequently both macroplacoids are connected by a thin, elongated cuticular strip (Fig. 36, arrowhead). In general, macroplacoids are strongly incised, the first at its middle, the second posteriorly (Figs 25, 35). However, these incisions are frequently indistinct, particularly in the second macroplacoid. In such cases, mainly on external side of each placoid, a small thickening occurs in a place of incision (Fig. 28, 37, arrowheads). In rare cases the incisions and thickening on the second macroplacoid are not discernible.

Claws small and stumpy, of shape between *Isohypsibius*- and *Hypsibius*-type, particularly the external claws, with more resemblance to *Hypsibius*-type, though somewhat modified. External and internal claw on each leg of distinctly different size (Figs 38, 43, 46, 48, 57). The difference in size between corresponding claw on the first and the hind pair of leg is very small, however, the youngest instars have these claws almost or, rarely, of the same length. In two specimens the claws on the first leg were even slightly longer than those on the fourth leg. Main branches, compared to the remaining part of claws short, and with peculiarly shaped accessory spines. Lunules absent, no cuticular bars between the claws and their bases.

Claw bases fairly narrow, the claws moderately constricted just above the base (Figs 38, 44). Secondary branches relatively large, wide at the base but thin (Figs 38, 41), reinforced by fold-like thickening along the upper edges (Figs 38, 40, 44). A transverse bar-like thickening in the middle of claw base (Figs 44; 49: arrowhead, 52, 58, 59), often hardly discernible, provides additional strengthening of the claw. The upper (anterior) edge of the secondary branch and the lateral side of the claw's base of the external and internal claw form a more or less distinct right-angle (Figs 38, 41, 43). However, this angle is often unclear, and there may be a curved transition between the two sides, rather than angular, and/or the angle between them is only slightly obtuse (e.g., Figs 49, 51, 53). Main branches also relatively wide and thin (Figs 41, 43, 53, 55), but only short, compared to the claw's basal part. The main and secondary branch of the internal claws are



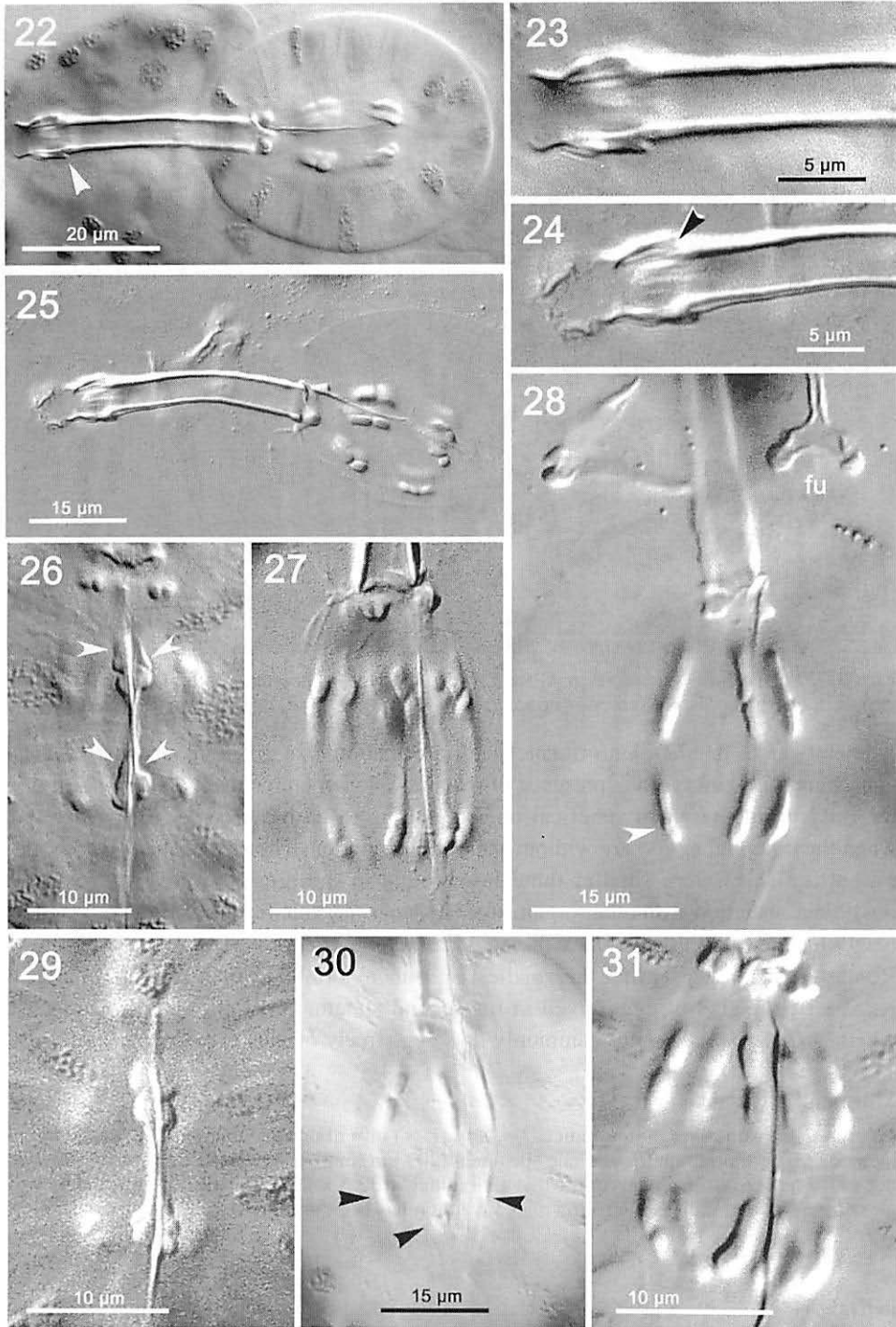
Figs 13-21. *Hypsibius klebelsbergi* MIHELČIČ. – 13-15, mouth cone with mouth opening; 16, teeth (mucrones) in the mouth cavity; 17, protruded anterior region (mouth cavity, stylet sheaths) of the mouth tube; 18, fragment of dorsal cuticle, at the level of muscle attachment; 19, hind part of the body; 20, cloacal region; 21, cloacal slit (explanations in text).

solidly fused, the branches' bases not being flattened at the point of divergence (Figs 41, 42). As a result, the branches are probably inflexible. Branches of external claws are fused differently. The base of the main branch is greatly flattened and distinctly thinner than the remaining part of the branch (Figs 40, 45, asterisk). Moreover, the branch is located slightly laterally on the inner side of the basal part of the claw, i.e., at the side facing the internal claw (Figs 40-42). Thus, a part of the fused unit of both branches occurs slightly below the upper edge of the secondary branch (Figs 40, 42, 51, 56). This distinctly narrowed main branch base and its slight shift in relation to the rest of claw form a hinge between the two branches (Figs 40, 43, 45, asterisk), giving them independent movement (e.g., Figs 42, 44).

Accessory spines (= points) of main branches large and unusually flattened (e.g., Figs 41, 43, 44, 45, 56). The spines form along the anterior (dorsal) part of the main branch in a characteristically wide, somewhat shield-like structure (Figs 39, 45; 50, arrowhead) of unknown functional significance. No comment was made about the shape of the spines, but was illustrated with *SEM* micrographs by KRAUS (1977: Figs 10, 12). They have been described and drawn in DASTYCH (1993), based on poorly preserved type material. MIHELČIĆ'S (1959) original description erroneously noted the absence of these structures. The base of the accessory spines is relatively short, i.e., not located on the whole arc of the main branch. The base is relatively high, being easily visible as a characteristic swelling on the upper curvature of the claws' main branches, when the claw is viewed from the side (e.g., Figs 48, 53, 54, 57, arrowheads; see also DASTYCH 1993: Figs 2, 5). When viewed frontally (*LM*) and dependent on the depth of plane of focus, the main branch and both the spines resemble either a triangle with a wide base standing on its apex (Fig. 56) or a short letter "T". In the latter, the horizontal part represents accessory spines, the vertical one being the main branch (Fig. 47). The tips of the spines on claws I to III are set some way back from the tips of the main branch (Figs 40, 41); on hind legs the tips are much more closely placed to one another (Figs 43, 45). A not dissimilar flattened accessory spines, but forming with main branch a structure termed "paddle-like" by CHANG & RHO 1996, was reported in the freshwater tardigrade *Isohypsiobius granditintinus* CHANG & RHO, 1996.

Eggs are sub-spherical or slightly ovoid and median sized. BOUIN'S fixation turned the eggs yellow but they were off-white in ethanol, which is presumed to be the natural colour (eggs were not extracted from the dried samples). The shell is relatively thin (0.8-1.3 μm) and without ornamentation (Fig. 61, arrowhead). In this study the eggs were found both within exuvium (two exuviae with seven and nine eggs, respectively; from a sample of macerated plant) or free (two samples from cryoconite holes: 12 eggs, four contained embryos, the latter with well discernible bucco-pharyngeal apparatus: Fig. 62, arrowhead) and three eggs (two with embryo) were found in a sample from the firm surface. Eggs not in exuviae (i.e., presumed to be freely laid on the substrate) were often covered with mineral particles and/or detritus (Fig. 60).

Information about the morphology of the egg shell of *H. klebelsbergi* and the method of their deposition has not previously been published. KRAUS (1977) provided two statements in his thesis: a) the absence of eggs in his material (*l.c.*, p. 159) and b) the presence of three eggs, described as "colourless, without processes and of 40 μm in



Figs 22-31. *Hypsibius klebelsbergi* MIHELČIČ. – 22, 25, bucco-pharyngeal apparatus, lateral view; 23, 24, anterior apophyses of the mouth tube, lateral view; 26-31, variability of placoids (all DIC; explanations in text).



Figs 32-37. *Hypsibius klebelsbergi* MIHELČIČ. – 32, buccal apparatus, lateral view; 33, 35-37, variability of placoids; 34, anterior apophyses of the mouth tube, lateral view (all *PHC*).

diameter” (*l.c.*, p. 172). Unfortunately no information was given about whether these eggs were with embryo; the presence of recognisable structures in an embryonate egg is the only proof that such spherical objects belong to tardigrade species, particularly when the potential egg(s) are without shell ornamentation. The size of the eggs reported by KRAUS (*l.c.*) were smaller than those found in the present study, suggesting a possible association with other organisms. However, the rule for predicting the length of newly hatched tardigrades (“3 x the eggs diameter”: HALLAS 1972, DE SMET & VAN ROMPU 1994) suggests that the smallest specimens (100-115 and 140 μm) found by KRAUS fit the size of eggs given in his second statement. At present it is not clear whether *H. klebelsbergi* not commonly lays eggs freely or into exuviae.

Morphometric data

Measurement values are in μm, indices (*pt*, *mpl*, *V*, *r*²) in %. For abbreviations see “Material and methods”. The morphometrics of one specimen (202 μm long) is separated from other data by a dot (●). The size of the pharynx of that individual is 30.6 x 25.2 μm. For the presentation of measurements and indices the following convention has been used:

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

Individuals

A) Measurements:

Body length	349.50 ± 98.58 (162-505) [55] * 28.2 ● 202
Buccal tube length	29.80 ± 3.32 (24.3-36.0) [55] * 11.13 ● 26.1
Stylet supports attachments	19.25 ± 2.01 (15.8-22.5) [55] * 10.46 ● 15.8

Buccal tube width (external)	4.14 ± 0.63 (3.2-5.0) [16] * 15.19 • 3.6
Buccal tube width (internal)	2.94 ± 0.65 (2.3-4.5) [16] * 21.94 • 2.7
Macroplacoid 1 length	5.60 ± 0.50 (5.0-6.6) [15] * 9.2 • 5.4
Macroplacoid 2 length	4.32 ± 0.60 (3.6-5.4) [13] * 13.79 • 4.5
Macroplacoid row length	13.72 ± 2.65 (10.8-18.9) [21] * 19.33 • 11.7
Claw external 1 length	9.75 ± 1.09 (8.1-12.6) [19] * 11.21 • 9.5
Hind claw (4) (= ext.) length	10.96 ± 1.37 (9.5-13.5) [16] * 12.48 • 9.5
Hind claw (4) main branch length	8.43 ± 1.10 (6.3-10.8) [25] * 13.02 • 7.2
Hind law (4) base height	5.25 ± 1.32 (3.6-8.1) [14] * 25.20 • 3.6
Claw internal 4 length	6.96 ± 0.69 (6.3-8.6) [11] * 9.89 • 7.2

B) Indices

<i>Pt</i> stylet supports	64.61 ± 1.93 (60.5-69.7) [55] * 2.99 / 92.6 • 60.5
<i>Pt</i> buccal tube width (ext.)	14.39 ± 1.45 (11.7-16.1) [16] * 10.05 / 27.6 • 13.8
<i>Pt</i> buccal tube width (int.)	10.44 ± 1.33 (8.3-12.2) [16] * 12.77 / 60.6 • 10.3
<i>Pt</i> macroplacoid 1 length	19.91 ± 1.33 (18.2-21.4) [15] * 5.64 / 72.0 • 20.7
<i>Pt</i> macroplacoid 2 length	15.44 ± 1.92 (12.1-17.9) [13] * 12.64 / 26.7 • 17.2
<i>Pt</i> macroplacoid row length	47.96 ± 4.21 (42.0-56.8) [21] * 8.79 / 88.7 • 44.8
<i>Pt</i> claw 1 (ext.) length	34.55 ± 3.18 (29.4-40.7) [19] * 9.20 / 43.8 • 36.4
<i>Pt</i> hind claw (4) length	39.13 ± 2.22 (35.1-43.4) [16] * 5.67 / 79.3 • 36.0
<i>Pt</i> hind claw (4) main branch length	29.12 ± 2.01 (25.6-32.4) [25] * 6.92 / 68.4 • 27.6
<i>Pt</i> hind claw (4) base height	17.66 ± 3.03 (13.8-25.0) [14] * 17.17 / 65.2 • 13.8
<i>Pt</i> internal claw (4) length	25.65 ± 1.61 (23.5-27.6) [11] * 6.26 / 61.5 • 27.6
<i>mpl</i> index	78.24 ± 7.11 (66.7-85.7) [13] * 9.09 / 40.1 • 83,3

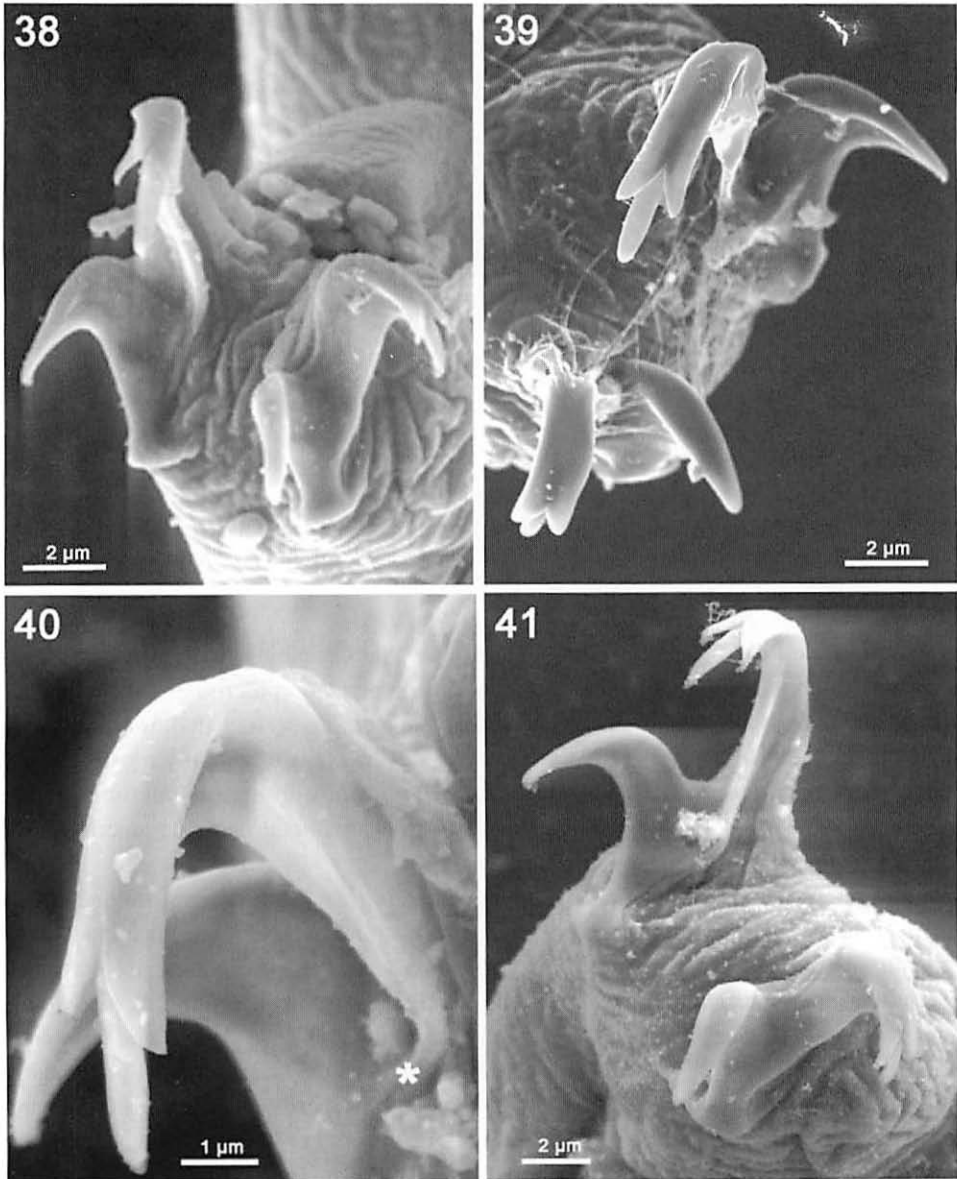
Eggs (*n* = 31)

Eggs found in two exuviae (*n* = 16; seven and nine eggs, respectively, 54-61 x 46-55 µm, all without embryos: from a sample of phanerogam) were smaller than the eggs found free. Eggs presumed to be free laid (*n* = 15) were in three samples, two from cryoconite holes, one from firm; seven eggs with embryo measure 62-90 x 57-86 µm.

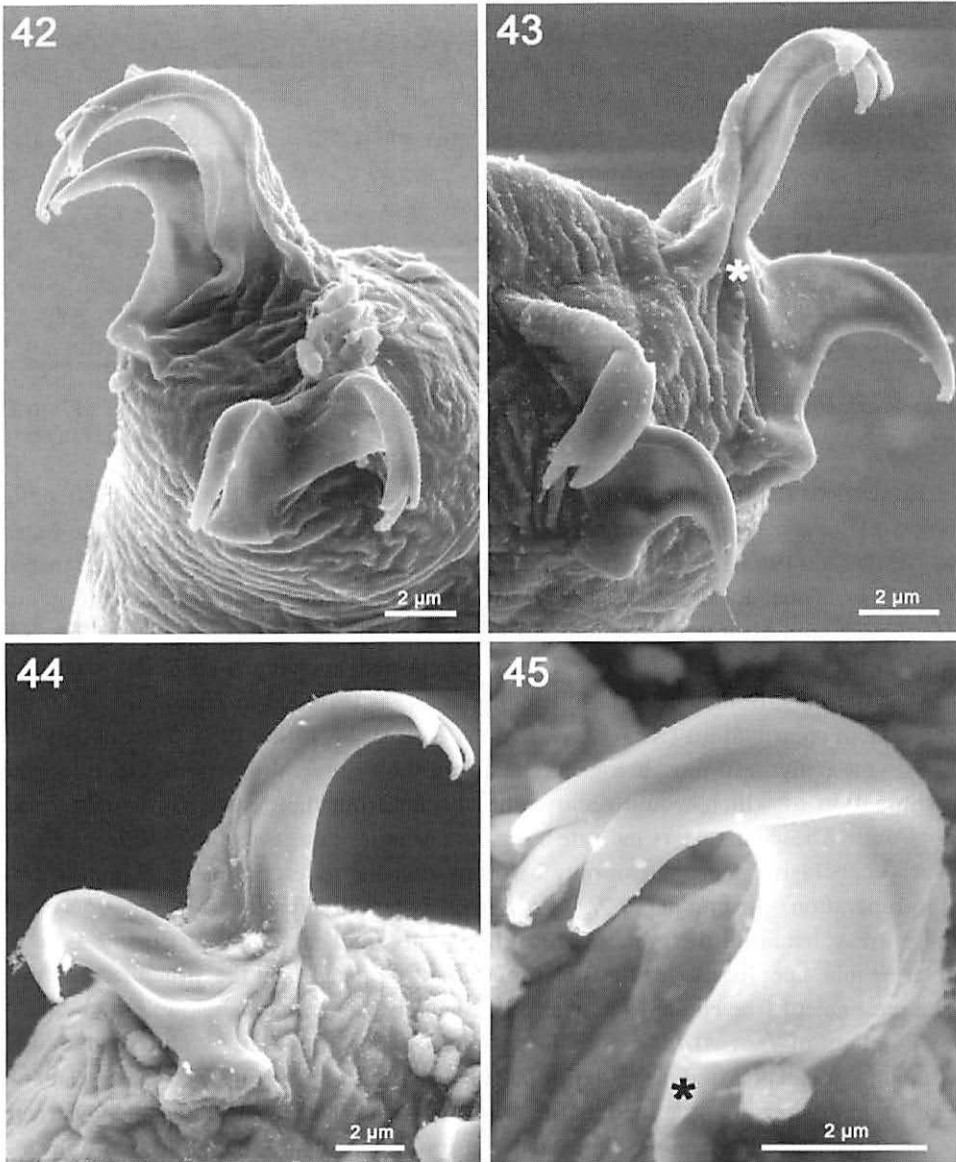
Reproduction strategy. – There is no published information on the reproduction strategy of *H. klebelsbergi*. The highly pigmented epidermis totally conceals gonads, making it impossible in either KRAUS (1977) or the present studies to recognize females by observation of mature oocytes in ovaries, which are normally mostly clearly visible in other tardigrade species. KRAUS (*l.c.*) did not observe copulation among cultured or freshly collected animals, and by default assumed parthenogenetic reproduction a possible strategy for this species.

In order to answer the question of reproductive strategy, 283 orcein-stained specimens were examined with respect to gender. However, no of the animals examined had a gonad with clearly defined mature oocytes or spermatozoa. The gonads in almost all were filled with undifferentiated (germ) cells of variable diameter (2.5-4.5 µm). Only three specimens had cells in the gonad of different size; two individuals with two larger cells (*c.* 10 µm) and in the third two cells of *c.* 25 µm diameter. The larger cells presumably represent immature oocytes. No testes with well defined spermatozoa were found in any of the squashed preparations. However, in four specimens the gonad was caudally divided in two parts, which is interpreted here as two male gonoducts (Fig 63). The testes contained a variable number of spermatids (Figs 63, 65: arrows, 64). [In tardigrades the single testis is always terminated by two gonoducts, an ovary by one (e.g.,

NELSON 1982, BERTOLANI & REBECCHI 1999)]. The two gonoducts indicate the presence of male organs, so that a bisexual (amphimictic) reproduction mode in *H. klebelsbergi* can be assumed. In hermaphroditic species a solitary gonad (ovotestis) is terminated as in females (e.g., REBECCHI *et al.* 2000). Therefore, the above findings in *H. klebelsbergi* suggest the absence of parthenogenesis and hermaphroditism - at least in the population examined here. The total lack of mature oocytes in screened material is strange and needs an explanation which can be obtained by the further study on fresh material.

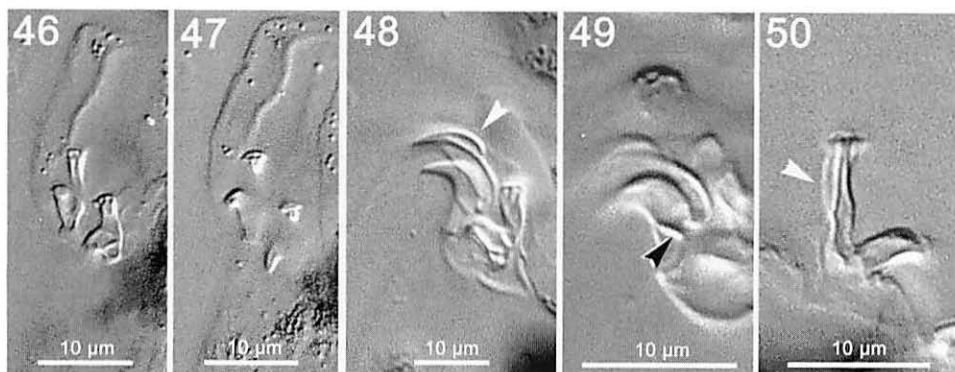


Figs 38-41. *Hypsibius klebelsbergi* MIHELČIĆ. – 38, 39, claws of leg I; 40, external claw of leg II; 41, claws of leg II.



Figs 42-45. *Hypsibius klebelsbergi* MIHELČIČ. – 42, claws of leg III; 43, claws of leg IV; 44, external (= hind) claw IV; 45, main branch of external claw IV.

Taxonomic status. – The generic status of *H. klebelsbergi* became confused when MIHELČIČ (1959, 1963) placed the species in two different genera (at that time subgenera), *Hypsibius* EHRENBERG, 1848 and *Isohypsibius* THULIN, 1928, based on the claw structure. Originally the claws were described as being similar to those of *Hypsibius oberhauseri* (now *Ramazottius o.*), “...but stronger and at the base fused” (*l.c.* 1959) [“kräftiger und am Grunde verwachsen”]. In a later supplement to the description, MIHELČIČ placed the species “...without difficulties, based on claws, into the genus *Hypsibius* EHRENBERG, 1848 and subgenus *Isohypsibius*” (*l.c.*, 1963: from German), but without providing comment or explanation.



Figs 46-50. *Hypsibius klebelsbergi* MIHELČIČ. – Claws of leg IV (46) in a low and high (47) focal plane; 48, claws of leg I; 49, claws of leg II; 50, claws of leg IV (all DIC; explanations in text).

RAMAZZOTTI (1968) described a similar species from the Himalayan cryoconite holes, *H. janetscheki*, and compared it with *H. klebelsbergi*, but did not cite the paper by MIHELČIČ (1963). He characterized the type of the claws for the new taxon as that of *Hypsibius*. Thus, RAMAZZOTTI (1972) listed both species as members of the genus *Hypsibius*, and although he cited MIHELČIČ (1963), did not comment on the *Isohypsibius*-status for *klebelsbergi*. This was reported unchanged in RAMAZZOTTI & MAUCCI (1983). Both species have been reported as included in *Hypsibius* also in all subsequent contributions (KRAUS *l.c.*, DASTYCH 1993, MCINNES 1994, THALER 1999).

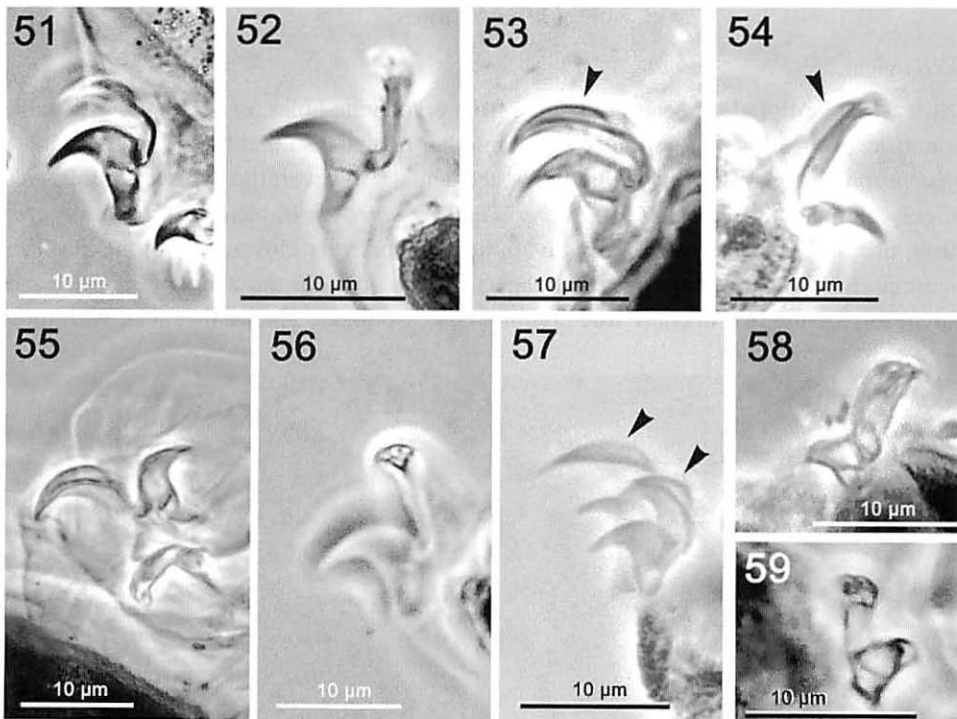
Present analysis of morphological characters shows several unreported features, the combination of which would remove the species from the genera *Isohypsibius* and *Hypsibius*. These characters indicate a separate generic status for *H. klebelsbergi*. However, a conclusive decision can only be taken after the redescription of *H. janetscheki* (in preparation). In this paper *H. klebelsbergi* is tentatively placed in *Hypsibius*, but there are substantial differences from this genus (and *Isohypsibius*) in the type of anterior tube apophyses (for the stylet muscle attachments) and the structure of claws. The presence of free laid eggs may also be a feature (see remarks below).

Members of *Isohypsibius* have the apophyses shaped as flat, symmetrical ridges, without any incision in their posterior part (e.g., THULIN 1928: Figs 23B, PILATO 1974: Fig. 2). In *Hypsibius*, as defined by BINDA & PILATO (1986: Fig. 1D), the apophyses are symmetrical, arc-like, and have a caudal incision which shapes them into the form of a so-called “semilunular hooks” [however, see the different shape in BERTOLANI 1982: Fig. 47 B, for the type species *H. dujardini* (DOYÈRE, 1840)]. In *H. klebelsbergi* the apophyses are asymmetrical, flat (i.e., not arc-like) and their deep caudal incision forms a terminal tooth, similar to the barbs on an arrowhead. This type of apophyses resembles that recently described for *Acutuncus*, a “sharp hook” (PILATO & BINDA 1997).

Defining recognisable claw type (e.g., THULIN 1928; MARCUS 1936: Figs 229, 230) for some eutardigrades is often difficult. The problem occurs particularly in the Hypsibiidae when the form of the claws is intermediate between the so-called *Isohypsibius*- and *Hypsibius*-types, i.e. when the main defining criterion, “arc-like” as against “right-angle” shape for the basal part of claw is obscured. Claws of *H. klebelsbergi* represent this intermediate form, which is closer to that of *Hypsibius*, as indicated by more “arc-like”

shape of the basal claw (when seen in profile), and is further suggested by the frequent occurrence of an “obtuse-angle shape” of the claw base, while this resembles more the “arc-shape” recognized originally as characteristic for *Hypsibius* (THULIN, MARCUS *l.c.*; then however for the type species *Hypsibius oberhaeuseri* (DOYÈRE, 1840); now *H. dujardini*) (comp. e.g., Figs 41, 51, 53 vs. 40, 43, 52). However, these differences have some elements of subjectivity resulting from the orientation of the observed claw base, and the starting point for measuring these angles (compare e.g., MARCUS 1936: Figs 229, 230). Furthermore, the *Hypsibius*-type of claws in *H. klebelsbergi* would in part indicate a different shape of base for the main branches (“solid” vs. “hinge-like”) between external and internal claw and the distinct size difference. The claws of *H. klebelsbergi* have marked individual trait that differs from the *Hypsibius* norm, i.e., a unique shape of strongly flattened accessory spines and a strong curvature of the main branches. The general small size of the claws and their short main branches are also distinctive. (A more detailed discussion on these types of claws is in preparation).

Recent observations of *H. klebelsbergi* have indicated that the eggs are both free laid and laid in exuviae, thus creating questions about the strategy this species uses in egg laying. Other eutardigrades that lay eggs direct on the substrate do not lay smooth shelled eggs, therefore the variation in egg laying seen in *H. klebelsbergi* may represent,



Figs 51-59. *Hypsibius klebelsbergi* MIHELČIĆ. – 51, external (hind) claw of leg IV; 52, external claw of leg IV; 53, external claw of leg IV; 54, external claws of leg I; 55, external claws of leg IV; 56, accessory spines on external claws of leg IV; 57, claws of leg II; 58, internal (anterior) claws of leg IV; 59, internal claw of leg IV (all PHC; Fig. 57: KOH; other explanations in text).

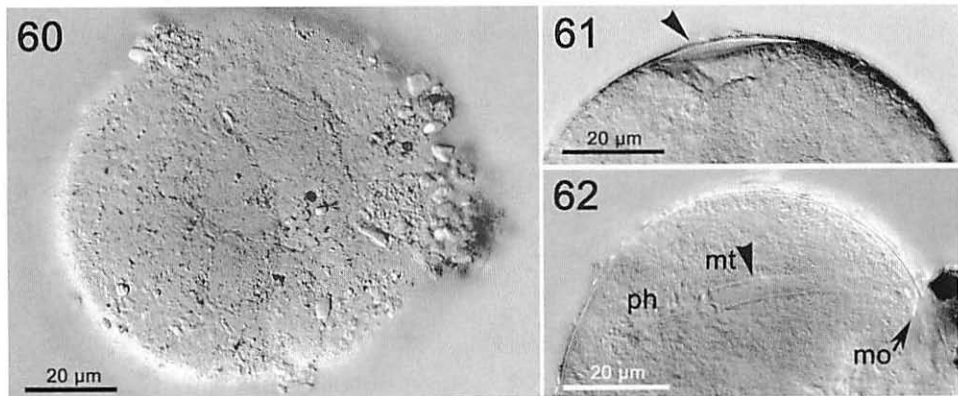
a) behavioural aberrancy, b) an artefact of the processing (e.g., the tearing of the exuviae) or c) exemplifies a real biological peculiarity of the species. *Ramazzottius* Binda & Pilato, 1986, *Hebesuncus* PILATO, 1987 and *Acutuncus* PILATO & BINDA, 1997, are the only genera of the Hypsibiidae to deposit free laid eggs and the shell ornamentations is always species-specific. In other taxa in the family the eggs are laid into exuviae and have always smooth shell. However, occasionally ornamented, normally free-laid eggs, are found in exuviae, e.g., in *Ramazzottius oberhauseri*-group, *Acutuncus antarcticus* (RICHTERS, 1904), *Macrobotus hufelandi*-group (the latter of the Macrobiotidae) and some other taxa. Moreover, *A. antarcticus* has also been reported with shells almost devoid of ornamentation (thus being very similar in the shape and thickness to that of *H. klebelsbergi*), and often covered by mineral and organic particles. The strategy of egg deposition in *H. klebelsbergi* needs further studies, but if the prevailing strategy does prove to be that of free-laid eggs, then it will add weight to the arguments for taxonomic discreteness of this glacier dweller.

It is the authors' opinion that the redescription of *H. klebelsbergi* (and probably *H. janetscheki*) indicates that this species does not belong to either *Isohypsibius* or *Hypsibius* genera. However, further study is required before the exact phylogenetic relationship and generic status of this species can be confirmed.

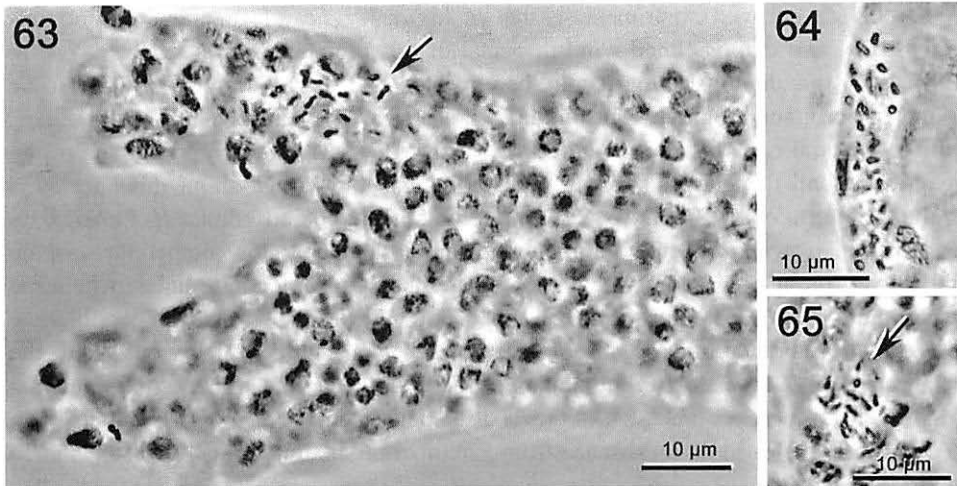
General notes

Ecological

At least 12 tardigrade species are known from cryoconite holes, excluding questionable identifications. Most are normally found in other habitats, so that the presence of these species on glaciers is either facultative or accidental. However, the two very similar and highly pigmented species, *H. klebelsbergi* and *H. janetscheki*, have only been reported from the glacier surface or, very rarely, in habitats that very closely border the glacier's ice margin (the former species, this paper). In our opinion, the available data clearly suggests that the two taxa are true cryobionts, i.e., obligate glacier dwellers, with most



Figs 60-62. *Hypsibius klebelsbergi* MIHELČIČ. – **60**, surface of an egg with mineral and organic particles; **61**, fragment of egg shell; **62**, fragment of egg with buccal apparatus of an embryo (all DIC, Bf).



Figs 63-65. *Hypsibius klebelsbergi* MIHELČIČ. – 63, fragment of testis with germ cells and spermatids (gonoducts partly torn off); 64, 65, spermatids in gonoducts (all *PHC*, *alo*; explanations in text).

occurring in cryoconite holes or similar microhabitats on the ice surface (but see GRØNGAARD et al. 1999). This has already been indicated by STEINBÖCK (1957), MIHELČIČ (1963), KRAUS (1977), DASTYCH (1993) and THALER (1999). However, the hypothesis must be confirmed by further comprehensive faunistic surveys in periglacial zones and generally in other non-glaciated regions in the Alps, the Himalayas, other high mountain ranges and polar regions. Nevertheless, the literature on Alpine tardigrades, although scanty when considering the vast areas covered by mountain ranges, has never mentioned the characteristic and easily recognizable blackish tardigrade *H. klebelsbergi*, in non-glaciated areas (e.g., EHRENBURG 1853, 1854, 1860, HEINIS 1908, MENZEL 1914, FRANZ 1950, FRANZ & MIHELČIČ 1954, VAJ 1956, RAMAZZOTTI 1956, MAUCCI 1974, 1982, MANICARDI & BERTOLANI 1987, ROBOTTI 1972, BINDA 1974, PILATO & REBECCHI 1992). The absence of the species from non-glacial habitats was confirmed recently by KRAUS (*l.c.*: p. 161, 169, 171, 176), who examined limnic and terrestrial habitats in the periglacial area of the Rotmoosferner glacier, as well as glacial cryoconite habitats. In a glacier foreland KRAUS found “only very rarely” (*l.c.*, p. 45) individuals of *H. klebelsbergi* in a flowing meltwater, but provides no further details. Interestingly, in bryophyte- and phanerogam cushions that had only recently arrived on the ice surface, KRAUS (*l.c.*) found four semi-terrestrial tardigrade species, but never *H. klebelsbergi*. Nor was *H. klebelsbergi* found in material from a rocky crest (Liebener Rippe) located very close to the glacier. In the sample of a greatly macerated phanerogam sampled in this study (including roots), it is presumed that the material had been present on the glacier ice for a long time. The presence of live individuals of *H. klebelsbergi*, but only the macerated shells of eggs of *Macrobotus richtersi* Murray, 1911 and a member of *Macrobotus harmsworthi*-group in this sample suggests the macrobiotids dwelled in the tuft before the plant was transported on the glacier surface, but died out before (or during) the period that the macerated plant remains were colonised by *H. klebelsbergi*.

KRAUS (*l.c.*) proved experimentally that *H. klebelsbergi* can form anhydrobiotic tuns (Tönnchen) and demonstrated wind dispersal of this propagule on the glacier (*l.c.*, p. 176). With this demonstration it may be expected that this tardigrade would also be found in periglacial habitats, but this does not appear to occur. Therefore, the distribution potential of *H. klebelsbergi* does not assist the survival and reproduction of the species beyond the glacier habitats. The biological knowledge about this species is limited (STEINBÖCK 1957, KRAUS *l.c.*), but it might be hypothesised that *H. klebelsbergi* has a very narrow temperature optimum range and is adapted to low temperatures which prevails on the ice surface, particularly in waterbodies of cryoconite holes, where the temperature averages about 0.1 °C (e.g., STEINBÖCK *l.c.*, KRAUS *l.c.*). This possible low temperature tolerance may be only one of a number of factors which would limit the occurrence of *H. klebelsbergi* in habitats of the periglacial zone, where environmental conditions are more variable. KRAUS (*l.c.*) also suggested extreme cold-stenothermy in *H. klebelsbergi*, based on its absence from a number of small (warmer) pools examined around the glacier.

KRAUS (*l.c.*) reported the most abundant populations of *H. klebelsbergi* occurred in the 250-300 m wide belt of cryoconite holes in the ablation zone, which was located about 20 m below the snow (firn-line) border. In spring and summer, as winter accumulation of snow melts, old frozen cryoconite holes reappear, and as the firn-line recedes, new cryoconite habitats are created. Below that ablation belt and nearer the snout of the glacier, cryoconite material accumulates into larger ice bowls, basins and grooves and at the tip aggregates with rocky debris where it becomes dry. KRAUS (*l.c.*, p. 39) noted that this dry cryoconite, particularly the organic debris containing potential propagules (= tuns) of *H. klebelsbergi*, can be wind transported to higher altitudes, where finding favourable sites on a glacier, individuals are able to continue their life cycle. Evidence for this is supplied by finding *H. klebelsbergi* on small snow-free ice surfaces at higher altitudes, i.e., above 3000 m a.s.l., where they are exposed to high level of solar radiation and strong winds during the alpine summer (KRAUS, *l.c.*, 39). However, these populations were less abundant than those of the main populations at lower altitude in the glacier's ablation zone.

These observations provided by KRAUS (*l.c.*) largely validate the hypothesis that *H. klebelsbergi* is an obligate cryobiotic dweller. The cyclical transport of animals through meltwater downwards and by wind-borne tuns to higher altitudes is, in our view, the best explanation of the sustained presence of this tardigrade on glaciers. This does not negate the view of GRØNGAARD *et al.* (1999) that cryoconite holes are dead-end habitats. On the contrary, we think that the remarks by the latter authors (*l.c.*) to a large degree support KRAUS' hypothesis. Nevertheless, evolution of these peculiar habitats on ice surface has several parallels to that of other short-living ecosystems (= merocoenoses), such as, e.g., tree-holes or dung-pats.

GRØNGAARD *et al.* (1999) generally consider tardigrades reported from cryoconite holes as accidental (fortuitous) colonists and suggest that also *H. klebelsbergi* and *H. janetscheki*, both known only from glacial habitats "more likely..., as these other tardigrades... also occur in englacial and periglacial habitats". In the light of the study by KRAUS (*l.c.*) the authors do not share this opinion, though the supposed cryobiotic status of the two species can only be confirmed through additional studies on high mountain tardigrades.

The limited literature on the Himalayan tardigrades (for review see DASTYCH & KRISTENSEN 1995) does not report dark, highly-pigmented eutardigrade (*H. janetscheki*) from periglacial habitats, and no such specimens were found in material from the periglacial zone at the Annapurna Base Camp (Annapurna South: Central Nepal, unpublished data, H.D.). Interestingly, the dark-pigmented heterotardigrade *Echiniscus ehrenbergi* DASTYCH & KRISTENSEN, 1995, described from bryophytes of the periglacial zone of the Khumbu Glacier (NE Nepal), has a pigmented cuticle, particularly the dorsal plates, but not the epidermis, as in *H. klebelsbergi* and *H. janetscheki*.

KRAUS (*l.c.*) analysis of *H. klebelsbergi* was based on measurements that differ from the current taxonomic standards and therefore it is not possible to perform direct comparisons. Nevertheless, it is interesting to note that he recorded a slight increase in size between the main branches of the external claws from legs I to IV (5-13 μm to 5-14 μm length; $n = 570$). These morphometrics, though not explained by KRAUS (*l.c.*), were confirmed by the present observations and are a trend which is rather rare in tardigrades. Normally, the length of main branches typifies the size of a particular claw, which is characterized by positive allometric growth. The abundant data ($n = 570$) provided by KRAUS (*l.c.*) demonstrated no increase in size at the minimal values for legs I to IV (5 μm) and a slight increase in size for the maximal values for legs I to IV (from 13 to 14 μm : = 7.1 % ratio). In the present material these difference in the size of claws are also low, i.e., 14.7 % ratio for claw I ($n = 19$) and even less, 6.7 %, for the hind claw IV ($n = 16$). This short and almost uniform claw size for all pairs of legs, as well as the peculiar shape of accessory spines and the presence of short main branches, may be an adaptation in *H. klebelsbergi* for locomotion on icy surfaces. KRAUS (*l.c.*) observed individuals of *H. klebelsbergi* moving easily on icy surfaces, though they suffered problems (as other tardigrades) with movement on a smooth, glass surface. To our knowledge, there are no papers describing the submerged structure of ice surface in the glacial ablation zone at microscopic level, so that it is too early for speculation on the functional morphology of the claws of *H. klebelsbergi* in relation to the above substrate. Indeed, there is almost no published information about the functional adaptation of the claws of any member of Tardigrada for moving on various kinds of substrate (but see GREVEN & SCHÜTTLER 2001, SCHÜTTLER & GREVEN 2001), thus further study is required to understand how these possible adaptations could assist the animal's movement over ice or through meltwater currents.

Using statistical analysis KRAUS (*l.c.*) was able to differentiate between 16-17 in-stars (ecdyses) within *H. klebelsbergi* and reported the absence of eggs in exuviae. An interesting observation by KRAUS (*l.c.*) was that in culture exuviae rose to the surface of the water after deposition and did not remain on the bottom of culture vessels, the latter pattern being usual for tardigrades. This is an phenomenon that previously has not been observed and it was KRAUS' (*l.c.*) opinion that this was the reason for the lack of exuviae in samples collected from the bottom of cryoconite holes. Results from the present study would tend to support this view (only two exuviae in 62 samples), indicating a hydrophobic character of the cuticle in this species.

Abundance

KRAUS (*l.c.*) noted that *H. klebelsbergi* was most abundant in cryoconite holes in late summer and autumn, shortly before winter freezing of the habitat. While the sampling for this study was qualitative rather than quantitative, the data obtained provided some information on the population dynamics. Altogether 3843 specimens were observed in 15 BOUIN'S or ethanol preserved (i.e., not dried) samples from cryoconite holes on the Rotmoosferner glacier (\bar{x} = 256.20, SD = 235.08, V = 91.8 %, range: 18-766 individuals per pooled sample). This confirmed KRAUS' (*l.c.*) notes about the abundance of *H. klebelsbergi* in individual cryoconite holes (0.5-4.0 cm diameter, depth 1-15 cm) which was given as up to 75 tardigrades (\bar{x} = 20.69, SD = 22.03, $min-max$ = 0-75 individuals, V = 106.5 %: calculated here from his data). The abundance of tardigrades in static or meltwater were given as: five individuals / 100 ml of static water over small groves in the ice surface and 85 to 360 individuals / 100 ml respectively in weak or strong meltwater currents (KRAUS, *l.c.*). From these studies he estimated (*l.c.*: p. 45) a daily outflow of *c.* 10 cm³ of biomass of *H. klebelsbergi* (*c.* 24 x 10⁶ specimens) from the glacier through melted snow and ice water.

The large numbers of *H. klebelsbergi* observed in the randomly sampled microhabitats for this study (cryoconite on firn, macerated plant, gravel and sand) indicated a broad distribution of the species within the locale of the glacier surface and edge. Indeed, only two of 49 samples in the area of locality "A" (silt and one cryoconite hole) contained no tardigrades. With the exception of a few rotifers, no other metazoans were encountered in these studies, indicating that *H. klebelsbergi* is an important faunistic element in cryoconite holes of the ablation zone of the glacier Rotmoosferner (KRAUS, *l.c.*, present study).

Accompanying tardigrade fauna

In cryoconite holes on the Rotmoosferner and several other sampled glaciers KRAUS (1977) found only *H. klebelsbergi*. However, in the present study a single shrunken specimen of *Ramazzottius oberhaeuseri*-group was found in a sample from a cryoconite hole at locality "A". Although this genus has never been reported from glaciers, the species appears to be a taxon already extracted from several lichen and moss samples growing on silicate rock at Kirchenkogel Mt, in close vicinity to the Rotmoosferner glacier (coll. K. THALER, 15 August 2001). Its presence in cryoconite hole is considered an accidental and was probably a wind-borne 'particle'.

In cryoconite holes in the Austrian Alps two other tardigrade species (*Macrobotus harmsworthi* MURRAY, 1907 and *Hypsibius augusti* MURRAY, 1907) have been found with *H. klebelsbergi* (see MIHELČIĆ 1963). However, the latter (*l.c.*) provide no location details beyond "Gletscher des Großglockners" nor any diagnostic details for these tardigrades. Neither species examined by MIHELČIĆ can now be identified with any certainty as almost all MIHELČIĆ'S material has been lost to science (see DASTYCH 1993). In addition, both taxa have been restudied in the recent period and no longer confine to the taxonomic identifications that MIHELČIĆ would have recognized (see e.g., BERTOLANI 1976).

Another species (*Hypsibius janetscheki* RAMAZZOTTI, 1968) known only from one record from cryoconite holes in the Himalayas, has also been reported as occurring together with the widely distributed tardigrade *Hypsibius convergens* URBANOWICZ, 1925. However, this latter species belongs to a group of confused taxa which urgently needs revision and RAMAZZOTTI'S identification should be verified.

Distribution

A literature search has shown that only the Austrian glaciers have been examined for the presence of tardigrades in the Alps, and *H. klebelsbergi* was found on Gurgler Ferner, Rotmoosferner, Gaißbergferner, Niederjochferner and Marzellferner (type locality) (all in the Ötztal Alps), Sultztalferner (the Stubai Alps), "glaciers of the Großglockner Mt" (the Tauern range), Federbjett Kees and Gefrorene Wand Kees (the Zillertal Alps) (STEINBÖCK 1957, MIHELČIĆ 1959, 1963, KRAUS 1977). Such data as is available suggests a wider distribution for this species on other glaciers in the Alps, but further study is required to validate this hypothesis. The only record of *H. klebelsbergi* from outside Austria was for Greenland (GRØNGAARD et al. 1992), but was based on misidentification (see GRØNGAARD et al. 1999).

KRAUS (*l.c.*) considered the Himalayan species *H. janetscheki* as a synonym of *H. klebelsbergi*, which would make *H. klebelsbergi* an extreme cold-stenotherm species with a disjunctive distribution that might be regarded as a relict of a wider distribution during the last ice age. However, the synonymy of the two species (see also RAMAZZOTTI 1972, RAMAZZOTTI & MAUCCI 1983, DASTYCH 1993, THALER 1999) must be confirmed by a redescription of *H. janetscheki* and critical comparison of both taxa.

To our knowledge, no tardigrade species has been identified from cryoconite holes in the southern hemisphere as yet, the latter structures known also from the Antarctic (e.g., WHARTON et al. 1981). One can presume that at least one tardigrade species, *Acutuncus antarcticus* (earlier: *Hypsibius a.*), a broadly distributed taxon in that region, will be found in cryoconite holes. It has a circum-Antarctic distribution in non-glaciated areas, is a characteristic form for freshwater, but occurs also in other habitats (DASTYCH 1991). The species has recently been found in various small waterbodies in Robertsollen nunataks, western Dronning Maud Land (DASTYCH & DRUMMOND 1996).

Check-list of tardigrades reported from cryoconite holes

A list of published records of tardigrade taxa found in cryoconite holes is presented below in chronological order. Doubtful identifications are marked with asterisk (*):

- Von DRYGALSKI (1897), Greenland: **Macrobotus hufelandi* SCHULTZE, 1883;
 JENSEN (1928), Greenland: **Macrobotus* sp.;
 STEINBÖCK (1936), Greenland: **Macrobotus echinogenitus* RICHTERS, 1904 (= algal (?) material reported by WITTRÖCK (1855); see also GRØNGAARD et al 1999);
 STEINBÖCK (1957), the Austrian Alps: "blackish" tardigrades (= *H. klebelsbergi*);
 MIHELČIĆ (1959), the Austrian Alps: *H. klebelsbergi* (coll. O. STEINBÖCK);
 MIHELČIĆ (1963), the Austrian Alps: *H. klebelsbergi*, **Macrobotus harmsworthi* MURRAY, 1907, **Hypsibius augusti* MURRAY, 1907 (possibly *Pseudobiotus a.*);
 RAMAZZOTTI (1968), Nepal: *Hypsibius janetscheki* RAMAZZOTTI, 1968, **Hypsibius convergens* (URBANOWICZ, 1925);
 KRAUS (1977), the Austrian Alps: *H. klebelsbergi*;

- DASTYCH (1985), West Spitsbergen: *Hypsibius dujardini* (DOYÈRE, 1840), *Hypsibius cf. arcticus* (MURRAY, 1907), *Diphascon recamieri* RICHTERS, 1911;
- DE SMET & VAN ROMPU (1994), West Spitsbergen: *Isohypsibius granulifer* THULIN, 1928, *D. recamieri*;
- GRØNGAARD *et al.* (1992), Greenland: **H. klebelsbergi* (= *Hypsibius* sp.: see GRØNGAARD *et al.* 1999), *Diphascon scoticum* (MURRAY, 1905);
- GRØNGAARD *et al.* (1999), Greenland: *Diphascon alpinum* MURRAY, 1906 (*sensu* PETERSEN 1952), *D. scoticum*, *D. recamieri*, *H. cf. arcticus* (egg), *H. dujardini*, *H. cf. dujardini*, *Isohypsibius* sp.;
- DASTYCH, KRAUS & THALER (this paper), the Austrian Alps: *H. klebelsbergi*, *Ramazzottius oberhaeuseri*-group.

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