

# *Cryobiotus roswithae* gen. n., sp. n., a New Genus and Species of Glacier-Dwelling Tardigrades from Northern Norway (Tardigrada, Panarthropoda)

*Cryobiotus roswithae* gen. n., sp. n., eine neue Gattung und neue Art gletscherbewohnender Bärtierchen aus Nordnorwegen (Tardigrada, Panarthropoda)

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**Summary:** *Cryobiotus* gen. n., a new genus that currently includes four morphologically very similar Palaearctic species of cold-stenothermic, cryobiontic tardigrades [*Cryobiotus klebelsbergi* (Mihelčič, 1959), *C. janetscheki* (Ramazzotti, 1968), *C. thaleri* (Dastych, 2004) and *C. roswithae* sp. n.] is erected. Members of the new genus (earlier included into the genus *Hypsibius* Ehrenberg, 1848) inhabit cryoconite holes on glacier surfaces within the ablation zone. *C. klebelsbergi*, the type species of *Cryobiotus* gen. n., is known from the Alps, *C. janetscheki* and *C. thaleri* are known from one single locality on the Nare Glacier at Ama Dablam Mtn in the Nepal Himalayas, *C. roswithae* sp. n. (this article) from a single locus in the northern part of Norway. These species resemble members of *Hypsibius* Ehrenberg, 1848 and *Acutuncus* Pilato & Binda, 1997. However, some morphological characters and their specific combination (mainly the shape of anterior apophyses of the mouth tube and the type of claws) indicate that they belong to a separate phyletic line. Moreover, species of the genus *Cryobiotus* gen. n. are distinct by their unusually dark and dense (UV-protective?) pigmentation. The new species, *C. roswithae* sp. n., resembles the Himalayan *C. janetscheki*, but mainly differs from this species by its distinctly longer and less stumpy claws, particularly on the fourth pair of legs.

**Key words:** Tardigrada, *Cryobiotus roswithae* gen. n., sp. n., glacier-dwellers, northern Norway, Palaearctic

**Zusammenfassung:** Die in dieser Arbeit neu beschriebene Gattung *Cryobiotus* gen. n. umfasst zurzeit vier paläarktische Arten von kalt-stenothermen, kryobiontischen Tardigraden [*Cryobiotus klebelsbergi* (Mihelčič, 1959), *C. janetscheki* (Ramazzotti, 1968), *C. thaleri* (Dastych, 2004) und die neue Art *C. roswithae* sp. n.]. Die Arten der neuen Gattung bewohnen Gletscheroberflächen innerhalb der Ablationszone und leben dort in Kryokonitlöchern. *C. klebelsbergi*, die Typusart von *Cryobiotus* gen. n., ist aus den Alpen, *C. janetscheki* und *C. thaleri* sind nur von einer einzigen Stelle am Nare-Gletscher beim Ama Dablam-Massiv im nepalesischen Himalaya und *C. roswithae* sp. n. ist ebenfalls nur von einer Stelle in Nordnorwegen bekannt. Diese Arten ähneln teilweise Taxa von *Hypsibius* Ehrenberg, 1848 und *Acutuncus* Pilato & Binda, 1997. Einige morphologische Merkmale und ihre spezifische Kombination (die Gestalt der vorderen Apophysen der Mundröhre, der Krallentyp) weisen jedoch darauf hin, dass sie zu einer separaten Stammeslinie gehören. Die Arten der neuen Gattung sind zudem durch ihre ungewöhnlich dunkle (UV-schützende?) Pigmentierung charakterisiert. *C. roswithae* sp. n. ähnelt stark der Art *C. janetscheki* vom Himalaya, unterscheidet sich jedoch von dieser hauptsächlich durch seine deutlich längeren und weniger stumpfen Krallen, insbesondere am vierten Beinpaar.

**Schlüsselwörter:** Tardigrada, *Cryobiotus roswithae* gen. n., sp. n., Gletscherbewohner, Nordnorwegen, Paläarktis

## 1. Introduction

Although the interest in polar and (high)-mountain Tardigrada started relatively early (e.g. EHRENBERG 1859, 1860; RICHTERS 1904 a, b, 1907; MURRAY 1907 a, b), studies concerning the presence of these animals in glacier habitats were conducted much later. The first described glacier tardigrade, *Hypsibius klebelsbergi* Mihelčič, 1959, was collected by Prof. STEINBÖCK in the Ötztal Alps, Tirol. STEINBÖCK (1957) noted that the colour of these animals was “blackish” and passed them on to Dr. Franz Mihelčič, who described this species in detail. *H. klebelsbergi* represents an obligate glacier inhabitant, which observation is confirmed by the absence of this species from periglacial habitats and other mountain regions (e.g. DASTYCH et al. 2003; DASTYCH 2015; ZAWIERUCHA et al. 2019). Two other morphologically very similar species, *Hypsibius janetscheki* Ramazzotti, 1968 and *Hypsibius thaleri* Dastych, 2004, live exclusively on glaciers, as both were reported from a Himalayan glacier to occur together in a sole location (e.g. RAMAZZOTTI 1968; JANETSCHEK 1990). Although re-described (DASTYCH 1993, 2004 a,b; DASTYCH et al. 2003), the precise taxonomic status of these specimens remained unclear. Therefore I proposed at that time to consider these animals altogether as “*Hypsibius klebelsbergi* group” (DASTYCH 2004 b). It should also be noted that the knowledge on the biology of these somehow enigmatic tardigrades is still either very limited (*H. klebelsbergi*) or practically non-existent. Until now some of the molecular characteristics concerning these tardigrades are known only for *H. klebelsbergi* (for an overview see DABERT et al. 2015). In 2007 I found in a pooled cryoconite sample from a glacier in northern Norway some tardigrade specimens being phenotypically very similar to the species of the “*Hypsibius klebelsbergi* group”. The morphological analysis and comparison of these specimens with members of this

group showed that the Norwegian material represents a new species, which is described in the present article. Since the taxa of the “*Hypsibius klebelsbergi*- group” are distinctly different from those of the genus *Hypsibius* I also propose herein a new genus for this complex of species.

## 2. Material and Methods

The material for this study comes from the Glacier Gihcejeiekna in northern Norway (for locality details see 3.2). The sediment containing tardigrades was collected from cryoconite holes in the glacier ablation zone and processed in the field by methods used in DASTYCH et al. (2003). Samples of cryoconite sediment from several holes were pooled, decanted (volume ca 8 cm<sup>3</sup>), fixed with 70% ethanol and examined in the laboratory several weeks later. The sediment contained numerous rotifers, remnants of more or less deteriorated bryophytes (chiefly their leaves), several small fragments of arthropod cuticles, a dead and partly deteriorated small aphid and a beetle of the genus *Bembidion*. Only five tardigrade specimens of the new species have been found in the sample and one tardigrade specimen of the “*Ramazottius oberhauseri* group”. From these five animals three specimens were mounted in Faure’s medium each on a separate microslide. After examination including measurements, one specimen was transferred from Faure’s mountant into lactic orcein and strongly squashed for further measurements and photomicrography. As this specimen was greatly damaged it was afterwards disposed. The remaining two slides with mounted specimens were designated as type material. The two last specimens were processed for SEM, i.e. gradually dehydrated, critical-point-dried and carbon-coated. After this procedure only one specimen was properly dried and stretched for examination and

imaging, the other specimen was damaged and disposed as well. The strong epidermal pigmentation in this group of tardigrades hides internal organs, making them hardly visible, if at all, unless to squash the animal. As the processed specimens were preserved in ethanol, it was even more difficult to prepare suitable slide preparations.

The specimens described here were compared with type material of *Hypsibius kelebelsbergi* Mihelčič, 1959, *H. janetscheki* Ramazzotti, 1968 and *H. thaleri* Dastych, 2004 (now all included into the new genus: described below) housed in the Zoologisches Museum Hamburg. Moreover, some additional specimens of *H. kelebelsbergi*, *Acutuncus antarcticus* (Richters, 1904) and *Mixibius saracenus* Pilato, 1992 have been also analysed. Furthermore, relevant morphometric data from the re-descriptions of three species described from glaciers (DASTYCH et al. 2003; DASTYCH 1993, 2004 a,b) have been used in the differential diagnosis.

The tardigrades were examined and microphotographs were taken with the phase- and differential interference contrast microscopes Photomikroskop III and Axioskop 2 and SEM-micrographs with the scanning electron microscope LEO 1512 (all Fa. Zeiss). Measurements were taken with eyepiece micrometer under phase contrast. The morphometric indices and coefficients used are explained in DASTYCH et al (2003) and DASTYCH (2004, 2006, 2018). The external claw index (ECI) has not been calculated due to the poor preparation and the lack of some measurements. The terms ‘fore’ and ‘hind’ claws denote the anterior and posterior claws on legs IV (i.e. internal and external, respectively).

Figures 4, 14-14, 17, 20, 22, 25, 34-39 come from the holotype of the new species, the other images from paratypes.

Abbreviations used:

ar – artefact; cl – cloacal slit; da – dorsal apophysis; DIC – differential interference

contrast; ec – external claw; ey – eye dot; fc – fore claw; HBI – the hind claw base index; HFBrI – hind/fore claw main branch index; hc – hind claw; HFCl – hind/fore claw lengths index; ic – internal claw; ICI – internal claws index; LM – light microscope; MBI – claws main branch index; MPLI – microplacoid index; n – sample size; pa – pharyngeal apophyses; PHC – phase contrast; PT – whole mouth tube indices (see PILATO 1981); PT ss – stylet supports “anterior” index; SEM – scanning electron microscope; ss – stylet support; SSA – mouth tube anterior unit (the distance between stylet sheaths and stylet support insertion); taa – (mouth) tube anterior apophyses (= AISM: MARLEY et al. 2011); tpa – (mouth) tube posterior apophyses; va – ventral apophysis; ZMH – Zoologisches Museum Hamburg; I-IV – the first to fourth pair of legs;  $\bar{x}$  - (arithmetic) mean.

### 3. Results

#### 3.1. Description of the new genus

Eutardigrada Richters, 1926

Family Hypsibiidae Pilato, 1969

*Cryobiotus* gen. n.

**Diagnosis:** Median sized to large, dark-brown or blackish-brown pigmented hypsibiids with smooth cuticle (\*) and large eyespots. Internal organs largely hidden by dense pigmentation. Six peribuccal lobes around mouth opening, peribuccal papillae not observed. The anterior apophyses of the mouth tube (= taa) of “acute hook” type (“*Acutuncus* variant”: PILATO & BINDA 2010), i.e. each apophysis distally terminated with a short spine. Pharynx with two macroplacoids. Both claws on the legs of the modified “*Hypsibius* type” (termed here “*Cryobiotus* subtype”). The main (= primary) branches of the claws with accessory spines being markedly wide and flat. No lunules, no cuticular bars between

the claws and their bases. Most likely true cryobionts, i.e. obligate glacier dwellers. (\*) The cuticle is smooth in LM, but already at moderate SEM magnifications it appears wrinkled; at higher magnifications regularly distributed tiny pore-like structures become visible that also occur in the type species *Cryobiotus klebelsbergi* (not published).

Type species: *Hypsibius klebelsbergi* Mihelčič, 1959

Other included taxa: *Hypsibius janetscheki* Ramazzotti, 1968, *Hypsibius thaleri* Dastych, 2004 and *Cryobiotus roswithae* sp. n. (this article).

Etymology: *kerjós* (Gr.) = ice, frost, freezing; *bíos* (Gr.) = life.

Remarks: The taxa complex grouped now under *Cryobiotus* gen. n. has been earlier provisionally included into a “*Hypsibius klebelsbergi* group” (DASTYCH 2004 b) due to the specific phenotypical appearance of its members and their extreme habitat. It has been already suggested to elevate these species to a new sub- or generic rank (DASTYCH et al. 2003; DASTYCH 2004 b, KIEHL et al. 2007; GASTOREK et al. 2018). Within the Hypsibiidae the members of *Cryobiotus* gen. n. resemble mostly the monospecific genus *Acutuncus* PILATO & BINDA, 1997, due to the shared type of the buccopharyngeal apparatus (“*Acutuncus* variant”: PILATO & BINDA 2010). This organ in *Cryobiotus* taxa is slightly different from that in *Acutuncus antarcticus* by the lack of peribuccal papillae and the presence of a more pointed distal apex of the anterior apophyses. When re-examinining specimens of *A. antarcticus* from several Antarctic localities, I found that these apices (“hooks”) are more stumpy in this taxon as suggested by the schematical drawings in PILATO & BINDA (2010: fig. 15B; comp. also DASTYCH & DRUMMOND 1996: fig. 1) and are more similar to the ventral apophysis of *Mixibius* Pilato, 1992 drawn by the authors (PILATO & BINDA 2010: fig. 15D).

Nevertheless, the size and shape of claws, particularly their accessory spines, distinctly discern taxa of these genera.

The trend of (a small) decrease of the claw size on legs I-IV towards the body end found in *C. roswithae* sp. n. and in *C. janetscheki* is in contrast to the opposing trend in *C. klebelsbergi* and *C. thaleri*, where the size of claws increases towards the body rear (comp. DASTYCH: 2004 b: fig. 1 vs GREVEN et al. 2005: fig. 2, SATTLER et al. 2012: fig. 8.4, respectively; for discussion see DASTYCH et al. 2003: p. 95 and DASTYCH 2004 b: p. 174). Nevertheless, the trend increase of the size of claws towards the end of the body is more marked than the opposite trend. However, all taxa of *Cryobiotus* gen. n. appear to be characterized by a relatively small size difference between the claws on legs I and legs IV, a condition better known in *C. klebelsbergi*. This was already noted by KRAUS 1972 (for discussion see DASTYCH et al. 2003); this relative uniformity of claw size implies an adaptation of these animals for locomotion in their glacial habitat.

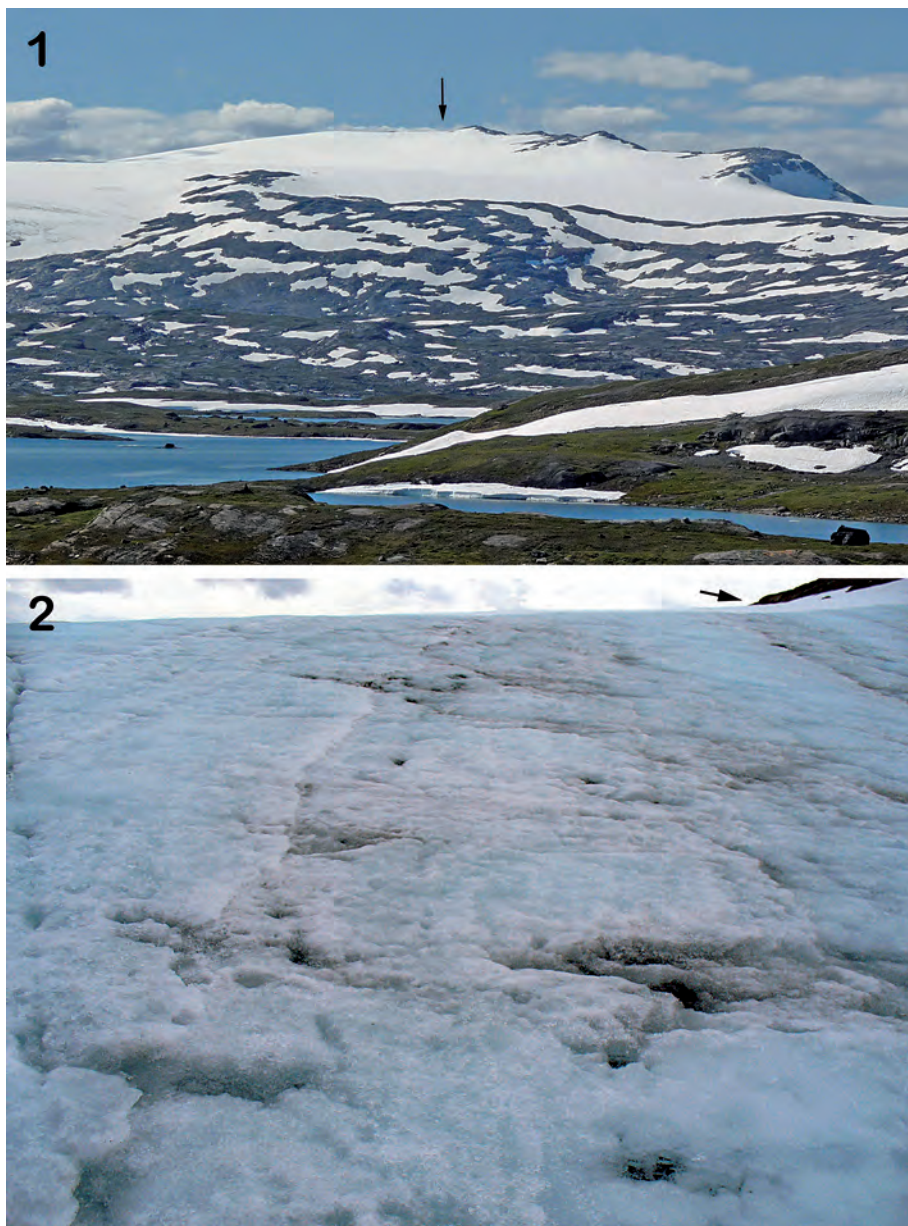
Distribution. Supposedly all members of *Cryobiotus* gen. n. are obligatory glacier dwellers inhabiting glacier surfaces in the ablation zone. This has been already evidenced for *C. klebelsbergi* by KRAUS (1972) and DASTYCH et al. (2003), and was recently confirmed from the Italian Alps by ZAWIERUCHA et al. (2019). Hitherto the taxa of *Cryobiotus* gen. n. have been reported only from the Palaearctic and are to be found in the Alps, northern Norway (this article) and Nepal Himalayas.

### 3.2. Description of the species

*Cryobiotus roswithae* sp. n.  
(Figs 4-45)

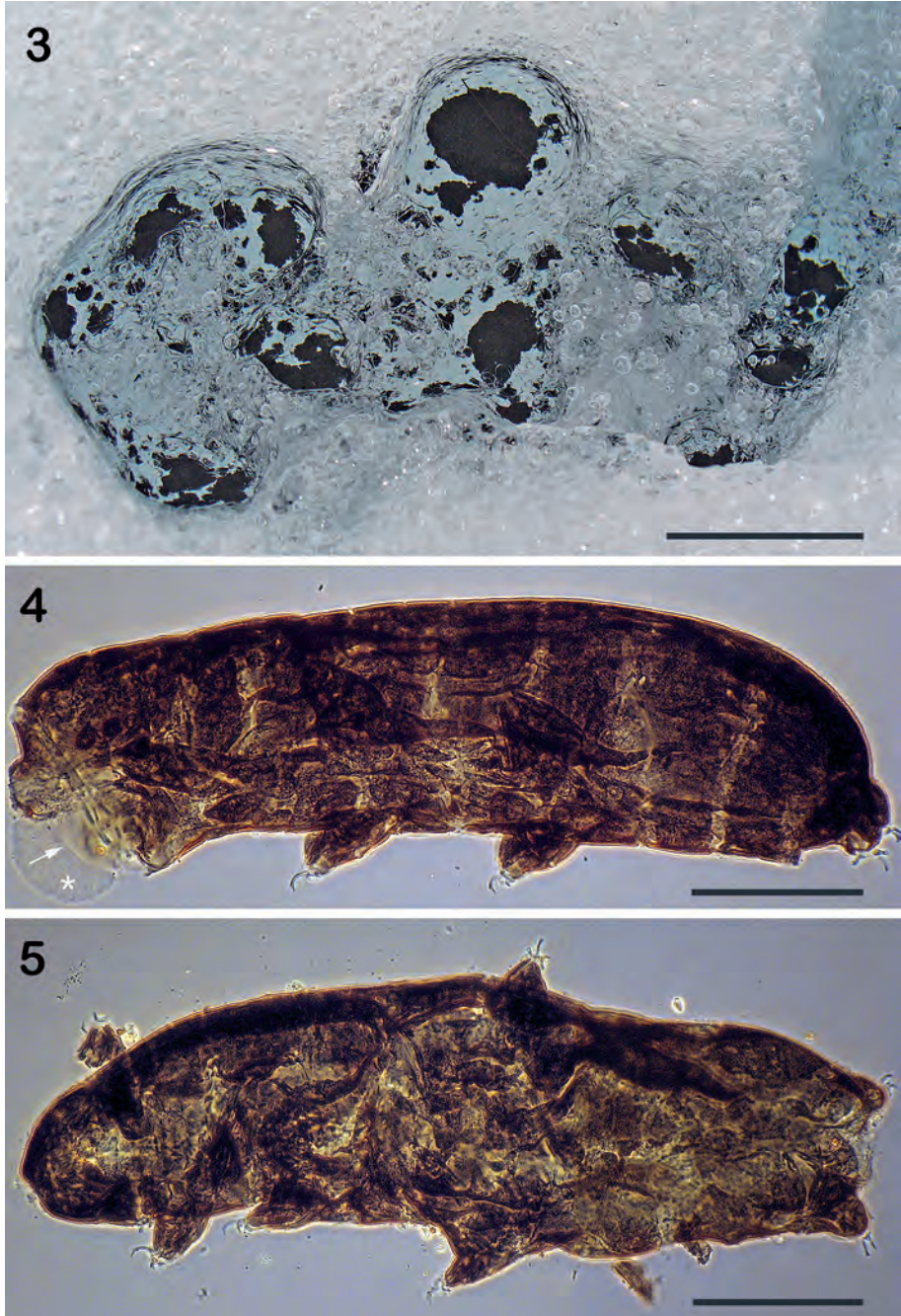
Holotype (Figs 4, 14-14, 17, 20, 22, 25, 34-39): Sex unknown, 493 µm, leg. H. DASTYCH, 5 August 2007. Mounted ventro-laterally on microslide (No. T 1453) in Faure's medium,





**Figs 1-2:** **1** The Gihccejekna Glacier, south-westward view (arrow: locus typicus of *Cryobiotus roswithae* sp. n.). **2** Study area showing cryoconite holes at the glacier pass (arrow: part of the Gihccejekna Range. Note the reddish colouring of some areas of the glacier surface with microbial communities. Figs 1-2: taken on 5 August 2007).

**Abb. 1-2:** **1** Der Gihccejekna-Gletscher, Blick nach Südwesten (Pfeil: Locus typicus von *Cryobiotus roswithae* sp. n.). **2** Untersuchungsgebiet mit Kryokonitlöchern am Gletscherpass (Pfeil: Teil der Gihccejekna-Bergkette). Man beachte zum Teil rötliche Färbung der Gletscheroberfläche durch mikrobielle Lebensgemeinschaften. Abb. 1-2: aufgenommen am 5. August 2007).



**Figs 3-5:** **3** Appearance of some sampled cryoconite holes. Note air bubbles under the thin layer of ice (scale bar ca. 20  $\mu$ m). **4** *Cryobiotus rosnitbae* sp. n., holotype, lateral view. Buccopharyngeal apparatus (arrow) partly squeezed out (asterisk: salivary gland). **5** Ditto, paratype, ventro-lateral view, specimen partly crushed. (Scale bars for Figs 4, 5: 100  $\mu$ m; both PHC. Fig. 3: taken on 5 August 2007.)



deposited in the Zoologisches Museum Hamburg (ZMH Acc. No. A-0002007), Centrum für Naturkunde, Universität Hamburg.

**Type locality** (Figs 1-3): Norway, Nordland, Ofoten, Glacier Gihcecejekna. The orographically right side of the ice pass at NW slope of a semi-nunatak within the small mountain range Gihcetjähkkå Mtn. (= Gihcecehkkå Mtn.) on the glacier (N 68° 01' 06.0", E 16° 47' 53.1"), 1330 m a.s.l., 5 August 2007, leg. H. DASTYCH. Flat glacier area of ca. 20 m<sup>2</sup> with cryoconite holes of ca. 5-20 cm in diameter. Pooled sample of cryoconite sediment from several holes.

**Paratypes:** Of the remaining four specimens (sex unknown), one animal mounted in Faure's medium (No. T 1452; Fig. 5) was designated as paratype and deposited in the Senckenberg Museum für Naturkunde, Görlitz, Germany (Acc. No. 01224), a further specimen has been disposed (see 2.) and two were processed for SEM. The stub with single specimen suitable for SEM is the second paratype, also deposited in the Zoologisches Museum Hamburg (ZMH Acc. No. A-0002008).

**Etymology:** Named in honour of recently deceased Mrs ROSWITHA DZIMBOWSKI for her enthusiasm towards the northern Scandinavian region Finnmark.

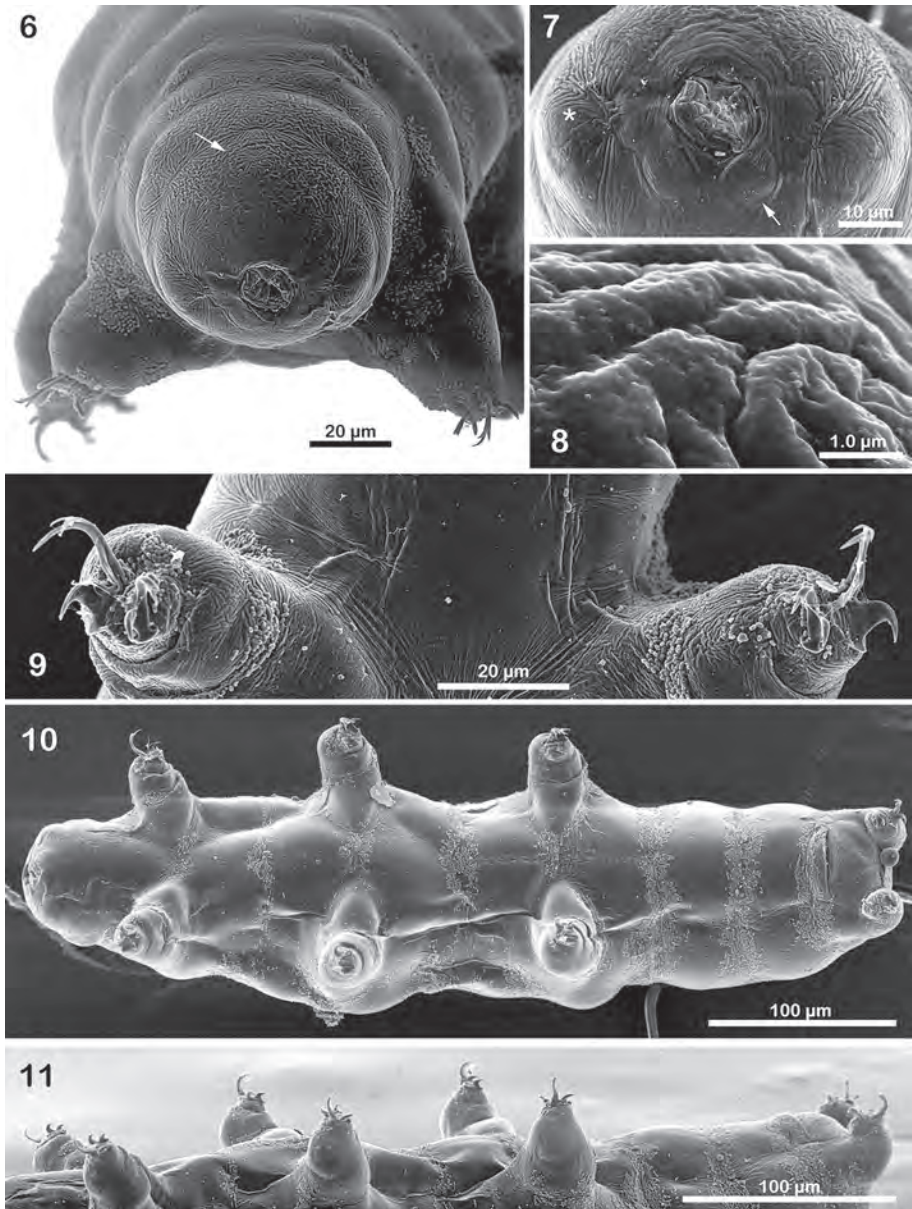
**Diagnosis:** As for the genus. Large, brown-blackish hypsibiid. Two macroplacoids, no microplacoid. Claws well developed, median-sized, decreasing slightly in size towards the body rear. Accessory spines on the main (= primary) branches of the claws well formed and wide.

**Description:** Body 493-515 (holotype 493) µm long, blackish-brown in ethanol preservation, dark-brown in Faure's mountant (Figs 4, 5). Eye dots large, blackish, up to 13 µm in diameter.

Cuticle smooth in LM. At moderate SEM magnification the cuticle is wrinkled (Figs 6, 7), at higher magnifications regularly distributed tiny pore-like structures become visible (Fig. 8). The head dorsally with some cuticular wrinkles arranged in a small circular area (diameter ca. 10 µm) as seen only with SEM (paratype) (Fig. 6, arrow). In a previous article a similar pattern was shown in some specimens of *C. kelebsbergi*, but not further commented (see DASTYCH et al. 2003, fig. 10). On both sides of the mouth opening occur symmetrically and slightly protruding cuticular areas (= "cheeks": Fig. 7, asterisk), possibly sensory fields. The cuticle around the mouth opening is collapsed (Fig. 7) due to the specimen's simplex-state. Therefore, from the usual six peribuccal lobes only four are discernible (Fig. 7, arrow).

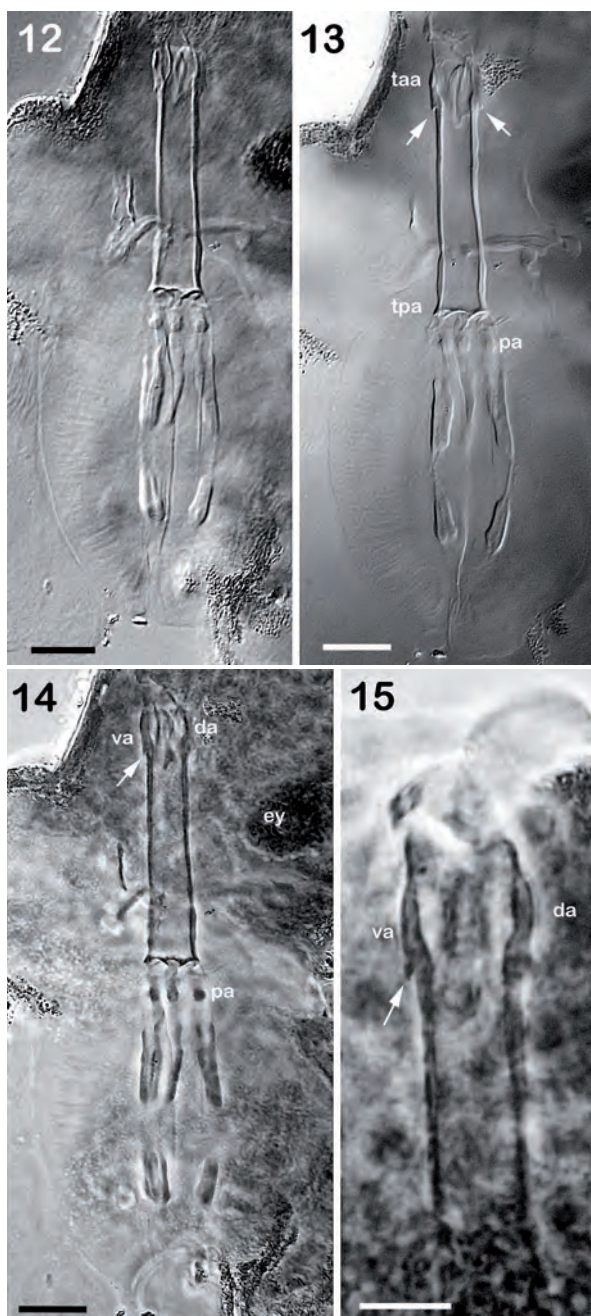
Buccopharyngeal apparatus well formed (Figs 12-14), ca. 20 % of the body length. It is well visible only in the holotype, but some of its characters (mouth cavity, pharyngeal apophyses) are partly displaced or damaged due to the squeezing of the organ (Fig. 4). Mouth cavity median-sized; details of its armature (mucrones, granules etc.) not visible due to its partial damage (Figs 12-15). Mouth tube relatively wide. The anterior apophyses of the mouth tube (Fig. 13, taa) of the modified "acute hook"-type (Figs 12-15) defined as "*Acutuncus*-variant" by PILATO & BINDA (2010). The dorsal and ventral apophyses terminated with a small, but distinct short and sharp spine (Figs 13-15, arrow). The apophyses slightly dis-

**Abb. 3-5:** 3 Erscheinungsbild einiger untersuchter Kryokonitlöcher. Man beachte die Luftblasen unter der dünnen Eisschicht (Maßstab ca. 20 cm). 4 *Cryobiotus roswithae* sp. n., Holotypus, lateral. Buccalapparat (Pfeil) teilweise herausgedrückt (Stern: Speicheldrüse). 5 Dito, Paratypus, zum Teil zerquetscht; ventro-lateral. (Maßstab für Abb. 4, 5: 100 µm; beide PHC. Abb. 3: aufgenommen am 5. August 2007.)



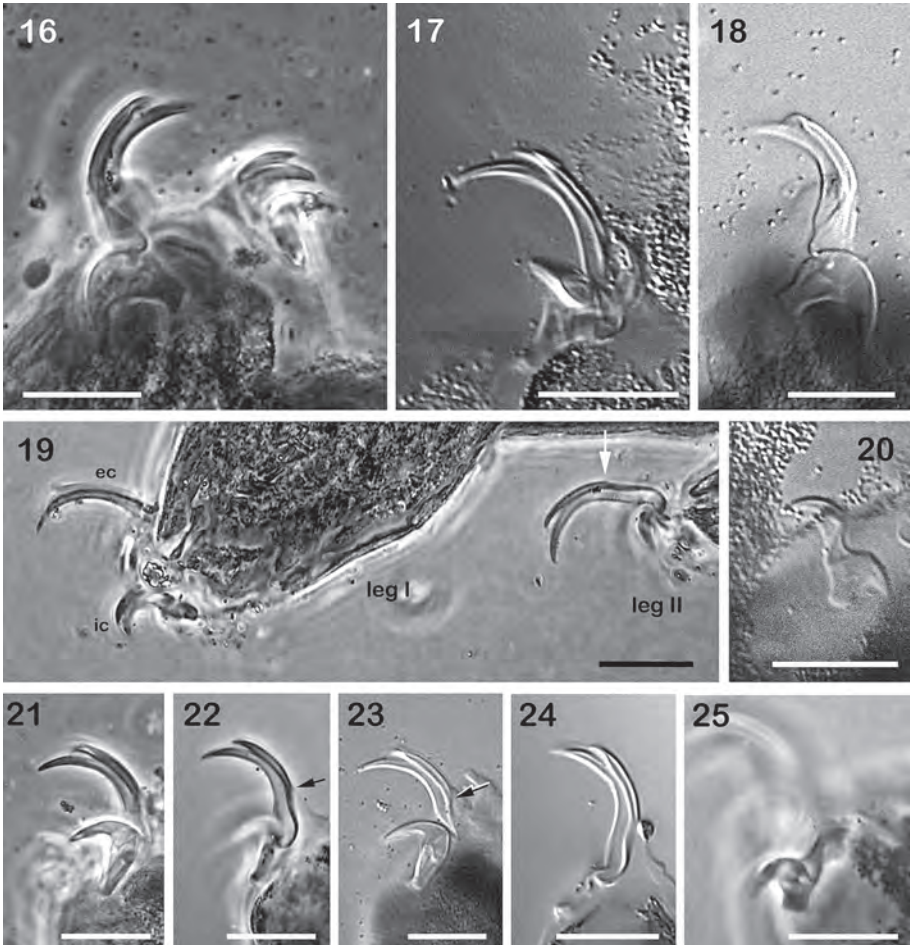
**Figs 6-11:** *Cryobiotus roswithae* sp. n. **6** Frontal view, simplex-state (arrow: roundish structure composed of cuticular wrinkles). **7** Mouth region, frontal view (asterisk: symmetrical lobe-like structure; arrow: peribuccal lobes). **8** Cuticular wrinkles with pore-like depressions. **9** The first pair of legs in ventral view. **10** Animal in ventral view. **11** Claws on legs I-IV, latero-ventral view. (SEM-images)  
**Abb. 6-11:** *Cryobiotus roswithae* sp. n. **6** Frontalansicht, Simplex-Stadium (Pfeil: rundliche Struktur aus Kutikulafalten). **7** Mundregion, frontal (Stern: symmetrische lappenartige Struktur; Pfeil: peribuccale Lappen). **8** Kutikularfalten mit kleinen Vertiefungen. **9** Beinpaar I, ventral. **10** Ventralansicht. **11** Krallen der Beine I-IV, lateral. (REM-Bilder)





**Figs 12-15:** *Cryobiotus roswithae* sp. n. **12-14** Buccopharyngeal apparatus, lateral view, holotype. **15** Anterior apophyses of the mouth tube lateral view, paratype (arrow: distal apophysis spine. Figs 12, 13: DIC-images; others = PHC). Scale bars: Figs 12-14 = 10  $\mu$ m, Fig. 15 = 5  $\mu$ m.

**Abb. 12-15:** *Cryobiotus roswithae* sp. n. **12-14** Buccalapparat, lateral. **15** Vordere Apophysen der Mundröhre, lateral (Pfeil: distaler Apophysendorn. Maßstab: Abb. 12-14 = 10  $\mu$ m, Abb. 15 = 5  $\mu$ m. Abb. 12, 13: DIC-Bilder; andere = PHC).



**Figs 16-25:** *Cryobiotus roswithae* sp. n. **16-18, 20** Claws, leg I. **19** Claws, legs I and II. **21-25** Claws, leg II (arrow: proximal part of the accessory spines of the main branch. Figs 20, 25: internal claw. Figs 16, 19, 21, 22, 25: PHC-images, others = DIC. Scale bars = 10  $\mu$ m).

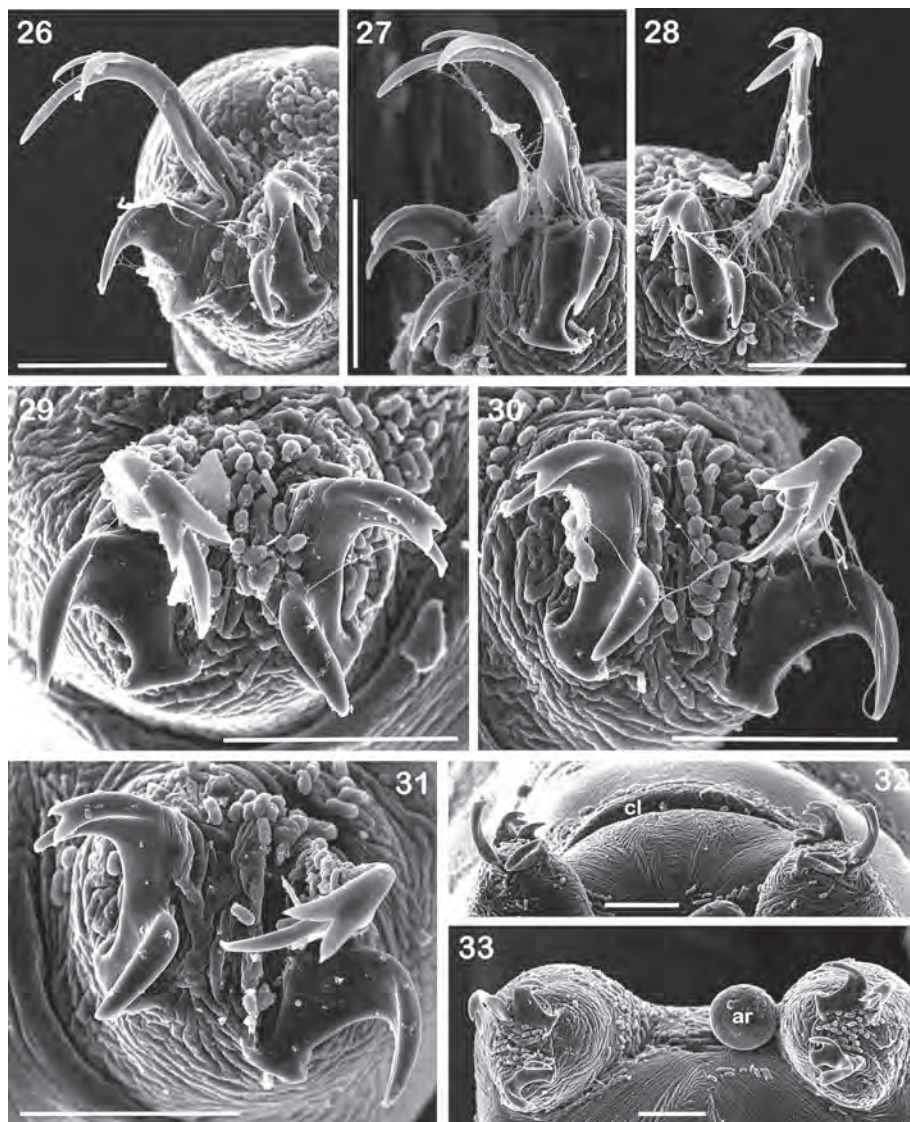
**Abb. 16-25:** *Cryobiotus roswithae* sp. n. **16-18, 20** Krallen, Bein I. **19** Krallen, Beine I und II. **21-25** Krallen, Bein II (Pfeil: proximaler Teil der Nebenspitzen. Abb. 20, 25: innere Kralle. Abb. 16, 19, 21, 22, 25: PHC-Bilder, andere = DIC. Maßstab = 10  $\mu$ m).

similar in shape when seen in profile (Fig. 14: da, va, respectively), in this case being a little concave in their middle. The posterior apophyses of the mouth tube (terminal thickening of the mouth tube: Fig. 13, tpa) tiny but distinct. Such tiny apophyses occur also in *C. klebelsbergi*, but were erroneously reported as absent (see DASTYCH et al. 2003, p. 82 and figs 25, 28). Pharyngeal apophyses (Fig. 14, pa) roundish, relatively small, with a diameter of ca. 1.6  $\mu$ m (holotype). They

are posteriorly shifted (preparation artefact) from the posterior apophyses (tpa). The stylet supports attached on the tube in a relatively posterior position; their pt index value (= PT ss) is 73-74 % (see morphometric data). The stylet's furca shaped as that in *C. klebelsbergi* (see DASTYCH et al. 2003: fig. 28, fu).

Pharynx subspherical, with two macroplacoids; microplacoids absent (Figs 12-14). Macroplacoids well developed, rod-shaped;





**Figs 26-33:** *Cryobiotus roswithae* sp. n. **26-28** Claws, leg I. **29-30** Claws, leg II. **31** Claws, leg III. **32-33** Claws on legs IV: the same claws shown from different angles (Figs 26-31, 33: ventral view, Fig. 32: ventral view, from behind). SEM-images. Scale bars: 10 µm).

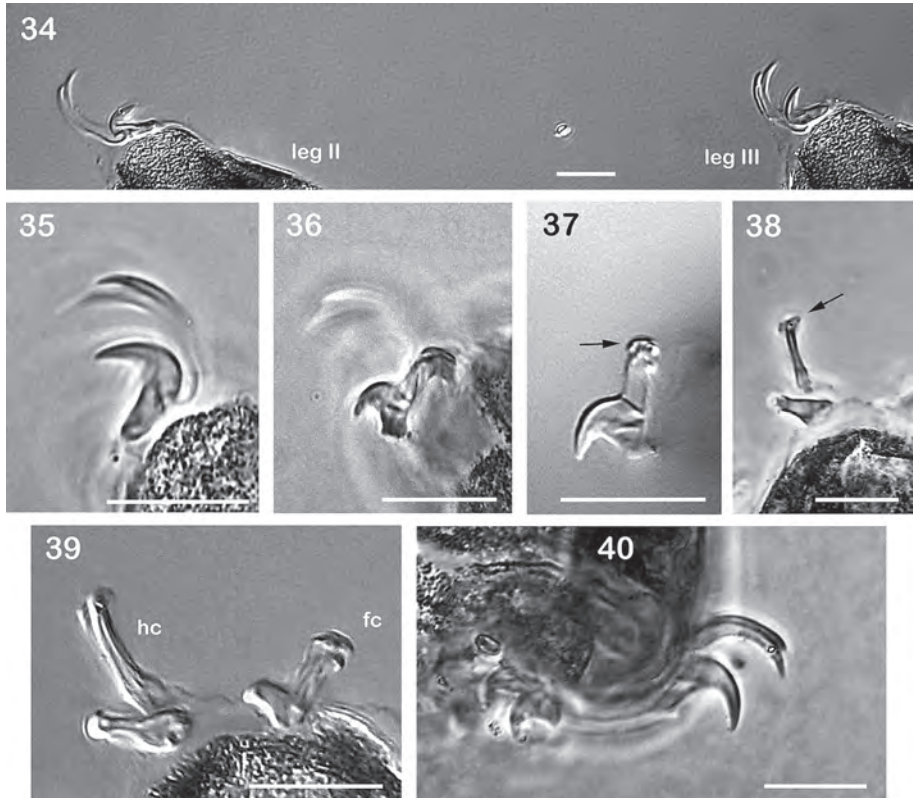
**Abb. 26-33:** *Cryobiotus roswithae* sp. n. **26-28** Krallen, Bein I. **29-30** Krallen, Bein II. **31** Krallen, Bein III. **32-33** Krallen, Beinen IV: dieselben Krallen aufgenommen aus verschiedenen Winkeln (Abb. 26-31, 33: Ventralansicht, Abb. 32: Ventralansicht, von hinten. REM-Bilder. Maßstab = 10 µm).

the first one relatively long, both of them without a distinct incision in the middle. Macroplacoids connected together with a very thin cuticular stripe (Fig. 13). The distance between the first macroplacoid

and the pharyngeal apophyses (pa) and that between both macroplacoids is relatively large (Fig. 14).

The claws in design as those of *C. klebelsbergi* (see DASTYCH et al. 2003), i.e. of a modified



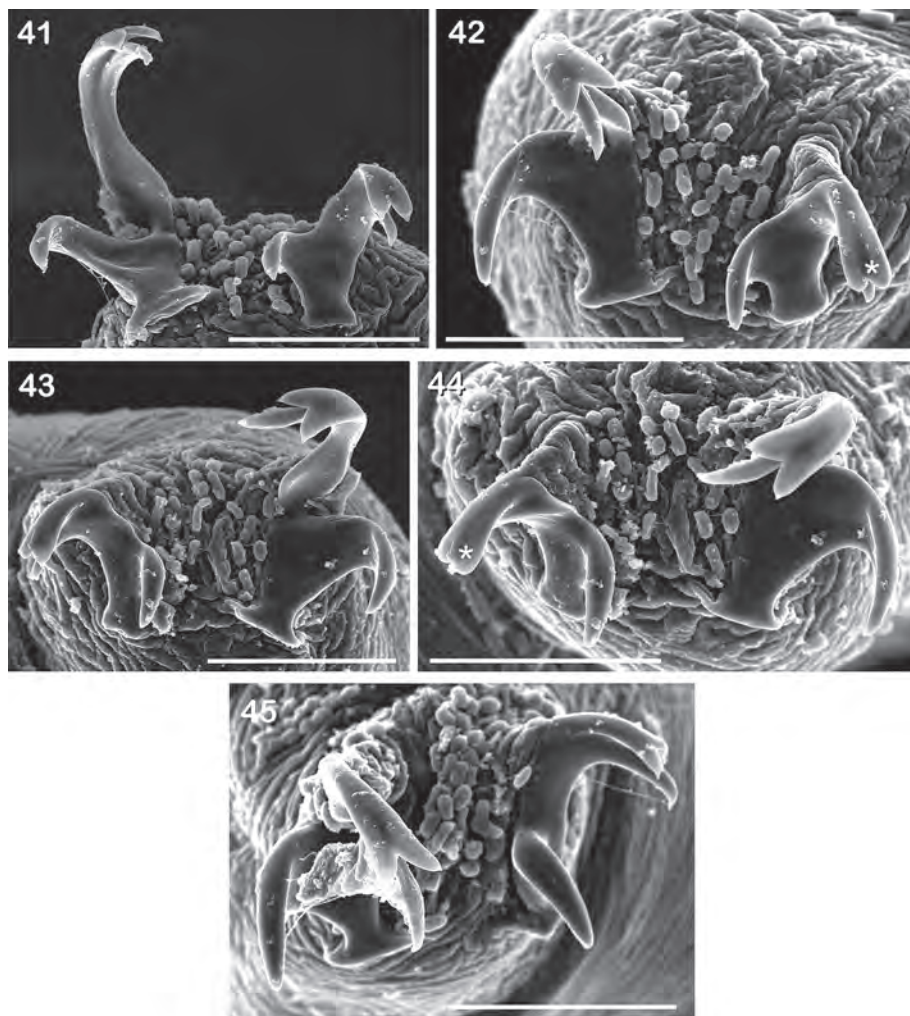


**Figs 34-40:** *Cryobiotus roswithae* sp. n. **34** External claws on leg II and III. **35** Ditto, leg III. **36-37** internal claw, leg III. **38** External claw, leg IV. **39-40** Claws on leg IV (Arrow: accessory spines, frontal view: Figs 36-37. Scale bars = 10 µm).

**Abb 34-40:** *Cryobiotus roswithae* sp. n. **34** Außenkrallen, Bein II und III. **35** Dito, Bein III. **36-37** Innere Krallen, Bein III. **38** Außenkrallen, Bein IV. **39-40** Krallen an Bein IV (Pfeil: Nebenspitzen, Vorderansicht: Abb. 36-37. Maßstab = 10 µm).

*Hypsibius*-type and termed here as „*Cryobiotus* - subtype“. They are of medium size; external claws distinctly larger than the internal claws; their main branches are provided with characteristic wide and flat accessory spines (e.g. Figs 21, 29, 35, 37, 38). The bases of claws relatively narrow, slightly constricted above their posterior edge and with strengthening transverse bare-like thickening in their middle (Figs 18, 23 35, 37, 41). The thickening is less discernible in external claws. Lunules absent, no cuticular bars between the claws and at their bases. The secondary branches of the claws bend strongly downwards and are sharply pointed (Figs 20, 26). The main

branches of external claws are visible in frontal plane as narrow (thin) rods (e.g. Figs 28, 38), being distinctly wider when seen in their sagittal plane (Figs 16, 18, 22). These branches are slightly longer on legs I and obviously thinner than those on the remaining legs. Between the main and secondary branch of external claws a kind of cuticular hinge occurs, allowing some deflections similar as that in *C. kelebelsbergi* (see DASTYCH et al. 2003: p. 84, figs 42-44, 52). The main branch and the secondary branch of internal claws solidly fused and therefore more inflexible (Figs 36, 37, 42). These claws are much stumper than the external ones.



**Figs 41-45:** *Cryobiotus roswithae* sp. n., claws on leg IV (Figs 41-42: the same claws shown from different angles; Figs 43-44 ditto). (Asterisk in Figs 42, 44: stumpy ends of the accessory spines on the main branches of the internal claw, SEM-images. Scale bars = 10  $\mu$ m.)

**Abb. 41-45:** *Cryobiotus roswithae* sp. n. Krallen am Bein IV (Abb. 41-42: dieselben Krallen aufgenommen aus verschiedenen Winkeln; Abb. 43-44 Dito). (Stern in Abb. 42, 44: stumpfe Enden der Nebenspitzen an den Hauptästen der inneren Krallen. REM-Bilder. Maßstab = 10  $\mu$ m.)

The accessory spines, particularly those on external claws, are fused with the main branches over a distance shorter than the half of the whole branch length (e.g. Figs 18, 22). The onset (= “base”) of this fusion (Figs 19, 22, 23, arrow) without any thickening or swelling. Such distinct swelling of accessory spines in their very proximal part occurs

in *C. klebelsbergi* (DASTYCH et al. 2003: figs 48, 51, 53). The fused accessory spines are markedly wide and flattened, particularly in their most distal parts. These fused spines terminate as a broad paddle-like structure, with relatively deep incision in its middle (e.g. Figs 26, 30, 43), creating distally two wide teeth (e.g. Figs 29, 31). Some of these teeth

were more or less rounded, forming a kind of roundish distal plate with small incision on the plate's edge (Figs 42, 44, asterisk). The apex of the main branch and its accessory spines resemble in the frontal plane a „T“ (Fig. 38, arrow). When viewed laterally, the wideness of the accessory spines gives the whole main branches a relatively thick appearance (Figs 16, 18, 32). The size of claws decreases only slightly towards the body rear (Figs 4, 5, 10, 11, 19), being thus slightly shorter on legs IV. The external claws on legs I are visibly longer and noticeably thinner than those on legs II-IV.

### 3.2.1. Morphometric data

Measurements are in  $\mu\text{m}$ , all indices in %. For abbreviations and definitions see 2, the first measurement concerns the holotype, the second behind the backslash the first paratype and the third (in brackets) that of disposed animal (see 2). Not measured (unfavourably positioned) structures are marked with “---”.

#### A) Measurements ( $\mu\text{m}$ )

Body length ca. 493/539 (515)  
 Buccal apparatus length 101.3/---  
 Pharynx (length x width) 58.5 x 49.5/---  
 Mouth tube length 36.9/39.4  
 SSA length (tube length above stylet supports) 27.0/29.3  
 Mouth tube width (external) 6.3/7.7 (7.2)  
 Mouth tube width (internal) 5.4/6.3 (5.4)  
 Macroplacoid row length 27.9/30.1  
 Macroplacoid 1 length 12.6/14.4  
 Macroplacoid 2 length 8.1/8.1  
 External claw 1 length 21.0/--- (17.1)  
 External claw 1 main branch length 15.5/15.3  
 External claw 1 base height ---/8.1 (8.1)  
 Internal claw 1 length 9.0/9.5 (9.5)  
 Internal claw 1 main branch length 5.0/5.0  
 Internal claw 1 base height 4.5/5.0 (5.4)  
 External claw 2 length 17.1/17.6  
 External claw 2 main branch length 13.5/14.4

External claw 2 base height 7.6/7.7  
 Internal claw 2 length 9.0/10.0  
 Internal claw 2 main branch length 4.5/---  
 Internal claw 2 base height 4.5/5.4  
 External claw 3 length ---/---  
 External claw 3 main branch length 11.7/13.1  
 External claw 3 base height 6.8/---  
 Internal claw 3 length 8.6/9.5  
 Internal claw 3 main branch length 4.5/5.4  
 Internal claw 3 base height 4.5/4.5  
 Fore claw (= int. 4) length 8.6/8.1  
 Fore claw main branch length 5.4/5.0  
 Fore claw base height ---/4.5  
 Fore claw secondary branch length ---/5.5  
 Hind claw (= ext. 4) length ---/18.4  
 Hind claw main branch length 11.7/11.3  
 Hind claw base height ---/8.1  
 Hind claw secondary branch length ---/7.2

#### B) Indices

1) PT = "pt indices" (PILATO 1981):  
 PT stylet supports (PT ss) 73.2 /74.4  
 PT mouth tube width (ext.) 17.1/19.5  
 PT mouth tube width (int.) 14.6/17.1  
 PT macroplacoid row length 75.6/76.4  
 PT macroplacoid 1 length 34.2/36.5  
 PT macroplacoid 2 length 22.0/20.6  
 PT claw 1 (ext.) length 56.9/---  
 PT claw 1 (ext.) main branch length 42.0/38.8  
 PT claw 1 (ext.) base height ---/20.6  
 PT claw 1 (int.) length 24.4/24.1  
 PT claw 1 (int.) main branch length 13.6 /12.7  
 PT claw 1 (int.) base height length 12.2/12.7  
 PT claw 2 (ext.) length 46.3/44.7  
 PT claw 2 (ext.) main branch length 36.6/36.5  
 PT claw 2 (ext.) base height 20.6/19.5  
 PT claw 2 (int.) length 24.4/25.4  
 PT claw 2 (int.) main branch length 12.1/---  
 PT claw 2 (int.) base height 12.2/13.7  
 PT claw 3 (ext.) length ---/---  
 PT claw 3 (ext.) main branch length 31.7/33.2  
 PT claw 3 (ext.) base height 18.4/---  
 PT claw 3 (int.) length 23.3/24.1  
 PT claw 3 (int.) main branch length 12.2/12.2  
 PT claw 3 (int.) base height 12.2/10.4  
 PT fore (= int. 4) claw length 23.3/20.6  
 PT fore claw main branch length 14.6/12.7



PT fore claw base height ---/11.4  
 PT fore claw secondary branch length ---/14.0  
 PT hind (= ext. 4) claw length ---/46.7  
 PT hind claw main branch length 31.7/26.7  
 PT hind claw base height ---/20.6  
 PT hind claw secondary branch length ---/18.3

## 2) Other indices

Macroplacoid index (MPLI) 64.3/56.3  
 Claws main branch index (MBI) 133.5/141.7  
 Internal claws index (ICI) 104.7/117.3  
 Hind claw base index (HCBI) ---/71.7  
 Hind/fore claw length index (HFCI) ---/44.0  
 Hind/fore claw main branch index (HFBrI) 73.5/71.7

Variability: not known.

Reproduction mode and ploidy: not known.

Molecular markers: not known.

### 3.2.2. Differential diagnosis

*C. roswithae* sp. n. represents the fourth morpho(species) within the complex of phenotypically very similar members of *Cryobiotus* gen. n. In two species, *C. klebelsbergi* and *C. thaleri*, the length of claws increases (slightly) from leg I to leg IV. This trend seems to dominate in Tardigrada, but is variously manifested in different taxa. The opposite trend, however, seems to be very rare, but occurs in *C. janetscheki* and *C. roswithae* sp. n. In these species the length of claws decreases slightly from legs I to legs IV, being visibly shortest on legs IV (MBI-index in *C. janetscheki*:  $\bar{x} = 140.0$ ;  $n = 7$ ; see DASTYCH 2004 a, p. 189), in *C. roswithae* sp. n. that index equals 132.5/141.7;  $n = 2$ ). In spite of that common character with *C. janetscheki*, the new species differs from the latter chiefly in the form of claws. In *C. roswithae* sp. n. claws are much more slender and distinctly less stumpy as those in *C. janetscheki*. Moreover, the proximal (basal) part of the main branches of external claws is markedly thinner in *C. roswithae* sp. n. compared to those in the former taxon.

Also the main branches, particularly those of external claws, are distinctly shorter, being additionally much more curved in *C. janetscheki* as those in the new species (see Figs 16-24 and DASTYCH 2004 a: figs 11-14, arrow and Figs 22, 23, this article).

Differences between these both taxa occur also in the location of their stylet supports. These are placed more anteriorly on the mouth tube of *C. janetscheki* (PT ss index:  $\bar{x} = 68.6$ ,  $n = 5$ ), but more posteriorly in *C. roswithae* sp. n. (PT ss = 73.2/74.4). Furthermore, the mouth tube in the latter taxon is markedly wider (PT = 17.1/19.5, vs.  $\bar{x} = 11.7$ ,  $n = 4$  in *C. janetscheki*), and main branches of external claws are distinctly longer (PT 42.0/38.8 vs. 21.4 in *C. janetscheki*). The same concerns the length of the main branches of the hind claw (PT 31.7/26.7 in *C. roswithae* sp. n., vs.  $\bar{x} = 18.9$ ,  $n = 5$  in the former species: comp. l.c. 2004 a, p. 189).

The decreasing size of claws on legs I to IV in *C. roswithae* sp. n. distinguishes the new taxon from *C. klebelsbergi* and *C. thaleri*. Moreover, the new species differs from *C. klebelsbergi* by its markedly longer and slender claws and not abruptly terminated accessory spines in their anterior parts. Therefore, the accessory spines do not form a distinct swelling on the main claw branch in the new species, such as in *C. klebelsbergi* (see e.g. DASTYCH et al. 2003: figs 48, 55). The stylet supports in *C. roswithae* sp. n. are located more posteriorly on the mouth tube as those in *C. klebelsbergi* (PT ss index: 73.2/74.4, vs.  $\bar{x} = 64.6$ ,  $n = 55$ , respectively). The second macroplacoids in *C. roswithae* sp. n. are shorter than those in *C. klebelsbergi* (MPLI index: 64.3/56.3, vs.  $\bar{x} = 78.2$ ,  $n = 13$ , respectively). Moreover, the mouth tube is wider in the new species as that in *C. klebelsbergi* (PT ext. width: 17.1/19.5, vs.  $\bar{x} = 14.3$ ,  $n = 13$ , respectively). Macroplacoids in *C. klebelsbergi* are usually incised distinctly in their middle, but far less, if at all, in the new species (DASTYCH et al. 2003: figs 25, 28-31; Figs 12-14, this paper).

*C. roswithae* sp. n. differs from *C. thaleri* by the fact that the claws on legs IV are the smallest, whereas those in the latter species are the longest. Moreover, the anterior apophyses of the mouth tube (taa) are more concave in profile in *C. thaleri* and less in *C. roswithae*. The distal (caudal) processes of the apophyses are usually spine-like in *C. thaleri*, vs. tooth-shaped and blunter in the new species (Figs 12-15; DASTYCH 2004 b: figs 7-12, 14-19). The mouth tube is wider in *C. roswithae* sp. n. (PT ext. width: 17.1/19.5, vs.  $\bar{x} = 11.6$ ,  $n = 38$ ). In the new species the external claws are distinctly longer than the internal ones on legs and their main branches are relatively shorter compared to their base heights as those in *C. thaleri* (HCBI index 71.7, vs.  $\bar{x} = 60.3$ ,  $n = 22$ , respectively).

Some morphological differences between *C. klebelsbergi* and *C. janetscheki* have been provided in an identification key (DASTYCH 2004 b).

**Distribution:** *Cryobiotus roswithae* sp. n. is hitherto known only from its locus typicus on the Glacier Gihccejekna (see 3.2), where as few as five specimens have been found. Interestingly, this species was not recorded in many pooled cryoconite sample collections from other Norwegian glaciers in the years 2007-2009, namely Austere Okstindbreen, Engabreen and Steindalsbreen. In the cryoconite holes of these glaciers, sampled between 180-850 m a.s.l., I only found numerous specimens of *Pilatobius recameri* (Richters, 1911), but no further tardigrade species.

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