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A new tardigrade species of the genus *Ramazzottius* BINDA & PILATO, 1986 (Tardigrada) from the nival zone of the Mont Blanc Massive (the Western Alps), with some morphometric remarks

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ABSTRACT. – A new tardigrade species, *Ramazzottius nivalis* sp. nov., from lichens inhabiting the nival zone in the Mont Blanc Massive (the Savoyer Alps, France), is described. The taxon differs from other congeners mainly by strikingly long thread-like processes on the eggshell, and very long light-refracting unit of markedly long external claws' main branches. Some remarks on morphometric indices of the buccal apparatus and claws are presented.

KEYWORDS: Tardigrada, taxonomy, *Ramazzottius nivalis* sp. nov., nival zone, the Mont Blanc Massive, the Western Alps, France, morphometrics

Introduction

Little is known about tardigrade fauna of the highest mountain group in the Alps, i.e., the Mont Blanc Massive. The first and only information is that of one of the pioneers of high altitudinal biological studies, Ch. G. EHRENBERG (1860). He reported amongst various microorganisms collected in the massive area (the nunataks of "Grand-Mulets-Felsen", "10 000' Höhe", = c. 3050 m), also on the two tardigrade species, *Echiniscus arctomys* EHRENBERG, 1853 and *Macrobiotus hufelandi* SCHULTZE, 1834.

Recently my colleague, MATTHIAS UPHUES, made available to me a small collection of bryophytes and lichens from a nival zone of the Mont Blanc massive. One of samples contained several individuals and eggs of a new species of the genus *Ramazzottius* BINDA & PILATO, 1986. This new taxon is described in the present paper, several new morphometric indices are introduced and some remarks on (eu)tardigrade morphometry are presented.

Material and Methods

A small, air-dried sample of lichens for this study comes from the Savoyer Alps in France. The tardigrades and the eggs were extracted by the method described by DASTYCH (1985) and examined with phase- and differential interference contrast microscopy (ZEISS "Photomikroskop III" and "Axioskop 2"). The material (11 specimens and two eggs) is mounted permanently on two microslides in FAURE'S medium and deposited at the Zoologisches Museum Hamburg and the Museum d'Histoire Naturelle, Geneva.

Measurements, including the length of the buccal tube, are taken as described in DASTYCH (2002). The (external) width of the mouth cavity is measured at the level of dorsal transversal ridges. The anterior part of buccal tube (= *SSA* unit) is the distance between the upper (dorsal) edge of the stylet sheaths and the anterior (fore) edge of the stylet support insertion, just at its point of attachment to the tube's wall. The posterior part of the tube (= *SSB*) is taken from the anterior edge of the stylet support to the posterior edge of the tube. This distance includes the terminal posterior apophyses of the tube, but excludes the pharyngeal apophyses. Thus, the value of *SSB* unit includes the width (thickness) of the stylet support base insertion. The length of claws comprises the accessory spines. The length of the light-refracting unit (= *LRU*) is the distance between the terminal (proximal) edge of poorly sclerotized basal part of the claw's main branch and the beginning of its solid (= "normally") sclerotized section (see e.g., Fig. 13, arrows). The border between the two parts is mostly very distinctly marked.

Most of the morphometric indices and coefficients are explained in DASTYCH et al. (2003), DASTYCH (2004a, b), other are commented in the § "Morphometric remarks". These indices are based on the morphometry of the buccal apparatus structures, claws and are listed below.

1) the **w**hole buccal **t**ube length **i**ndices (abbreviated here *"WTI*"; = "*pt* indices", see PILATO 1981) describe the (percent) ratio between the length of the whole buccal tube and that of other relevant structure (= length of structure x 100 / length of the buccal tube);

2) the **p**osterior buccal tube **u**nit **i**ndices (*"PUI*"), related to *WTI* and introduced here, describe the (percent) ratio between the length of buccal tube below the stylet support insertion (= *SSB*) and that of other relevant structures (*SSB* length x 100 / length of structure);

3) **m**acro**pl**acoid **i**ndex (*"MPLI*") characterizes the size ratio between the second and the first macroplacoid (= *mpl*2 length x 100 / *mpl*1 length);

4) the **e**xternal **c**laws' **i**ndex (*"ECI*") describes the length ratio between the first external claw and the hind claw (= external claw I length x 100 / hind claw length);

5) the **i**nternal **c**laws' **i**ndex ("*ICI*": not applied in this paper, but listed here for overview: see also DASTYCH 2004a, b) is the ratio between the first internal claw and the fore claw (= internal claw I length x 100 / fore claw length);

6) the claws' **m**ain **b**ranch **i**ndex ("*MBI*"), characterizes the size ratio between the external main branches of anterior and posterior claws, i.e., on the first and the fourth pair of legs (= the external claw I branch length x 100 / hind claw main branch length);

7) the **h**ind claw **b**ase **i**ndex (*"HBI"*; in DASTYCH 2004a, b = *"hcbi* index") defines the size ratio between the hind claw base and its main branch (= hind claw base height x 100 / hind claw main branch length);

8) a new indicator, the **h**ind claw **l**ight-**r**efracting **u**nit **i**ndex (*"HLRUI*") characterises the (percent) length ratio between the claw's main branch and its *LRU* (= *LRU* length x 100 / hind claw main branch length);

9) the **h**ind claw **s**econdary **b**ranch **i**ndex (*"HSBI"*), also introduced here, defines the (percent) length ratio between the hind claw secondary branch and the claw's base [= hind claw secondary branch length x 100 / hind claw base length (= height)].

For comparative analysis some other species of *Ramazzottius* were examined, mostly their type specimens, viz. *R. cataphractus* MAUCCI, 1974, *R. ljudimlae* BISEROV, 1998, *R. caucasicus* BISEROV, 1998, *R. andreevi* BISEROV, 1998, *R. ruperus* BISEROV, 1999, *R. subanomalus* (BISEROV, 1985), *R. baumanni* (RAMAZZOTTI, 1962), *R. tribulosus* BERTOLANI & REBECCHI, 1988, *R. oberhaeuseri* (DOYÈRE, 1840), *R. theroni* DASTYCH, 1993, and *R. velaamnis* BISEROV & TUMANOV, 1993.

Abbreviations used are: *DIC*- differential interference contrast, *ECI* - external claws' index, *EPI* - egg processes' index, *HBI* - the hind claw base index, *HLRUI* - the hind claw's main branch light-refracting unit index, *LM* - light microscope, *LRU* - light-refracting unit, *n* - sample size, *MBI* - claws' main branch index, *min-max* - minimum-maximum range, *mpl* - macroplacoid, *MPLI* - macroplacoids' index, *PHC* - phase contrast, *PT* - the whole buccal tube indices (comp. PILATO 1981; = *WTI* indices), *PUI* - indices of the posterior part of the buccal tube (= *PU* indices),, r^2 - coefficient of determination, *SSA* - the buccal tube anterior unit (the distance between stylet sheats and stylet support insertion), *SSB* - the buccal tube posterior unit (the distance between stylet support insertion and terminal posterior apophyses), *SD* - standard deviation, *ss* - stylet support, *V* - coefficient of variation, *WTI* - the whole buccal tube indices (= *pt* indices), *WT SSA* - stylet supports "anterior" index (= *pt ss*"), *WT SSB* - stylet supports "posterior" index, *ZMH* - Zoologisches Museum Hamburg, $\bar{x}$ - (arithmetic) mean.

Description of species

Ramazzottius nivalis sp. nov.
(Figs 1-23)

H o l o t y p e. – (Figs 1, 4); sex unknown, 229 μm long (coll. M. UPHUES, 30 Juni 2005), mounted on microslide in FAURE'S medium together with seven paratypic animals and two such eggs. The holotype is mounted dorso-ventrally, the apices of its both main branches of the hind claws point laterally the same direction (Fig. 4, arrowheads). The microslide (No. T966) has been deposited in the Zoologisches Museum Hamburg (ZMH Acc. No. A14/06).

T y p e l o c a l i t y. – The Savoyer Alps, the Mont Blanc Massive, the Cosmique Ridge (Arête des Cosmiques) at the Mt Aiguille du Midi, nival zone, E slope, 3707 m a.s.l. A small (c. 2 cm^2) sample of an orange-reddish crustaceous lichen from a silicate rock. Several rotifers in the sample, no other tardigrade species.

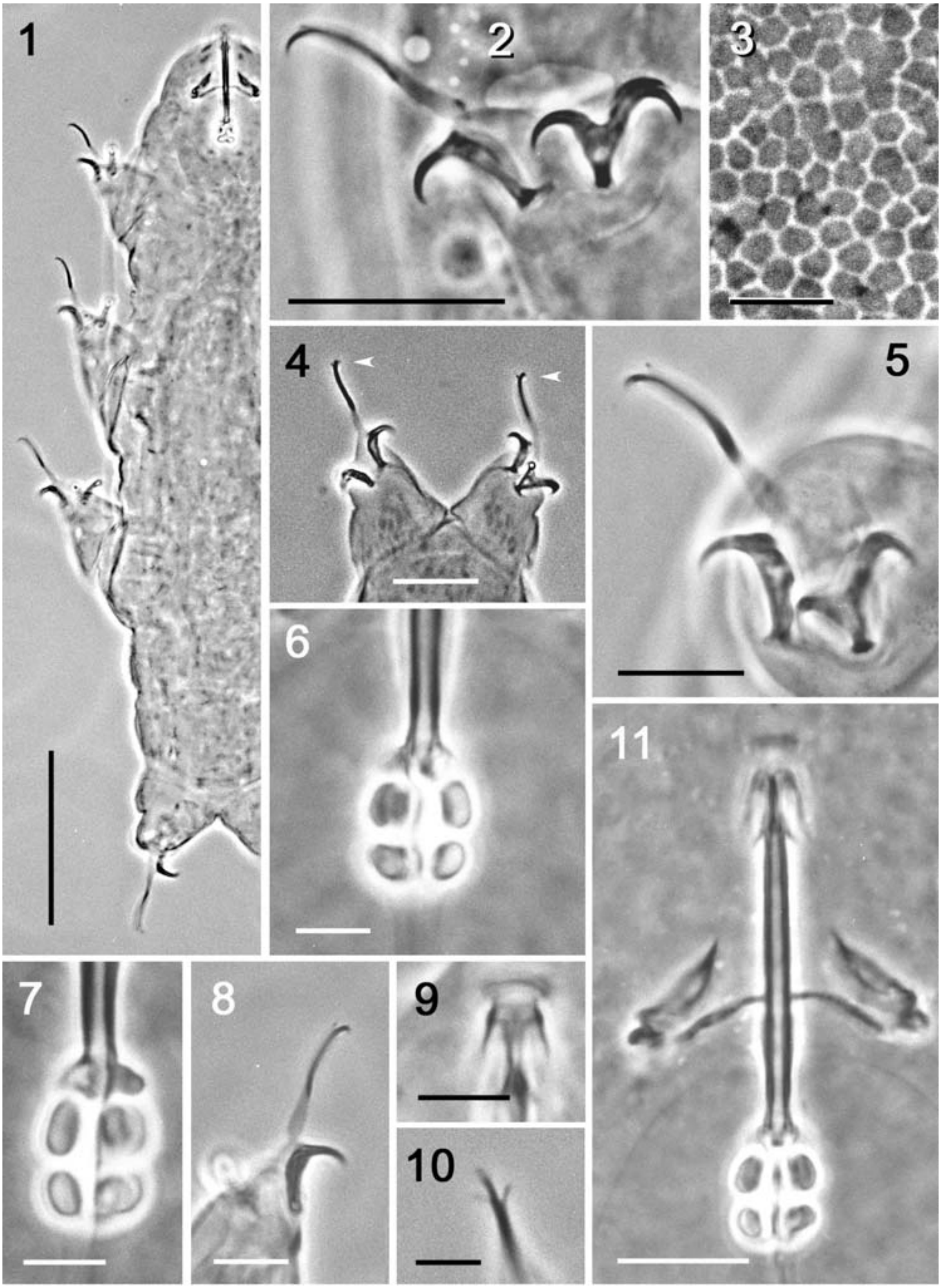
P a r a t y p e s. – Locality data as above: altogether ten animals, one non-embryonate egg (and remnants of another egg sticked to the dorsum of one individual) mounted in FAURE'S medium on two microslides (slide No. 1: seven paratypes and holotype (see above for Acc. No.); slide No. 2 (No. T965, ZMH Acc. No. A15/06): three paratypes). The latter microslide is deposited in the collection of the Museum d'Histoire Naturelle (Arthropodology & Entomology), Geneva, Switzerland.

E t y m o l o g y. – Named for the nival zone (*nix*, *nivis*, *nivalis*, L. = snow, snowy) where the species was found.

D i a g n o s i s. – Median sized *Ramazzottius* with granulated cuticle and very long main branches of external claws; the latter with strikingly long, light-refracting basal unit. Freely layed eggs, their small cone-shaped processes terminated with very long, thread-like tips. The processes and interprocess area smooth.

D e s c r i p t i o n. – Body, 204-358 μm long (holotype 229 μm) reddish, with more or less distinct chequered pattern, largest specimens with small clumps of dark-brown pigment. Dorsum, lateral sides of the body and legs sculptured, sculpture composed of indistinctly formed, slightly convex and polygonal thickenings (Fig. 3). The latter increasing modestly in size posteriorly, 1.5-2.0 μm wide, rarely up to 2.7 μm. Eye-dots absent, head elliptical sensory structures poorly visible.

Bucco-pharyngeal apparatus (Fig. 11) median sized. Ventro-anteriorly mouth opening. Mouth cavity median sized, some specimens dorsally and ventrally with two hardly visible granules representing lateral ridges; no other buccal structures visible in *LM*. Buccal tube narrow, with distinctly thickened wall below stylet support insertion. Terminal posterior apophyses of tube poorly developed, hardly visible. Pharynx spherical, or



Figs. **1-11**. *Ramazzottius nivalis* sp. nov.: **1**, animal in ventro-median view; **2**, claws II; **3**, dorsal sculpture (at the 3rd pair of legs); **4**, claws IV; **5**, claws II; **6-7**, pharyngeal armature; **8**, external claw III; **9**, mouth cavity and its region ; **10**, accessory spines, hind claw; **11**, bucco-pharyngeal apparatus (All *PHC*. Scale bars: Fig. 1: 50 µm, Figs 2, 4: 20 µm, Figs 3, 5, 8, 11: 10 µm, Figs 6, 7, 9, 10: 5 µm. Holotype: Figs 1, 4. Other explanations in text).

slightly sub-spherical, with relatively large pharyngeal apophyses (Fig. 16) and two small grain-like macroplacoids (Figs 6, 7, 11, 15, 16). First macroplacoid slightly longer than the second, indistinctly incised laterally in the middle of its length. Second macroplacoid, if at all, only with tiny lateral incision in its caudal part.

Claws moderately sized, but main (= primary) branches of external claws very long (Figs 1, 2, 5, 8, 12-14). Length of branches distinctly increasing posteriorly. A long part of basal unit of branches poorly sclerotized (Figs 5, 12-14). This section distinctly more pliable than remaining solid part of the branch and appearing as a strikingly long, differently light-refracting unit (*LRU*). The unit making *c*. 30-41 % (*n* = 9) of hind main branch. Border between two sections of the branch is mostly slightly oblique and sharply marked (Figs 12-14). Main branches of external and internal claws with thin but distinct accessory spines (Figs 10, 14), up to 3 μm in length. Secondary branch of external claws relatively long. All claws with poorly visible, lunula-like structures; the latter rather large on external claws. No transversal bar-like thickening below the claws' bases on legs I-III.

One whitish egg and a remnant of another one, both non-embryonated, have been found. The eggshell bears small cone-like processes, their apices are prolongated in strikingly long, thread-like tips (Figs 17-23). Processes sparsely distributed and their surface smooth. Bases of processes and interprocess area smooth.

Morphometric data

Measurements are in μm, all indices in %. Their values are presented in the following convention:

$\bar{x}$ ± *SD* (*min-max*) [*n*] * *V* (for measurements);
$\bar{x}$ ± *SD* (*min-max*) [*n*] * *V* / r^2 (for indices).

For the abbreviations and definitions see § "Material and methods". The morphometrics of the holotype (229 μm long) is separated from other data by a dot (●), its bucco-pharyngeal apparatus is 40.5 μm long, and pharynx 22.5 μm in diameter.

Individuals

A) Measurements (μm)

Body length	269.10 ± 42.21 (204-358) [11] * 15.7 ● 229.6
Mouth cavity (ext.) width	2.72 ± 0.51 (1.8-3.2) [6] * 18.8 ● 2.7
Buccal tube length	26.00 ± 3.18 (18.9-29.7) [9] * 12.3 ● 26.1
SSA length (tube above stylet supports)	16.20 ± 1.90 (12.2-18.5) [9] * 11.7 ●16.2
SSB length (tube below stylet supports)	10.20 ± 1.10 (7.7-11.3) [9] * 10.8 ● 9.9
Buccal tube width (external)	2.42 ± 0.33 (1.8-2.7) [8] * 13.8 ● 2.3
Buccal tube width (internal)	0.79 ± 0.13 (0.6-0.9) [8] * 15.9 ● 0.6
Macroplacoid row length	6.10 ± 0.90 (4.5-7.2) [9] * 14.8 ● 6.3
Macroplacoid 1 length	3.02 ± 0.50 (2.0-3.6) [9] * 16.7 ● 3.2
Macroplacoid 2 length	2.42 ± 0.41 (1.8-2.8) [9] * 17.0 ● 2.7
External claw 1 length	20.19 ± 1.78 (23.4-18.0) [8] * 8.8 ● 18.9
External claw 1 base height	8.10 ± 1.07 (6.3-9.0) [8] * 13.3 ● 7.2
External claw 1 main branch length	13.80 ± 0.37 (11.7-15.3) [9] * 8.2 ● 13.5
Hind claw (= ext. 4) length	27.10 ± 3 .23 (30.2-19.8) [9] * 11.9 ● 27.5
Hind claw base height	9.55 ± 1.54 (6.3-11.7) [9] * 16.1 ● 9.5
Hind claw main branch length	18.75 ± 2.07 (14.9-20.7) [9] * 11.1 ● 19.8
Hind claw main branch *LRU* length	6.60 ± 1.33 (4.5-8.6) [9] * 20.2 ● 7.2
Hind claw secondary branch length	7.60 ± 1.18 (5.4-9,0) [7] * 15.6 ● 7.2

B) Indices
1) *WTI* (the **w**hole **t**ube length **i**ndices) (= “*pt* indices”):

WT mouth cavity (ext.) width	9.93 ± 0.86 (9.1-11.3) [6] * 8.7 / 89.3 ● 10.2
WT SSA (tube above stylet supports)	62.83 ± 2.62 (59.7-67.8) [9] * 4.2 / 93.6 ● 63.8
WT SSB (tube below stylet supports)	37.97 ± 4.11 (27.6-40.74) [9] * 10.7 / 92.2 ● 37.9
WT buccal tube width (ext.)	9.39 ± 0.41 (8.6-9.2) [8] * 4.4 / 88.9 ● 8.6
WT buccal tube width (int.)	3.07 ± 0.43 (2.4-3.8) [8] * 14.2 / 38.5 ● 2.4
WT macroplacoid row length	23.21 ± 1.51 (20.4-25.8) [9] * 6.5 / 82.2 ● 24.3
WT macroplacoid 1 length	11.65 ± 0.70 (10.5-12.9) [9] * 6.1 / 92.7 ● 12.1
WT macroplacoid 2 length	9.29 ± 0.99 (7.4-10.3) [9] * 10.7 / 60.8 ● 10.3
WT claw 1 (ext.) length	78.71 ± 8.11 (72.4-95.3) [7] * 10.4 / 53.0 ● 72.4
WT claw 1 (ext.) main branch length	52.85 ± 6.83 (42.7-66.7) [8] *12.9 / 49.7 ● 51.7
WT claw 1 (ext.) base height	31.05 ± 2.15 (27.6-33.3) [7] * 6.9 / 75.9 ● 27.6
WT hind claw (= ext. 4) length	105.2 ± 5.53 (97.0-114.8) [8] * 5.3 / 83.2 ● 105.2
WT hind claw main branch length	76.69 ± 4.17 (66.6-78.6) [8] * 5.7 / 81.3 ● 75.8
WT hind claw base height	37.12 ± 2.69 (33.3-40.7) [8] * 7.3 / 89.1 ● 34.5
WT hind claw *LRU* main branch length	26.07 ± 3.49 (22.2-30.6) [8] * 13.4 / 52.8 ● 27.6
WT hind claw secondary branch length	28.15 ± 1.86 (25.4-30.3) [6] * 6.62 / 83.6 ● 27.1

2) *PUI* (the **p**osterior tube **u**nit length **i**ndices)

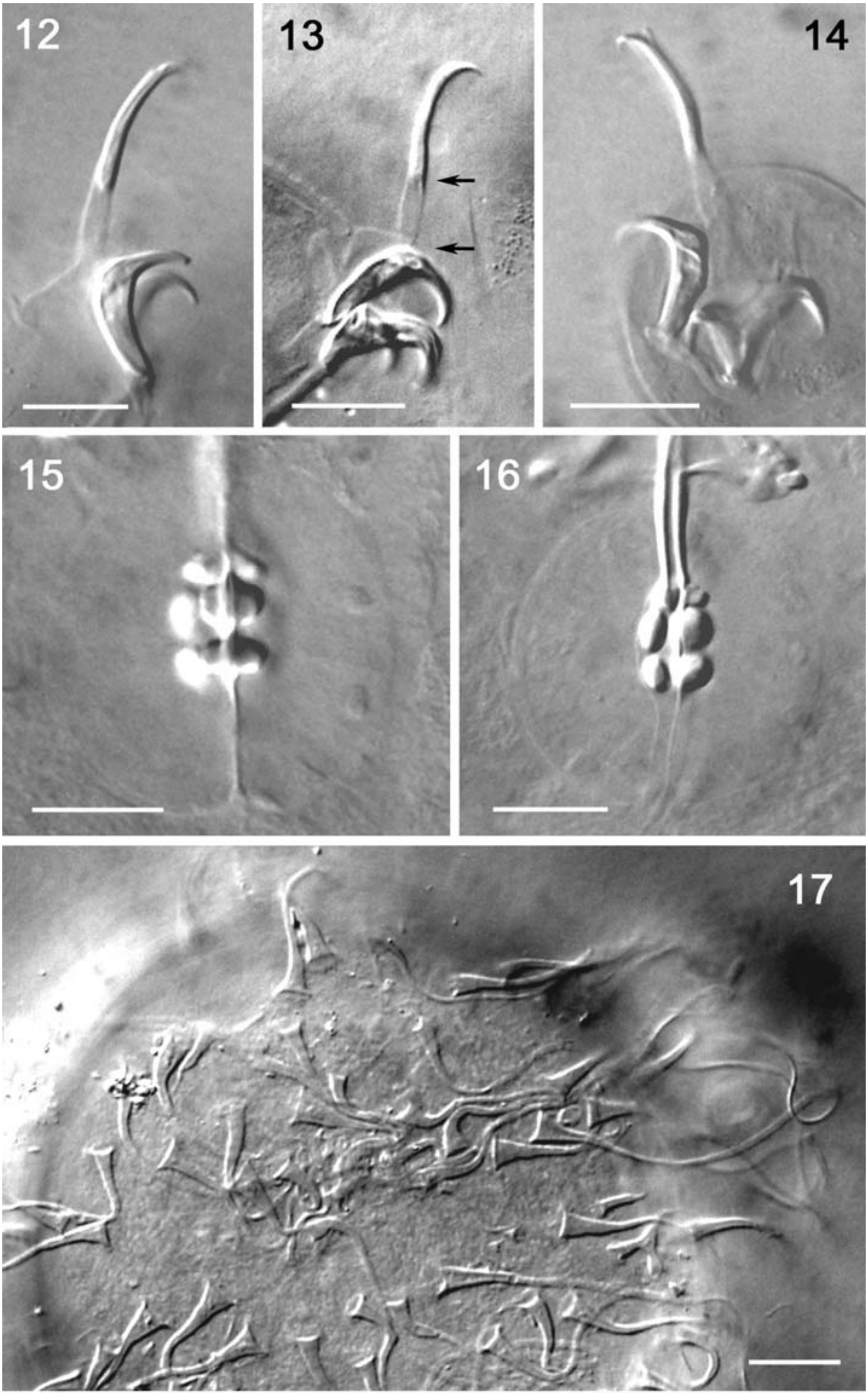
PU mouth cavity (ext.) width	26.05 ± 2.16 (24.0-29.2) [6] * 8.28 / 74.3 ● 26.1
PU buccal tube width (ext.)	24.09 ± 1.04 (22.7-25.0) [8] * 4.3 / 86.9 ● 22.7
PU buccal tube width (int.)	7.6 ± 1.01 (6.3-9.4) [8] * 12.9 / 33.4 ● 6.3
PU macroplacoid row length	59.92 ± 4.83 (52.4-66.7) [9] * 8.1 / 73.3 ● 63.6
PU macroplacoid 1 length	29.20 ± 3.24 (24.0-33.4) [9] * 11.1 / 81.1 ● 31.8
PU macroplacoid 2 length	23.80 ± 2.82 (19.0-27.3) [9] * 11.9 / 57.2 ● 27.3
PU claw 1 (ext.) length	200.70 ± 18.26 (187.5-235.3) [9] * 9.1 / 51.2 ● 190.9
PU claw 1 (ext.) base height	78.97 ± 4.33 (72.7-83.3) [8] * 5.5 / 76.3 ● 72.7
PU claw 1 (ext.) main branch length	141.00 ± 14.15 (118.2-164.7) [7] * 10.3 / 39.5 ● 163.3
PU hind claw length	268.90 ± 9.36 (257.1-281.8) [8] * 3.5 / 94.4 ● 277.2
PU hind claw base height	94.48 ± 7.93 (82.4-108.3) [8] * 8.4 / 80.9 ● 90.9
PU hind claw main branch length	186.50 ± 8.64 (175.0-200.0) [8] * 4.6/ 93.2 ● 200.0
PU hind claw main branch *LRU* length	71.08 ± 12.16 (57.1-94.1) [8] * 17.1 / 67.9 ● 72.7
PU hind claw secondary branch length	73.10 ± 5.54 (64.0-80.0) [6] * 7.6 / 76.1 ● 69.6

3) Other indices

Macro**pl**acoid **i**ndex *(MPLI)*	91.09 ± 8.40 (66.6-90.9) [9] *10.4 / 73.0 ● 87.5
External **c**laws **i**ndex (*ECI*)	76.22 ± 8.01 (67.2-90.9) [6] *10.6 / 50.3 ● 68.7
Claw **m**ain **b**ranch **i**ndex (*MBI*)	74.90 ± 8.74 (61.9-87.8) [8] *11.7 / 28.7 ● 68.2
Hind claw **b**ase **i**ndex (*HBI*)	50.78 ± 4.96 (42.4-50.1) [9] * 9.8 / 79.7 ● 48.0
Hind claw ***LRU*** **i**ndex (*HLRUI*)	34.77 ± 4.02 (30.3-41.3) [9] * 11.6 / 80.7 ● 36.4
Hind claw **s**econdary **b**ranch **i**ndex (*HSBI*)	74.54 ± 3.74 (66.7-77.0) [5] * 5.23 / 69.0 ● 75.8

Eggs (n = 2).
The egg is 73 x 68 µm in size, excluding the processes. The processes, including those on the remnants of another egg, are up to 39 µm long, at the base 1.8-3.6 (usually 2.3-2.7) µm wide.

V a r i a b i l i t y. – Individuals of *R. nivalis* sp. nov. show relatively little intra-specific variability, as judging from the 11 available specimens. Also in this species the *WT SSA* index (= *pt ss*) indicates a very high degree of correlation between variables, with values of *r squared* equalling 93.6 %. Such a high association also characterizes the posterior unit of the buccal tube described by *WT SSB* index, with values equal to 92.2 %. The distribution of processes on the eggshell in the two available eggs is markedly variable, compared to their relatively constant shape and length.



Figs. **12-17**. *Ramazzottius nivalis* sp. nov.: **12**, hind claw; **13**, external claw III; **14**, claws II; **15-16**, pharynx; **17**, processes of the eggshell (All *DIC*. Scale bars: 10μm).

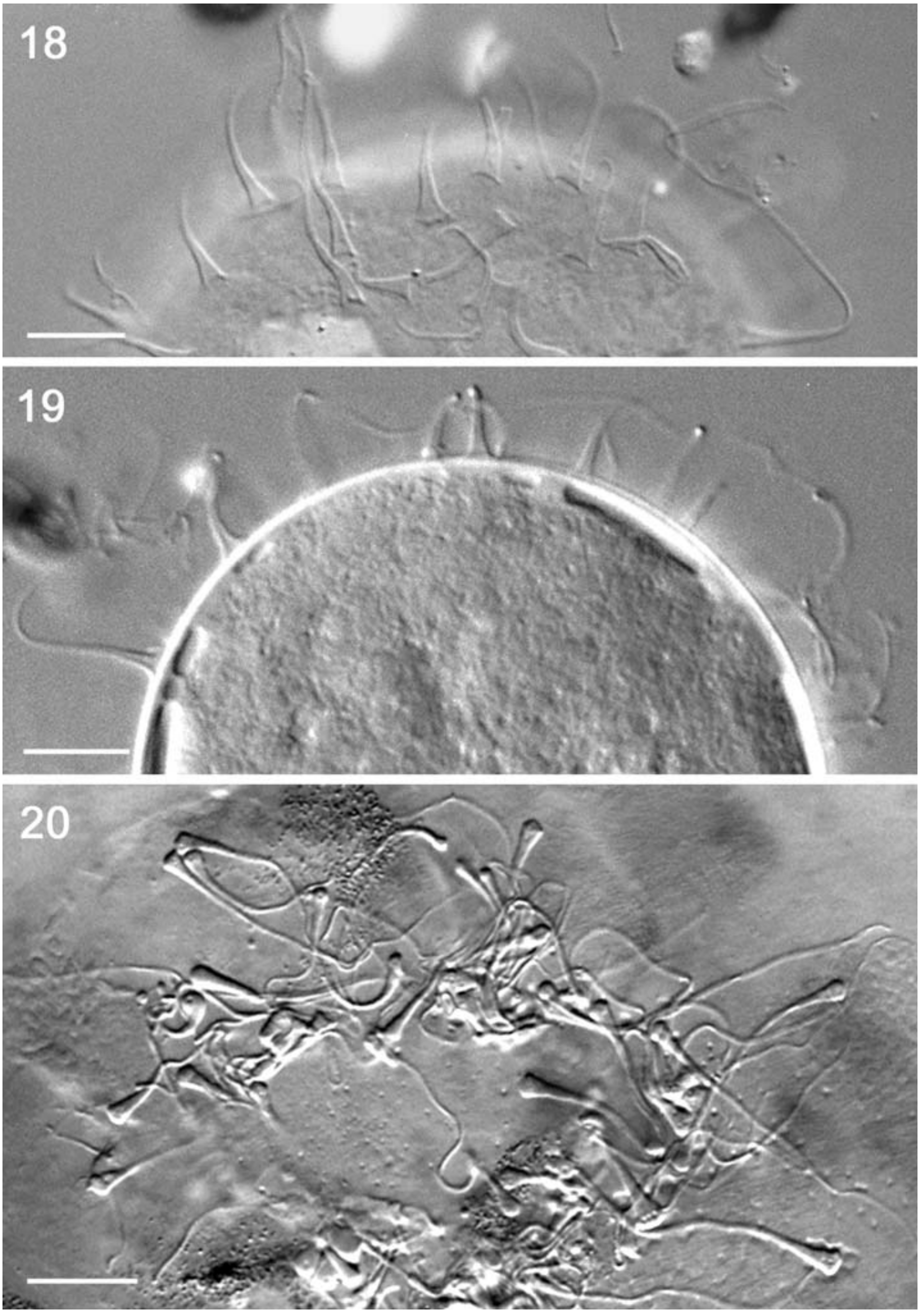
Differential diagnosis. – The new species can be easily separated from the 21 nominal taxa of *Ramazzottius* by the morphology of its eggshell with strikingly long and thread-like shaped processes, a character not known in any congener. Very long main branches of the external claws in *R. nivalis* sp. nov., resembling those in only two other species of the genus (*R. ljudmilae*, *R. cataphractus*), distinguish well the new species from the majority of other *Ramazzottius*.

The individuals of *R. nivalis* sp. nov. are most similar to those of *R. ljudmilae*, both species have accessory spines on the claws' main branches, external claws with markedly long main branches and narrow buccal tubes.

Nevertheless, the specimens of both taxa can be readily separated by the length of *LRU* (shorter in *R. ljudmilae* and decidedly longer in the new species (*HLRUI* index = 15.6% in *R. ljudmilae* holotype, 16.7 % in a paratype, but 30.3-41.3 % in *R. nivalis* sp. nov.: *n* = 9). Moreover, the new species has a distinctly wider mouth cavity [*WT* index, $\bar{x}$ = 9.9 (*n* = 6) vs. 6.3 (*n* = 5) in *R. ljudmilae*], wider (internal) tube [*WT*, $\bar{x}$ = 3.1 (*n* = 8) vs. 2.5 (*n* = 11)], shorter hind claws [*WT*, $\bar{x}$ = 105.2 (*n* = 8) vs. 119.2 (*n* = 11)], and longer secondary branches of hind claws [*HSBI*, $\bar{x}$ = 75.5 % (*n* = 5) vs. 52.6 % (*n* = 5), respectively] (comp. in part also BISEROV 1998, Table 3). Furthermore, in the new species macroplacoids are less incised and the bases of external claws are not (slightly) contracted in their middle, as in *R. ljudmilae*. The processes of the eggshell provide another well separating character. They are extremely long, thin and flexible in the new species, but short (no longer than 16 µm), truncate and stumpy in *R. ljudmilae*.

In the original description of *R. ljudmilae,* the range of the buccal tube length ("L buccal tube": "26.0-38.0" µm) and the length of *SSA* unit ("Ss": "16,7-25.5" µm) are provided, not the *WT SSA* index value (= "*pt* Ss"; see BISEROV 1998, Table 3). When calculating the *WT SSA* values, the range of this indicator equals 64.2-67.1 % (*n* = 11; in the holotype 67.6 %). In *R. nivalis* sp. nov the values are 59.7-67.8 % (*n* = 9) and 63.8 % for the holotype, respectively. The *WT SSA* index of the buccal apparatus of *R. ljudmilae* calculated from the original drawing (both sides are figured with slightly different length: BISEROV 1998, Fig. 5b) equals 59.8% on the left and 59.2 % on the right side of the tube. Thus, the total range of this index seems to be same for both species, making it irrelevant for discrimination.

R. nivalis sp. nov. differs clearly from the very similar *R. cataphractus* by accessory spines on the main branches of claws which are present in the new species but absent in latter taxon. Moreover, *R. cataphractus* has distinct transverse, wide bars under the claws' bases on legs I to III, the bars are absent in the new species. Individuals of the new species have, compared to *R. cataphractus,* narrower mouth cavity [*WT*, $\bar{x}$ = 9.9 (*n* = 6) vs. 13.7 (*n* = 7)], slightly narrower mouth tube [(*WT* ext., $\bar{x}$ = 9.4 (*n* = 8) vs. 10.7 (*n* = 11)], shorter hind claws [*WT*, $\bar{x}$ = 105.2 (*n* = 8), vs. 131.4 % in a paratype of *R. cataphractus*], longer *LRU* [30.3-41.3 %, *n* = 9, vs. 21.2 and 22.2 % (paratypes of *R. cataphractus*)], and longer secondary branches of hind claws [*HSBI*, $\bar{x}$ = 75.5, (*n* = 5) vs. 66.8 %, (*n* = 9), respectively]. The values of the *WT SSA* index in *R. cataphractus* ($\bar{x}$ = 63.3, *n* = 5) overlapping those of the new species (and *R. ljudmilae*), are also of little use for differentiation between these taxa.



Figs. **18-20**. *Ramazzottius nivalis* sp. nov.: **18-20**, processes of the eggshell (All *DIC*. Scale bars: 10 µm).

The egg processes in *R. cataphractus* are extremely variable in their shape and length (see DASTYCH 1985: Fig. 19, Plate XII f-j), but are never strikingly long and thread-like as in the new species. However, one should note that the original description of

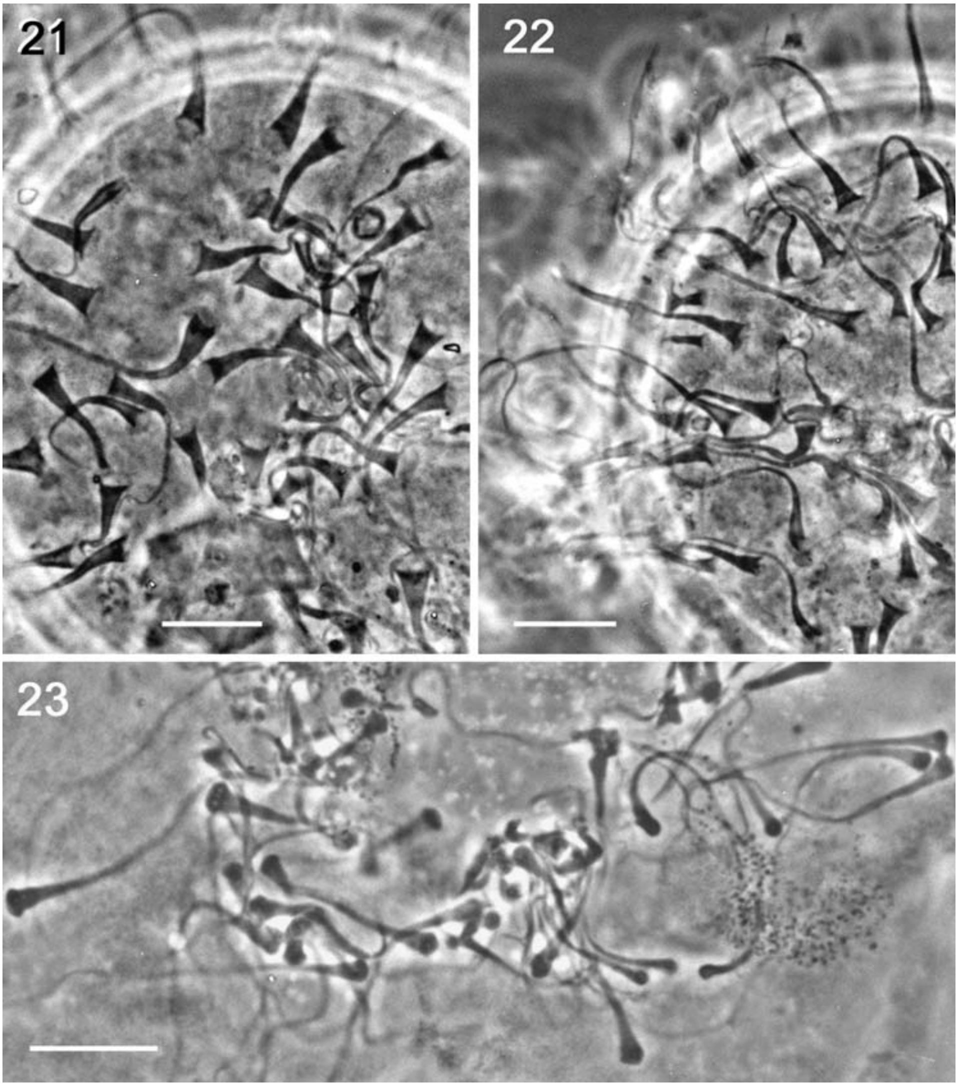
R. cataphractus only includes data on the morphology of animals. Their eggs are not described from the type locality (a vicinity of the mountain shelter Franz Joseph Haus in the Hohe Tauern Mts: comp. MAUCCI 1974) as they should be for the good characterization of the species.

M o r p h o m e t r i c r e m a r k s. – A widely used morphometric index in eutardigrades, so-called "stylet support insertion index", introduced by PILATO (1981) (= "*pt* stylet support", "*pt ss*", "*ssi*"; "*WT SSA*" in this paper) became a standardised characteristics in the descriptions of new taxa. It is also used for comparison between different populations (e.g., BISEROV 1990a, b). The index describes the length ratio between the buccal tube and its *SSA* unit and is considered as (one of) the most important indicator. It is assumed that the index is characterized by a low intraspecific variability (e.g., PILATO 1981, BERTOLANI & REBECCHI 1993; but see KINCHIN 1994) and a high association (correlation) between its both variables (e.g., DASTYCH 2005). However, the application of the index (and other indices as well) is often seriously restricted, but this fact is rarely discussed in the literature (e.g., KINCHIN 1994, 1996). Some of such restrictions are pointed out below. As a rule, one should carefully interpret all morphometric data.

1. A low variability of the *WT SSA* index (= *pt ss*), as measured by the coefficient of variation (= *vc, V*: e.g., BERTOLANI & REBECCHI *l.c.*, DASTYCH et al. 2003, respectively) often results from under-representation of juveniles in the sample data. This is quite common in taxonomic practice, e.g., BERTOLANI & REBECCHI, *l.c.* excluded "...small specimens, less than 270 µm..." from their statistical analysis of the *M. hufelandi*-group. The values of the index increase with the increasing age of animals (e.g., in *Macrobiotus, Ramazzottius, Hypsibius* EHRENBERG, 1848). Thus, the calculated results depend on the ratio of a particular age group (instars) in a given sample population. Consequently, a lack or under-representation of juvenile ("small") forms distort the morphometric characteristics of the species under consideration.

2. The range (*min-max*) of the *WT SSA* index, often used for discrimination between species, proved to vary considerably. The range is directly dependent on the number of measured adults and juveniles. The values of the index frequently overlap in similar (closely related?) taxa. A taxonomic decision is particularly unreliable when the index mean ($\bar{x}$) is calculated from different (often very limited) numbers of individuals and from specimens with unspecified body size (for variability of the latter factor see KINCHIN 1994, 1996).

3. The values of the *WT SSA* are more or less dependent on the mounting medium and its age, random measurement error, curvature of the buccal tube, orientation of the latter in the medium. Although these factors influence the result of measurements (thus the index values), there is, if at all, very little known about the role of each component (see e.g., KINCHIN 1994, 1996). Considering the curvature of the tube, the measuring error will be smaller in *Macrobiotus* or *Isohypsibius* due to relative straightness of the structure in these genera, but the error will remain larger in e.g., many *Ramazzottius*, *Hypsibius* and *Calohyopsibius* THULIN, 1928, where the tube is decidedly stronger curved. Thus, for



Figs. **21-23**. *Ramazzottius nivalis* sp. nov, processes of the eggshell (All *PHC*. Scale bars: 10 µm).

the genera with markedly curved tube, the application of the *PU* indices introduced in this paper (= *PUI*: see § "Material & methods") can be an important complementation of the *WT SSA* index. The *PU* indices are based on the posterior part of the tube which is relatively straight and less influenced by an error in measuring.

4. The values of the *WT SSA* index (and other ones?) are also sex-dependent, as demonstrated by BISEROV (1994) for *Macrobiotus seychellensis* BISEROV, 1994 and *Doryphoribius korganovae* BISEROV, 1994. Strangely, the author has not commented the phenomenon. The difference of the index values between males and females in these species is distinct, though the limited number of individuals studied (*l.c.*). These values may even be interpreted as those of two closely related but different taxa, when considering

some descriptions of new species in current taxonomic literature. It is possible that published *WT SSA* (= *pt ss*) values show an average of unknown proportion of males and females in a given sample. It should be noted that for the majority of tardigrade species it is not known if they are uni- or bisexual. One can suppose that morphometric dissimilarities might be a rule in bisexual (amphimictic) tardigrades. If so, then they would represent a separate quantitative character for females and males in the species considered.

5. It is not known if there are any differences in the values of morphometric indices in various cytotypes of the same species. The latter phenomenon has been reported in many tardigrade species (see e.g., BERTOLANI 1975, 1982, REBECCHI & BERTOLANI 1988, BERTOLANI & REBECCI 1993, BERTOLANI et al. 1987). Although some qualitative divergences between different cytotypes have already been documented (e.g., in eggshell morphology between uni- and bisexual populations of *Xerobiotus pseudohufelandi* (IHAROS, 1966): see BERTOLANI et al. 1987), no morphometric comparison of animals is available as yet.

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