The tardigrade *Hebesuncus conjungens* (Thulin, 1911) in the Alps, with notes on morphology and distribution (Tardigrada)

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(With 18 figures)

**Abstract**

The arctic-alpine tardigrade *Hebesuncus conjungens* (Thulin, 1911) is redescribed, based on type material and specimens from the Ötzal Alps (Nordtirol). The study focuses on the morphology of the buccal apparatus, claws and eggs, including SEM analysis. Some aspects of the biology and distribution of the species are discussed. The species is new for Austria.

**Introduction**

Members of the genus *Hebesuncus* Pilato, 1987 belong to cold-dwelling, widely distributed but relatively rarely recorded tardigrades, occurring in polar regions and various mountain ranges worldwide. Three species of *Hebesuncus* have been described: *H. conjungens* (Thulin, 1911), *H. schusteri* (Dastych, 1984) and *H. ryani* (Dastych & Harris, 1994). The latter two taxa are known only from the Antarctic, while *H. conjungens* has been reported from both the northern and southern hemisphere.

Several specimens and eggs of *H. conjungens* were found in bryophytes and lichens from the subnival zone of Mt. Festkogel (the Ötzal Alps, Nordtirol, Austria). As the knowledge of the morphology of this species still has many gaps, it was considered to revise the information on some of its poorly known or undescribed characters. Finally, several aspects of the biology and distribution of the taxon are discussed.

**Material and methods**

Bryophyte and lichen samples were collected in paper envelopes and air-dried, the tardigrades and their eggs then being extracted by the method described by Dastych (1985). The specimens are mounted on six microslides in Faure’s medium or polyvinyl-lactophenol and have been deposited in the Zoologisches Museum Hamburg (ZMH Acc. No A26/02). Photomicrographs were taken with a ZEISS "Photomikroskop III", SEM micrographs with LEO 1525. For SEM examination specimens were washed, transferred to hot Bouin’s medium, dehydrated in ethanol, critical-point-dried and gold-coated.
Figs 1-5. *Hebesuncus conjungens* (Thulin): 1 - animal in lateral view; 2, 3 - claws of leg III; 4, 5 - claws of leg IV (Fig. 3: DIC; the other: PHC. Explanations in text, all scales in μm).
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The comparative material from Thulin’s Collection examined here contained three microslides, two of them labelled as type material. However, due to the lack of original information on some important abbreviations on the slide labels (R. M. Kristensen, pers. comm.), we considered only the microslide with the inscription “n. sp.”, as that one which bears type specimens.

Two morphometric indices have been employed. The index “pt ss” describes a size ratio between the length of the buccal tube and that of the insertion of the stylet supports (Pilato 1981); its modification, termed the “ptd” index (Pilato 1998), is based on the length of the only anterior unit of the buccal tube, i.e. its rigid part (= mouth tube). The mouth tube has been measured from the stylet sheaths to its first horizontal stria, the diameter of the buccal tube is taken just above the level of insertion of stylet supports. “External claw IV” means the most posteriorly located claw on leg IV (i.e. the hind one), homologous to external claws I-III.

The following abbreviations are used in the text: DIC - differential interference contrast, LM - light microscope, n - sample size, PHC - phase contrast, min-max - minimum-maximum, SD - standard deviation, SEM - scanning electron microscope, V - coefficient of variation (in %), x - (arithmetic) mean.

Description
Family: Hypsibiidae Pilato,1969
Genus: Hebesuncus Pilato, 1987

Hebesuncus conjungens (Thulin, 1911) (Figs 1-18)

Hypsibius conjungens: Thulin (1911), Figs 20-20b; n. sp. Type material: originally not listed. Locus typicus: Sweden, “Degerfors, Kiruna”, no other data. Examined here: three syntypes mounted on microscope slide, sex undetermined; one syntype in the simplex-stage. The slide labelled with ink: "Hypsibius conjungens / 3 ex. / n. sp. / Thulin det." and "Sm. 43 / Sverige / glyc-gelatine". One (left) label with additional, different handwriting: “Type material”. Deposited in the Zoologisk Museum, Copenhagen.

Other material examined: (A) Austria, the Ötztal Alps, Obergurgl: Mt. Festkogel. Mosses and lichens from silicate rocks of the summit area (subnival zone), 3030-3035 m a.s.l., 23.8.2000, coll. K. Thaler. Five samples: Cetraria lichen (12 specimens), Stereocaulon lichen (6 + 2 eggs), moss (2), moss with Alectoria lichen (8) and a not identified lichen sample (9 specimens); (B) data as above, moss sample from silicate rock under summit, c. 2800 m a.s.l., 24.6.2001 (1 specimen); (C) microscope slide from Thulin’s Collection, labelled: “Hypsibius conjungens / 3 ex. / Thulin / 1928: 6 / Thulin det” and “Abisko. Lapland./ Lav på klippeblok (= lichen on rock) / glyc-gelat. 1943”. One (left) label with additional, different handwriting “Type material”. Three specimens, two of them with two eggs, each provided with processes. (In our opinion these individuals do not represent type material); (D) as above; the slide labelled only on one side: “Sm. 43? / Hypsibius conjungens / Thulin / 7 ex. / 1913(14) / Glyc.gel.”

Body relatively stout, with medium sized legs and small claws (Fig. 1). The specimens examined are 128-263 μm long, pale-pink or whitish, some of the largest individuals with small clumps of brown pigment. Eye-dots small, composed of several brownish-black pigment granules. Cuticle smooth, in the rear of the body of larger specimens slightly wrinkled; no cuticular “pores".
The mouth opening located on the well formed mouth cone is directed ventro-anterioirly (Figs 1, 12) and is surrounded by six distinct lobes (Fig 13: asterisk), a structure till now not reported in the genus. Just outside the opening occurs a narrow, more or less plicate cuticular ring (Fig. 13: arrowhead). This ring, due to its location and folds, slightly resembles a ring of lamellas found in the Macrobiotidae. In the *simplex*-stage the mouth opening has six distinct notches around (Fig. 12).

Buccal apparatus medium-sized, 55 μm long (this measurement and those below, were taken in a specimen from Mt. Festkogel, being 241 μm in length). Buccal tube relatively long but narrow, pharynx spherical, with distinct pharyngeal apophysis and two small macroplacoids (Figs 1, 6, 8). The anterior dorsal and ventral apophyses of the mouth tube asymmetrically placed (Fig.7: arrowheads), shaped generally in agreement with Pilato's (1987) description. In the present material, however, the tube wall at the ventral apophysis is distinctly thicker (Fig. 7: arrow) compared to that illustrated by the author (l.c., 1987: Fig. 4). External diameter of tube 1.8 μm,

Figs 6-11. *Hebesuncus conjungens* (Thulin): 6, 8 - bucco-pharyngeal apparati; 7 - mouth tube, lateral; 9 - egg; 10, 11 - fragment of egg (Figs 6, 11: DIC; the other: PHC).
Figs 12-17. *Hebesuncus conjungens* (Thulin): 12 - habitus, frontal view; 13 - mouth opening; 14 - claws of leg IV; 15 - egg; 16-17: egg processes and interprocess area.
Fig. 18. *Hebesuncus conjungens* (Thulin): fragment of egg.

internal 0.9 μm. The tube with a characteristic horizontal striation (Figs 6-8) occurring from its mid-section to nearly its posterior end. The striation, well visible in LM only at the magnification 1250 times, is best developed in the mid-section of the buccal tube. The structure provides the tube with greater flexibility. In the present material the striation never reaches the pharyngeal apophyses, as figured by Pilato (1987: Figs 4, 5).

Stylets well developed, with “typical” furca. Stylet supports inserted anteriorly on buccal tube, i.e. at less than 50% of the buccal tube length (Figs 1, 6). Thus, “pt ss” index equals $\bar{x} = 42.50$ ($SD = 1.700$, min-max = 39.5-44.5, $V = 4.00$, $n = 7$). As a result, the unit between the stylet supports and the anterior pharynx wall is markedly long. The “ptd ss” index is $\bar{x} = 69.07$ ($SD = 2.008$, min-max = 65.2-73.2, $V = 2.91$, $n = 12$; in two syntypes 68.0 and 68.5, respectively). The “ptd external claw IV” index is equal $\bar{x} = 53.22$ ($SD = 3.434$, min-max = 47.6-59.1, $V = 6.45$, $n = 12$; in two syntypes 53.7 and 56.0), the “ptd” index for the main branch of the external claw IV is $\bar{x} = 40.10$ ($SD = 2.634$, min-max = 35.7-43.8, $V = 6.57$, $n = 12$; in two syntypes 33.3 and 36.0). Terminal apophyses of the buccal tube small, barely visible and located at large pharyngeal apophyses.

Pharynx spherical, medium sized (27 x 29 μm), with distinct, somewhat conical pharyngeal apophyses and two small corn-like macroplacoids
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(Figs 1, 6, 8). Microplacoid lacking. The apophyses 1.8 μm long, distinctly shorter than the first macroplacoid, the latter with a small external constriction at its middle (Figs 6, 8: arrowheads). This constriction is only well discernible in LM at the magnification 1250 times. The second microplacoid without constriction, and only slightly smaller that the first macroplacoid. Macroplacoid row 5.4 μm, the first macroplacoid 2.3 μm, the second 1.8 μm long, both c. 2.0 μm wide.

Legs relatively short and without cuticular bars at the claws' bases. Claws small and stout, without lunules (Figs 2-5, 14). Their main branches with large and off-standing accessory spines, which are particularly well developed on internal claws (Figs 2, 3, 14). Bases of main branches of external claws with a characteristic refractive zone (Figs 2-5, 14), in LM resembling an "empty" part of the branch (e.g. Fig. 3: arrowhead). This part has a somewhat collapsed appearance in SEM preparations (Fig. 14: arrowhead). A similarly structured fragment occurs in the upper part of the claw base, at the secondary branch (e.g. Fig. 4: arrowhead). These refractive units appear to enhance the flexibility of the main branches. Internal claws mostly distinctly sub-superficially sculptured (Figs 2, 14: arrows). External claw I, 8 μm long and claw IV, 12 μm in length. The refractive zone 2 μm long in the main branch of 8 μm length.

Eggs small, whitish, and laid freely on substrate. The egg examined in LM (SEM) is 74 (52) μm in diameter with processes, 63 (43) without them. The egg shell is covered with greatly elongated conical (or even spine-like) flexible processes tapering to rather sharp tips (Figs 9-11, 15-18). The processes are 5.5-8.0 μm long and 1.3-2.5 μm wide at the base. Interprocess area almost smooth, with tiny, sparsely distributed pits (e.g. Figs 16, 17: arrowhead, 18: arrows), not definable by LM.

The characters observed in material from Thulin's Collection agree well with those from Mt. Festkogel, except for the striation of the pharyngeal tube, not recognized in glycerio-gelatine mounted specimens.

**Biology and ecology**

The mode of reproduction is not known. The most comprehensive data on the ecology of H. conjungens are still those by Dastych (1980, 1987, 1988), based mainly on material from the Tatra Mts. Owing to its preferred occurrence in subnival and alpine zone, H. conjungens represents there an eualpine element and dominates in bryophytes and lichens from rocks, indicating its lithophilous character. The taxon was collected exclusively on non-carbonate, acid or (pH) neutral rocks such as granite, gneiss, quartzite etc. (1690 specimens in 100 samples: Dastych 1980), being totally absent from carbonate (basic) rocks like limestone, dolomite or marl. This indicates distinct acalciphilous character of the species and its indicatory role for non-carbonate rocky habitats. In the Tatra Mts H. conjungens represents an euryhygric and photophilic form with a strong predilection for southern, i.e. warmer and rapidly drying slopes (SW, S, SE) and open habitats and was absent in samples from cold, northern slopes (N, NE; see Dastych 1980: Fig. 350).
Material from Mt. Festkogel confirms the eualpine character of *H. conjungens* and its predilection for non-carbonate type of bed-rock. Also most recent data (Newfoundland: Collins & Bateman 2001, Greenland: Peters & Dumjahn 1999) and some other publications reporting the species (e.g. Dastych 1984, 1987, Kathman 1990, Szymanska 1994), where the kind of bed-rock was originally not specified or it could be later reconstructed, confirm also the acalciphilous character of *H. conjungens*. As far as we know, two records of *H. conjungens* from Slovenia and Italy, reporting individuals and eggs from mosses at the entrance of the caves San Canziano (= Grotten von St. Kanzian, Škocjanske Jame) in the Karst and Spluga delia Preta near Verona (Bertolani 1941, Mascardini & Bertolani 1963, respectively), might be the only reports of *H. conjungens* from calciferous rocks. Nevertheless, the kind of bed-rock was originally not specified by the authors. In our opinion the presence of the species on calciferous bed-rock should be confirmed.

**Distribution**

An attempt of a synthesis of distribution of *H. conjungens* is provided by Dastych (1980), based on literature data and autecological information about the species from the Tatra Mountains (*i.e.*, Figs 230, 231, 232). The taxon is classified there as a boreal-mountain element with a Holarctic range. The species was then, however, also reported outside this region, *i.e.* from the Andes (Chile: Ramazzotti 1962, Argentina: Mihelčič 1967, 1971). Subsequently Dastych (1988) transferred the species to the arctic-alpine sub-element, considering the latter as part of a larger, northern-mountain element (which included also the boreal-mountain one).

*H. conjungens* has also since 1980 been reported from several localities throughout the Holarctic, a part of the review being provided by McInnes (1994). Moreover, the species was recorded in New Zealand (Horning *et al.* 1978, Nelson & Horning 1979), Patagonia (Maucci 1988) and the Antarctic (Utsugi & Ohyama 1991, 1993, Hiruta & Ohyama 1995). Records of *H. conjungens* published after a survey by McInnes (*i.e.*) or not listed there, are from Iceland (Maucci 1996), Greenland (Maucci 1996, Peters & Dumjahn 1999), Barentsøya (Svalbard: Van Rompu & De Smet 1991), Scotland (Morgan & Lampard 1986), Russia (Biserov 1991, 1996a, b, 1999), Canada (Axel Heiberg Island: Szymanska 1994; Newfoundland: Collins & Bateman 2001) and Argentina (Rossi & Claps 1989). However, in the light of recent descriptions of two new congeners very similar to *H. conjungens*, *i.e.*, *H. schusteri*, and *H. ryani* from Antarctica, records of *H. conjungens* outside the Holarctic should be verified and the identifications based also on the species-specific morphology of the egg shell. The opinion is here expressed that the reports of *H. conjungens* from Antarctica represent misidentification.

To our knowledge, *H. conjungens* has only been reported from the Alps by Ramazzotti (1945: Trentino-Alto Adige) and Robotti (1972: Gran Paradiso). However, the species probably occurs widely at higher altitudes in the Alps, dwelling in bryophytes and lichens on open, non-carbonate rocks.
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The taxon is new for fauna of Austria. In Germany it was recorded for the first time in Schwarzwald (Black Forest) by R. Schuster (Feldberg, 1300 m a.s.l., in moss on non-calciferous rock, May 2001) (unpublished pers. comm. to H. D.).

Comments

The original description of *H. conjungens* by Thulin (1911) lacks a differential diagnosis. However, the illustrations (l.c.: Figs 20, 20a) show a markedly elongated unit of the buccal tube between the stylet supports and anterior wall of the pharynx. Moreover, Thulin (l.c.) suggested an intermediate status of *H. conjungens* between *Hypsibius pallidus* Thulin, 1911 and *Diphascon oculatum* Murray, 1906, due to the flexible (pharyngeal) tube in the former taxon. Original description of *H. conjungens* lacks also data on the characteristic striation of the tube, since the structure is not recognizable in glycerogelatine mounted specimens. The striation was mentioned for the first time by Cuénot (1932) ("une légère ondulation") in material from the Vosges. Cuénot was also the first to describe and illustrate eggs (l.c.: Fig. 73-1), their shell morphology and mode of deposition free on the substrate. In 1972 Robotti re-discovered the striation which was the base for the transfer to *Diphascon*. Due to the strikingly short pharyngeal tube and the presence of egg processes in *D. conjungens* and *D. schusteri*, compared to other congeners in *Diphascon*, Dastych (1988) suggested a separate generic status for the two taxa. Independently, Pilato (1987) re-described the buccal apparatus of *D. conjungens* and *D. schusteri* and created for these species the new genus *Hebesuncus*. His generic diagnosis, however, did not include characters of egg morphology and mode of their deposition, which is different from most of the Hypsibiidae.

Taking into account the poor knowledge on the distribution of *H. conjungens*, particularly the boundary of its range and the insufficient information about its biology and ecology, it is still preferred to consider the taxon as an arctic-alpine sub-element, as originally suggested by Dastych (1988). At present such a consideration explains the evident borealpine type of disjunction of this species (*sensu* Holdhaus 1954; = northern-mountain, Dastych l.c.). A more thorough discussion on the borealpine disjunction of some tardigrades (including *H. conjungens*), as was given for bumblebees by Reinig (1965), would be premature. Nevertheless, when using the interesting proposal of Reinig (l.c.) and considering available data, *H. conjungens* could be placed well within one particular category suggested by Reinig (l.c.), namely of taxa with arctic-subnival or subnival-arctic disjunction, depending on the centre of origin of the species and the direction of its dispersion. Similarly, it is still considered as premature to speculate on the centre of origin of *H. conjungens* and *Hebesuncus* in general. However, the worldwide distribution of *Hebesuncus* suggests pre-Pleistocene age of its origin. The genus was tentatively placed in the Diphasconinae by Dastych (1992), then with some fragmentary notes on its phylogeny. It should be noted that so far no member of *Hebesuncus* has been reported from the Paleotropical and Australian Regions.
Zusammenfassung

Das arktisch-alpine Bärtierchen *Hebesuncus conjungens* (Thulin, 1911) (Hypsibiidae) wird nach Material vom Festkogel (Ötzaler Alpen, Nordtirol) und der Typenserie wiederbeschrieben und seine Ökologie und Verbreitung werden diskutiert. Die Art ist neu für die Fauna von Österreich.

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References


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