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Ramazzottius agannae sp. nov., a new tardigrade species from the nival zone of the Austrian Central Alps (Tardigrada)

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(with 41 figures)

Abstract

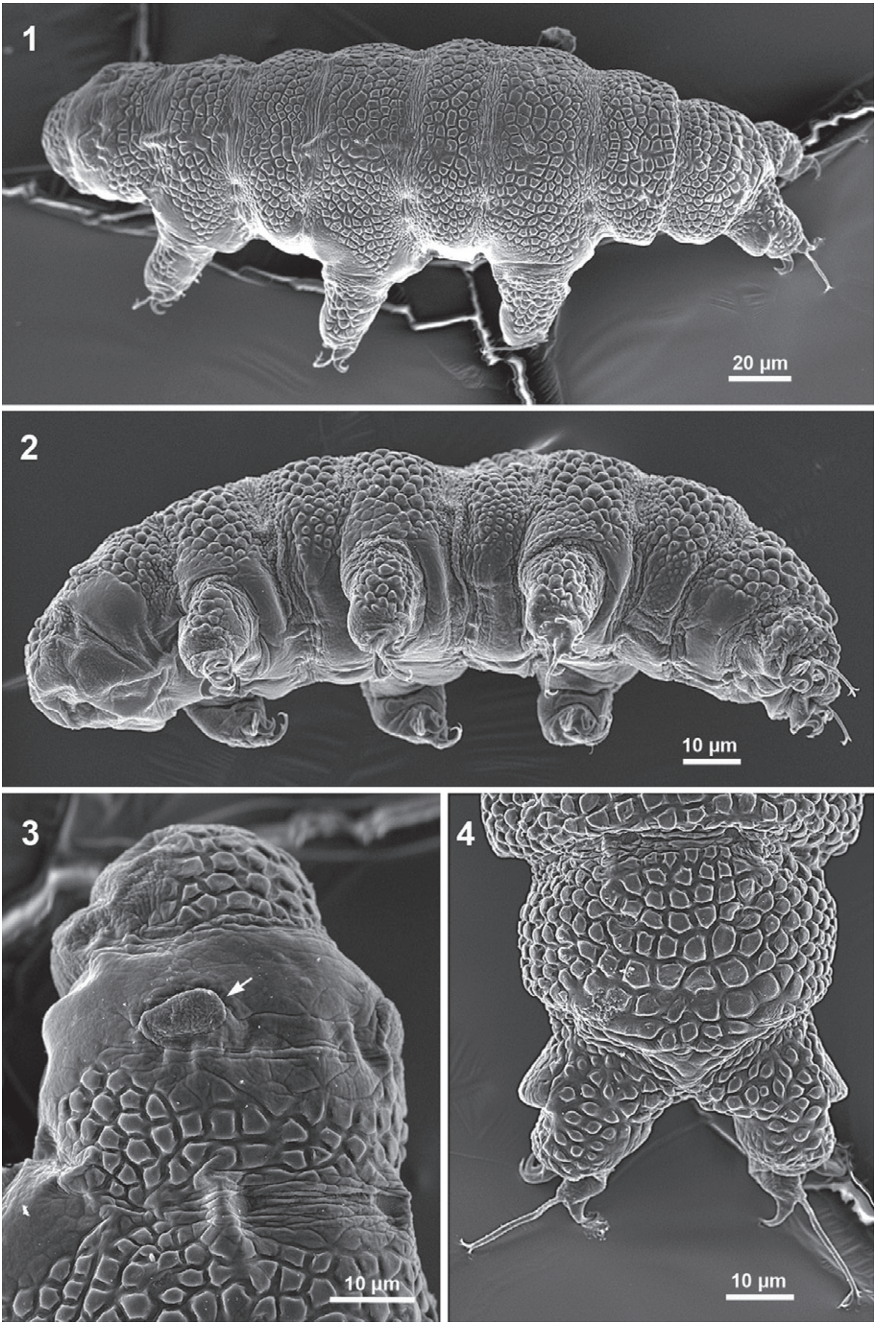
A new tardigrade species, *Ramazzottius agannae* sp. nov., from mosses, lichens and soil of the nival zone in the Ötztal- and Stubai Alps (Nordtirol, Austria), is described. The new taxon differs from other congeners mainly by the combined presence of strikingly large cuticular granulation, the long light-refracting unit of the external claws' main branches and morphology of the eggshell. Some new data on the similar taxon, *R. baumanni* (Ramazzotti, 1962), are provided.

Keywords: Tardigrada, taxonomy, *Ramazzottius agannae* sp. nov., nival zone, Central Eastern Alps, Austria.

Introduction

Tardigrades of the genus *Ramazzottius* Pilato & Binda, 1986 occur worldwide, being also a permanent component of polar and high-mountain microfauna. Several congeners, e.g., *R. cataphractus*, *R. montivagus, R. andreevi, R. ljudmilae, R. caucasicus* have been reported at an altitude above the timberline (Maucci 1974, Dastych 1983, Biserov 1997/98) and one taxon, *R. nivalis*, is known from the nival zone in the Western Alps (Dastych 2006). Recently the taxonomy of *Ramazzottius* has been partly revised (Biserov 1997/1998) and some new data on biology and presence of a cryptic species within *R. oberhaeuseri* (Doyère, 1840) have been reported (Faurby *et al.* 2008). *R. oberhaeuseri*, one of the first described tardigrades, is considered widely as 'omnipresent' and worldwide distributed species, but according to all new information, the records from high regions of the Alps (Calloni 1889a, b, Menzel 1914) need confirmation.

While working on alpine tardigrades I have encountered in the material from the nival zone of the Eastern Central Alps numerous specimens of a new remarkable species of *Ramazzottius*. In the present paper I describe this taxon and provide some new data on the morphology of its similar congener, *R. baumanni* (Ramazzotti, 1962).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Material and methods

The mosses, lichens and underdeveloped (mineral) soil for this study have been collected in the Ötztal- and Stubai Alps (Nordtirol). The samples come from three high-mountain localities and are listed at the description of the new species. The tardigrades and their eggs were extracted by the method described by Dastych (1985) and examined with phase- and interference contrast microscope (ZEISS "Photomikroskop III") and by scanning electron microscope (LEO 1525). For *SEM* examination specimens were washed, transferred either to hot Bouin's medium or hot 100 % ethanol, critical-point-dried and carbon-coated.

The morphometric indices and coefficients used in this paper are explained in Dastych *et al.* (2003), Dastych (2004a, b) and summarized in Dastych (2006). In the latter paper (*l.c.* 2006, p. 34) the formula for calculation of the "*PUI*" indices (= the **p**osterior **u**nit buccal tube **i**ndices) has been presented erroneously, i.e., it should read: "length of considered structure x 100 / *SSB* length" instead of: "*SSB* length x 100 / length of considered structure". Nevertheless, the values of *PUI* indices presented in that paper (*l.c.* 2006: p. 38) have been calculated in accordance with the *PUI* formula corrected above, thus all the data are valid for comparison with other taxa.

Abbreviations used are: *DIC* - differential interference contrast, *ECI* - external claws' 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, here applied: see below), *PUI* - indices of the posterior part of the buccal tube (= *PU* indices), *PVL* - polyvinyl-lactophenol, r squared (= r^2) - coefficient of determination, *SSA* - the buccal tube anterior unit (the distance between stylet sheaths and stylet support insertion), *SSB* - the buccal tube posterior unit (the distance between stylet support insertion and terminal posterior apophysis), *SD* - standard deviation, *SEM* - scanning electron microscope, *ss* - stylet support, *V* - coefficient of variation, *WTI* - the whole buccal tube indices (= *pt* indices: Pilato 1981), *WT SSA* - stylet supports "anterior" index (= *pt ss*), *WT SSB* - stylet supports "posterior" index, *ZMH* - Zoologisches Museum Hamburg, $\bar{x}$ - (arithmetic) mean.

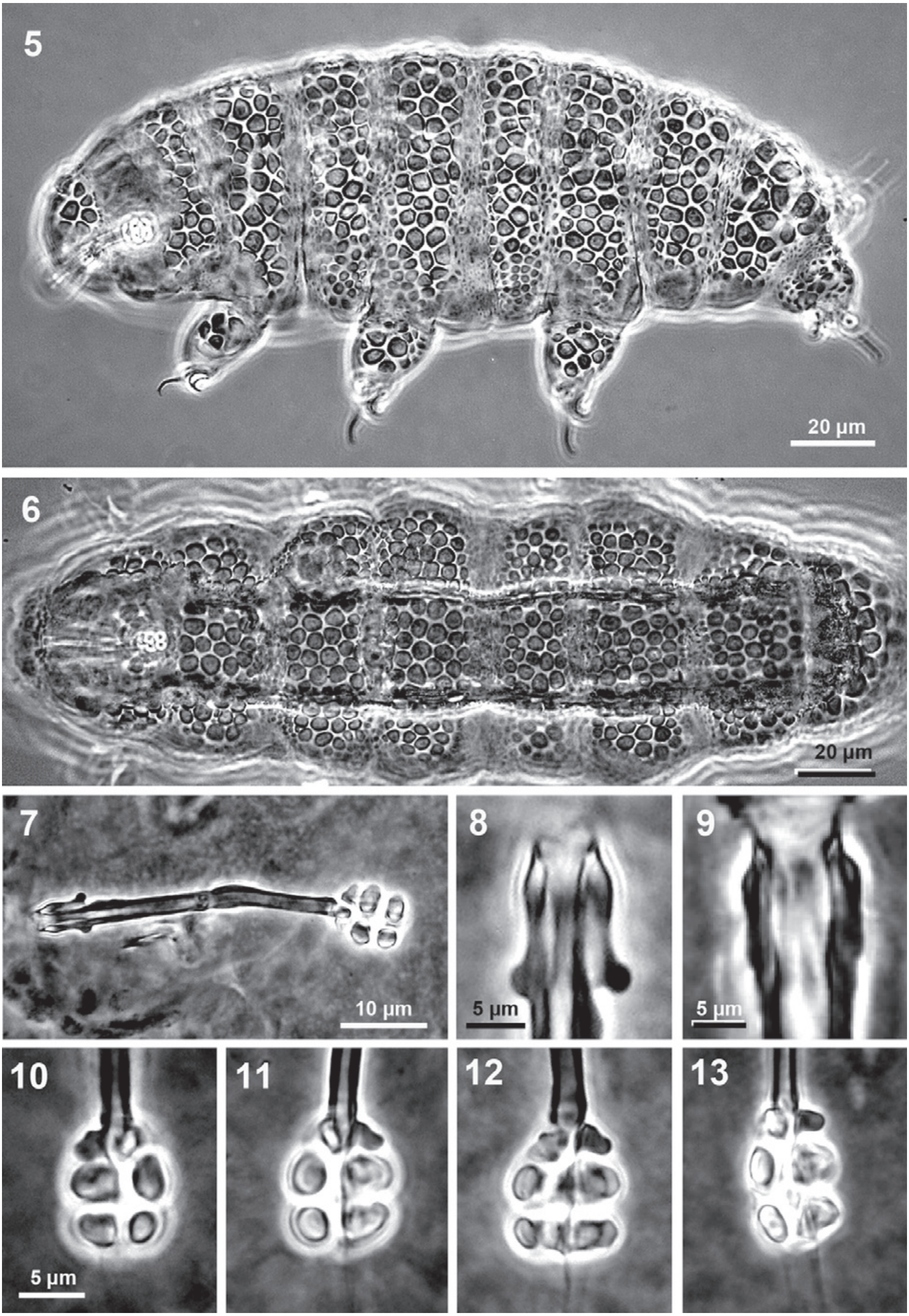
Description of species

Ramazzottius agannae sp. nov.

(Figs 1-41)

H o l o t y p e. – (Fig. 6); sex unknown, 279.0 µm long, coll. F. Stauder, 11 September 2006. The animal is mounted on microslide in Faure's medium together with one paratypic specimen (301.0 µm long) and three other tardigrade species (*Echiniscus wendti* Richters, 1903, *Hypsibius scabropygus* Cuènot, 1929 and *Minibiotus* sp.). The holotype is mounted dorso-ventrally and has dorsally two longitudinal, artificially formed cuticular folds (Fig. 6). The microslide (No. T1078, slide No. 1) has been deposited in the Zoologisches Museum Hamburg (*ZMH* Acc. No. A16/10).

Figs 1-4. *Ramazzottius agannae* sp. nov. **1**. animal in dorso-lateral and, **2**. latero-ventral view, **3**. head region with head organs (arrow), **4**. dorso-posterior part of the body.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TYPE LOCALITY. – The Ötztal Alps, the Ramolkamm Range, Mt. Zirmkogel (N 46° 52' 52'', E 10° 59' 19''), 3280 m a.s.l., summit, nival zone. Moss and some lichens with a small clump of soil, partly beneath stone. 11 September 2006, coll. F. Stauder.

P a r a t y p e s. – Altogether 67 paratypic animals and 9 such eggs mounted in Faure or *PVL* medium on 16 serially numbered microslides (Nos. 1-16). The *ZMH* Acc. No. A17/10 includes all paratypes, except for the paratype mounted with the holotype (see above).

(A). Mt. Zirmkogel, all locality data as above: one paratypic animal on slide No. 2, together with *E. wendti, H. scabropygus, Minibiotus* sp. (*PVL* medium);

(B). The Ötztal Alps, the Ramolkamm Range, Mt. Nörderkogel (= Mt. Nederkogel), (N 46° 54' 25", E 11° 00' 33"), summit area, nival zone, *c*. 3158 m a.s.l. Lichen *Umbilicaria* cf. *cylindrica* from unshaded silicate rock, 3 August 2008, coll. H. Dastych. Altogether 25 paratypic animals (including 2 males, 5 juveniles) and one such egg. They are mounted on six slides in Faure's and one slide in *PVL* medium, being sympatric with *H. scabropygus* and a specimen of *Ramazzottius* sp. The slides are numbered 3-9.

(C). The Stubai Alps, Mt. Lüsenser (= Lisenser) Spitze (N 47° 05' 17'', E 11° 06' 39''), 3230 m a.s.l., summit, nival zone, lichen *Umbilicaria* cf. *cylindrica* from a stone. 10 January 2009, coll. O. Nägele. Totally 29 paratypic animals (incl. two males) and eight paratypic eggs, mounted on six slides in Faure's and one in *PVL* medium. They co-occur with *E. wendti, H. scabropygus, Ramazzottius* sp., *Macrobiotus macrocalix* Bertolani & Rebecchi, 1993 and *Macrobiotus hufelandi*-group. The slides are numbered 10-16.

Five paratypes, exuvium and a paratypic egg (Mt. Nörderkogel, slide No. 5, Fig. 28) are lodged at the Museum d'Histoire Naturelle (Arthropodology & Entomology), Geneva, Switzerland. The remainder of type specimens deposited in the *ZMH*, including 10 non-type individuals mounted on *SEM* stub (Acc. No. A18/10).

ETYMOLOGY. – The new species is dedicated to my daughter, Agathe Anne. The specific name is a combination of letters, feminine in gender.

DIAGNOSIS. – Median sized *Ramazzottius* with strongly sculptured cuticle covered with large knobs ('granules'). Main branches of external claws long, with markedly long light-refracting unit (*LRU*). Freely layed eggs mostly with cone-shaped processes of small diameter, processes often of variable size and shape. Interprocesses and processes area smooth.

DESCRIPTION. – Body 162.0-425.0 µm long (holotype 279.0 µm), animals alive usually brownish-orange. Eye-dots absent. Dorsal and lateral sides of body and legs markedly sculptured. Ornamentation with large and mostly well formed hemispherical or flattened knobs (tubercles, 'granules'), often

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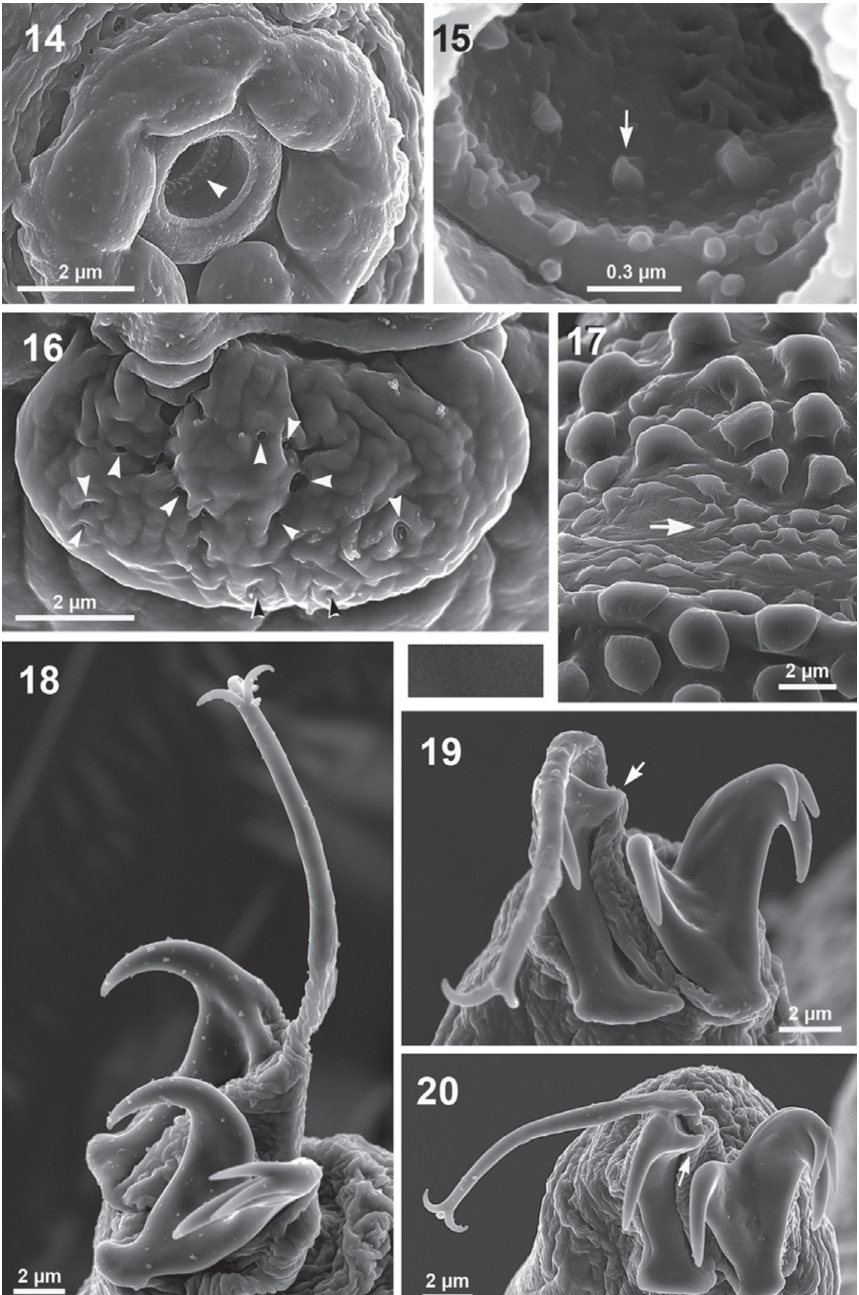
Figs 5-13. *Ramazzottius agannae* sp. nov. (5-8). **5**. juvenile, dorso-lateral view, **6**. holotype, dorsal ornamentation, **7**. buccopharyngeal apparatus, lateral view, **8**. buccal anterior apophyses, lateral view, **9**. *Ramazzottius* cf. *cataphractus* (the Antarctic), buccal anterior apophyses, lateral view; *Ramazzottius agannae* sp. nov., **10-13**: pharyngeal apophyses and macroplacoids. (Scale bars for Figs 11-13: 5 µm).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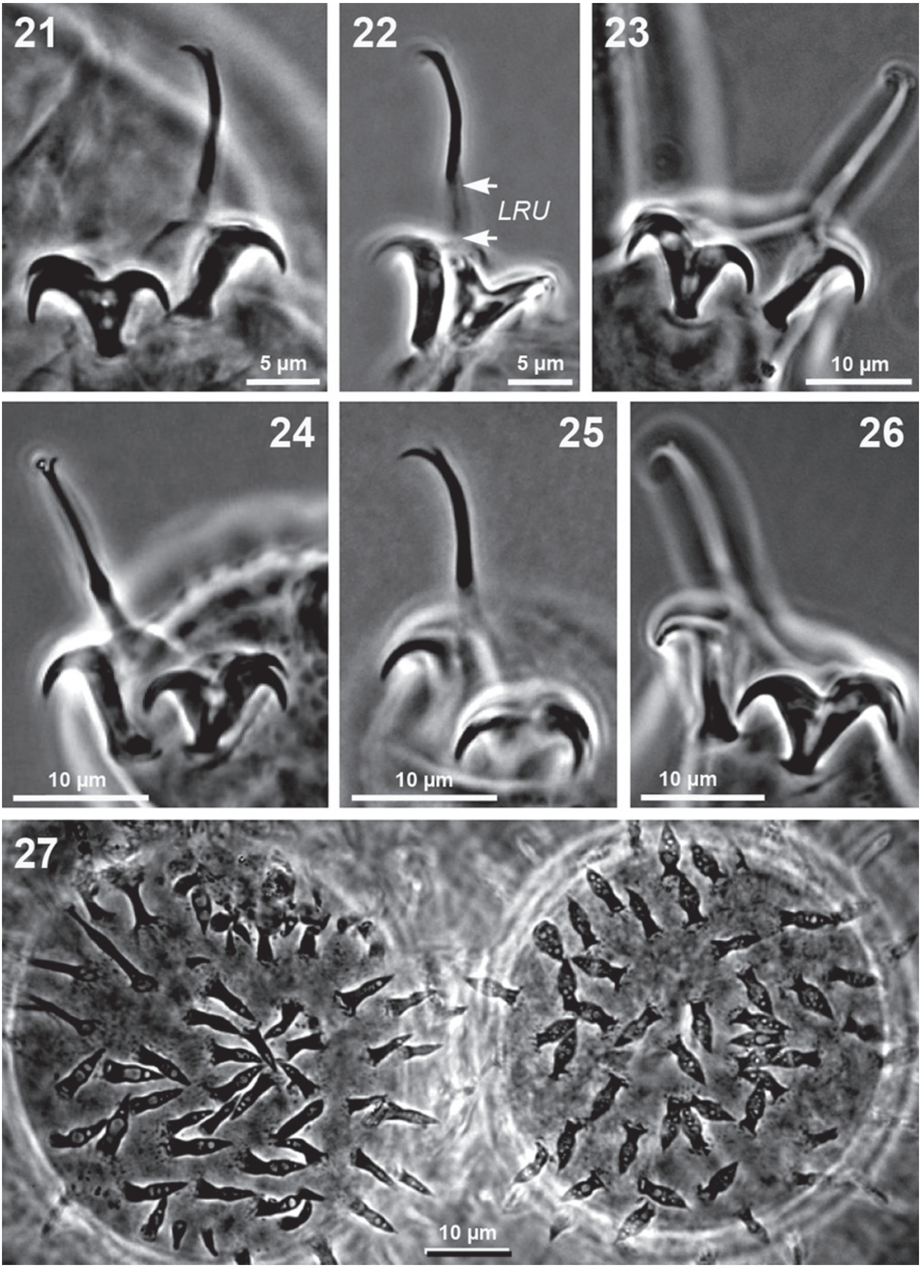
with polygonal shaped base (Figs 3-6). Knobs of similar size on dorsum, including head segment, usually 4.0-6.0 μm wide, rarely up to 9.0 μm and spread over whole dorsum. Largest knobs sometimes on head region, sometimes in posterior part of body and only slightly increase posteriorly. In area between pseudo-segments sculpturing distinctly smaller (Fig. 17, arrow) and granules usually oval. Sometimes, particularly in rear of animal, two or three knobs, rarely more, fused laterally at their bases and forming an elongated structure, up to 13 μm wide (in one case 18.0 μm). Head segment with distinct unsculptured transversal band that lacks granulation. Band with pair of head organs, i.e., elliptical, symmetrically located sensory (?) structures (Figs 1; 3, arrow). Surface of structures with tiny, sparsely distributed pores (Fig. 16, arrowheads). External and posterior sides of legs I-III with distinct knobs, these absent in frontal (anterior) area of legs. Knobs mostly covering whole legs IV, being particularly well developed on the legs' upper ("dorsal") side. Body venter smooth, but delicate granulation usually present ventrally in a region bordering with legs IV and in some areas at edges of pseudo-segments. External side of each leg IV with usually well developed papilla, mostly slightly oval and up to 12.0 μm in diameter and often with a distinct cuticular lateral swelling (Figs 1, 4). Papilla is located either on or close to lateral swelling. However, these both structures are frequently poorly discernible or absent.

Buccopharyngeal apparatus (Fig. 7) median sized, mouth opening ventro-anteriorly. Mouth cavity small, with ring of small granules (teeth) discernible only in *SEM* (Fig. 14, arrowhead), and four rather larger teeth, barely definable in *LM* (Fig. 15, arrow). Buccal tube narrow (Fig. 7), with distinctly thickened wall just below the insertion of stylets' supports. Buccal anterior apophyses dissimilar in shape (Fig. 8). Terminal posterior apophyses of tube tiny. Pharynx medium sized, spherical or slightly sub-spherical, with relatively large pharyngeal apophyses and two grain-like macroplacoids (Figs 7, 10-13). First macroplacoid mostly slightly longer than second, its lateral incisions, if present, barely visible. No lateral incision in caudal part of second macroplacoid. No microplacoids present.

Claws moderately sized, with long main (= primary) branch of external claws (Figs 4, 18). Claws and their branches increase in size posteriorly. Basal unit of main branches of external claws with long, poorly sclerotized part (Figs 18, 22, 24), this part more pliable than remaining solid unit. This pliable, differently light refracting unit (*LRU*) strikingly long and with distinct, sharply marked border (Fig. 22 *LRU*, arrows). Length of *LRU c*. 25-37 % (*n* = 55) of the hind main branch. Main branches with distinct but thin accessory spines (Figs 18, 25). Secondary branch of external claws moderately sized, with small, lateral tooth in its apical part (Figs 19, 20). All claws with noticeably widened bases. Claws' bases, particularly on IVth legs, often with

Figs 14-20. *Ramazzottius agannae* sp. nov. **14**. mouth region, **15**. armature of mouth cavity, **16**. head region with head organs, **17**. inter(pseudo)segmental granulation, **18**. claws of leg II, **19**. claws of leg III, **20**. claws of leg II. (Explanations in text).



Figs 21-27. *Ramazzottius agannae* sp. nov. **21**. claws of leg I, **22-23**: claws of leg III, **24-26**: claws of leg IV, **27**. processes on eggs.

lunula-like structures (visible in *LM*, Fig. 23). In *SEM* structures are absent or corresponding to cuticular folds due to artefacts. No transversal bar-like thickenings below claws' bases on legs I-III.

Eggs spherical, whitish. Eggshell mostly with relatively short conical, spear- or bullet-like processes (Figs 27-34, 37-41), usually slightly constricted above their bases and with sharp or blunt tip. Processes often like as long thorns, thin or thick rods or bars, sometimes expanded apically (Figs 32, 37, 40), formed like as short or long filaments or of intermediate shape. Many processes with one or more bubble-like structures inside (Figs 27, 28, 31). Processes, their bases and interprocess area smooth in *LM*. Light granular surface discernible in *SEM* might be artefact due to fixation (Fig. 39). Shape and size of processes and also distance between them often extremely variable, even on same egg (Figs 29, 33, 34). All found eggs non-embryonated.

Morphometric data

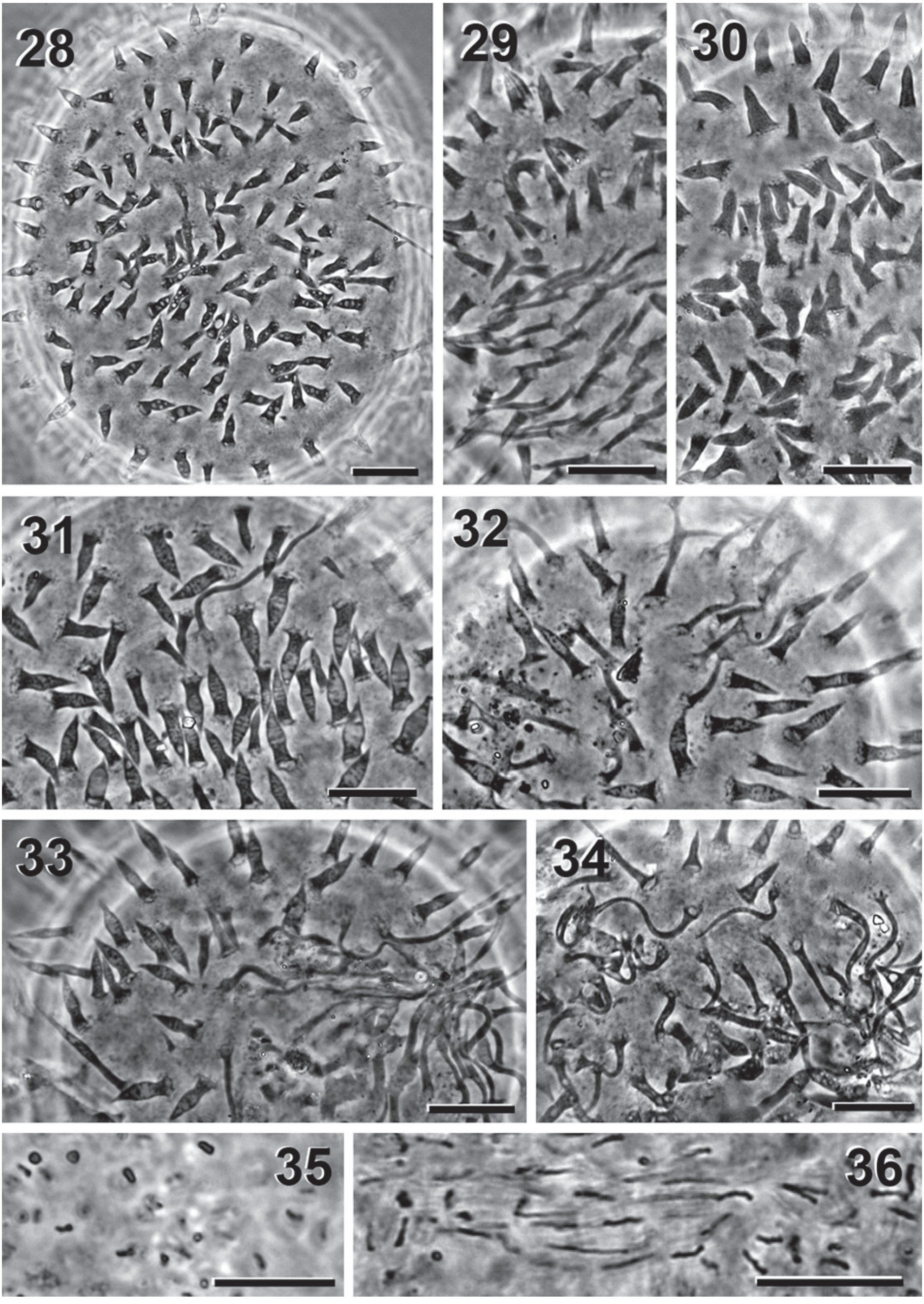
Measurements are in µm, all indices in %. Their values are presented in the following convention:

$\bar{x}$ ± *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" and Dastych (2006). The morphometrics of the holotype (279.0 µm long) is separated from other data by a dot (•), its bucco-pharyngeal apparatus is 45.0 µm long, and pharynx 24.3 µm in diameter.

A) Measurements (µm)

Body length	292.5 ± 68.91 (162.0-425.0) [61] * 23.6 • 279.0
Mouth cavity (ext.) width	2.55 ± 0.45 (1.6-3.6) [55] * 17.6 • 2.3
Buccal tube length	27.87 ± 3.66 (18.9-35.1) [48] * 13.1 • 25.7
SSA length (tube above stylet supports)	15.5 ± 1.88 (10.4-18.9) [50] * 12.4 • 13.5
SSB length (tube below stylet supports)	11.78 ± 1.75 (7.2-15.3) [53] * 14.9 • 11.7
Buccal tube width (external)	1.76 ± 0.25 (1.4-2.3) [57] * 14.3 • 1.8
Buccal tube width (internal)	0.83 ± 0.14 (0.6-1.4) [57] * 17.6 • 0.9
Macroplacoid row length	5.31 ± 0.82 (3.6-7.2) [57] * 15.6 • 5.4
Macroplacoid 1 length	2.50 ± 0.42 (1.4-3.6) [56] * 17.6 • 2.7
Macroplacoid 2 length	2.03 ± 0.40 (1.1-2.7) [56] * 19.6 • 2.3
External claw 1 length	18.74 ± 3.44 (12.6-24.3) [50] * 18.4 • 16.2
External claw 1 base height	7.44 ± 1.48 (5.0-9.9) [56] * 19.9 • 6.3
External claw 1 main branch length	11.99 ± 2.12 (8.1-16.2) [55] * 17.7 • 12.6
Internal claw 1 length	8.06 ± 1.60 (5.4-10.8) [52] * 19.9 • 9.0
Internal claw 1 base height	4.59 ± 1.17 (2.7-6.3) [35] * 25.9 • 4.5
Internal claw 1 main branch length	4.74 ± 0.91 (2.7-6.3) [33] * 19.2 • 3.6
Hind claw (= ext. 4) length	23.30 ± 4.24 (15.3-29.7) [54] * 18.2 • 22.5
Hind claw base height	8.90 ± 1.6 (5.4-11.7) [61] * 18.2 • 9.9
Hind claw main branch length	14.73 ± 2.62 (9.9-19.9) [59] * 17.8 • 14.4
Hind claw main branch *LRU* length	4.54 ± 0.87 (2.7-7.2) [55] * 19.2 • 4.5
Hind claw secondary branch length	6.02 ± 0.93 (4.1-7.2) [38] * 15.5 • 6.3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Fore (= int. 4) claw length	8.43 ± 1.77 (4.5-10.8) [54] * 21.0 • 9.9
Fore claw base height	5.27 ± 1.18 (2.7-6.8) [39] * 22.3 • 6.3
Fore claw main branch height	5.47 ± 1.07 (2.7-7.2) [37] * 19.5 • 4.5

B) Indices

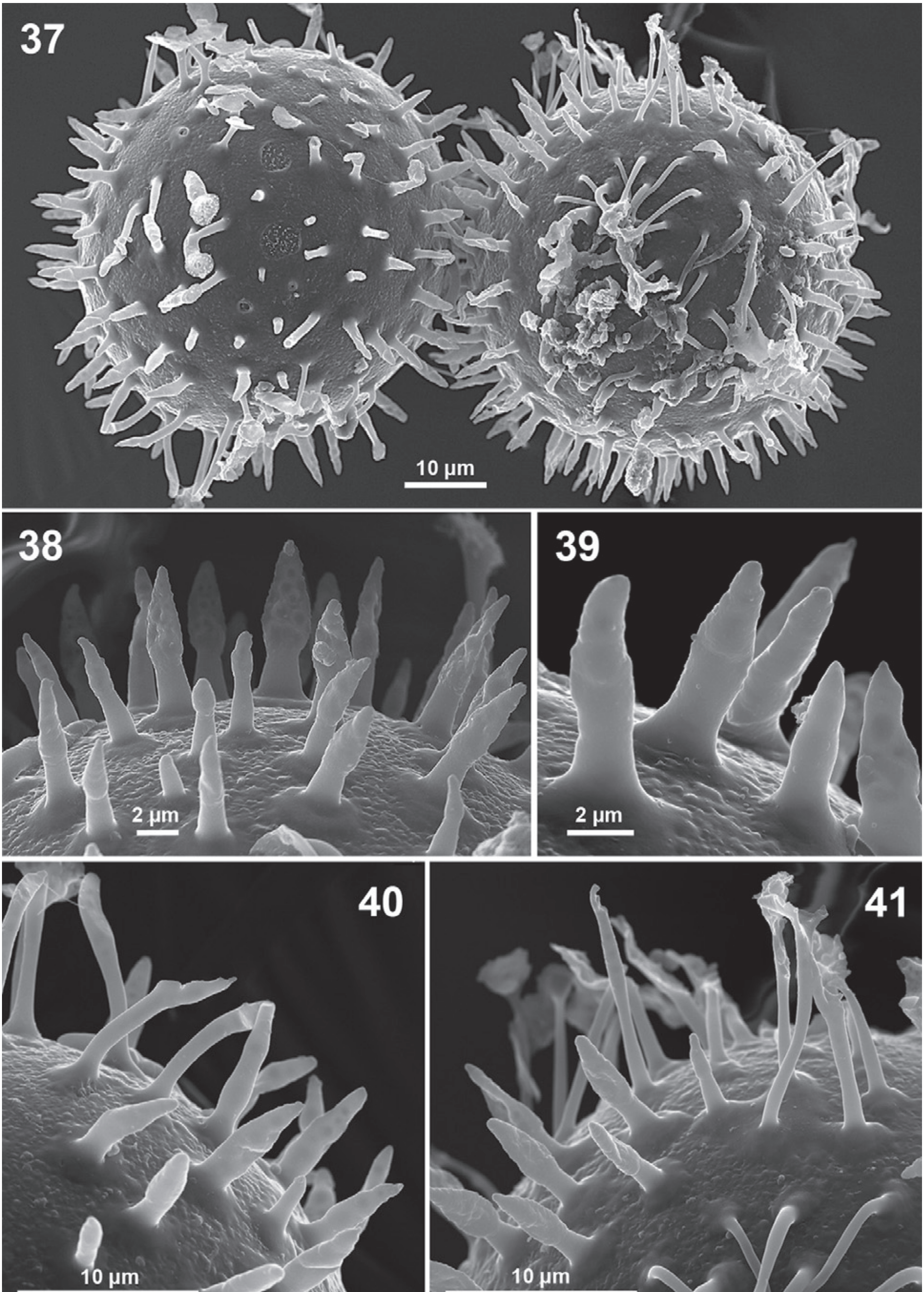
1) *WTI* (the **w**hole **t**ube length **i**ndices) (= "*pt* indices": Pilato 1981):

WT mouth cavity (ext.) width	9.23 ± 1.77 (6.5-12.5) [46] * 12.8 / 49.5 • 8.9
WT SSA (tube above stylet supports)	54.50 ± 2.20 (50.0-59.8) [47] * 4.1 / 91.2 • 52.5
WT SSB (tube below stylet supports)	42.45 ± 2.09 (36.7-46.7) [47] * 4.9 / 90.3 • 45.5
WT buccal tube width (ext.)	6.36 ± 0.71 (5.0-8.1) [47] * 11.3 / 40.0 • 7.0
WT buccal tube width (int.)	3.01 ± 0.36 (2.1-4.4) [47] * 12.1 / 44.9 • 3.5
WT macroplacoid row length	19.13 ± 1.77 (15.4-22.9) [47] * 9.3 / 60.3 • 21.0
WT macroplacoid 1 length	8.97 ± 1.14 (6.7-11.8) [47] * 12.7/45.7 • 10.5
WT macroplacoid 2 length	7.34 ± 1.07 (5.6-10.0) [47] * 14.6 / 42.2 • 8.9
WT claw 1 (ext.) length	67.79 ± 6.48 (56.3-83.0) [38] * 9.6 / 74.3 • 63.0
WT claw 1 (ext.) main branch length	42.83 ± 5.18 (31.3-53.3) [42] * 12.1 / 54.3 • 49.0
WT claw 1 (ext.) base height	26.81 ± 5.18 (18.7-33.3) [43] * 13.4 / 55.9 • 24.5
WT hind claw (= ext. 4) length	84.80 ± 8.87 (62.5-103.2) [40] * 10.5 / 68.6 • 87.5
WT hind claw main branch length	53.36 ± 5.74 (37.5-64.5) [45] * 10.8 / 62.6 • 56.0
WT hind claw base height	32.00 ± 3.68 (24.0-38.7) [47] * 11.5 / 62.5 • 38.5
WT hind claw *LRU* main branch length	16.35 ± 2.05 (11.1-20.6) [39] * 12.4 / 42.8 • 17.5
WT hind claw secondary branch length	21.28 ± 2.41 (15.6-26.7) [30] * 11.0 / 47.6 • 24.5
WT fore claw (= int. IV) length	30.70 ± 4.71 (19.2-40.0) [42] * 15.4 / 47.6 • 38.5
WT fore claw main branch length	20.03 ± 3.33 (9.4-26.7) [28] * 16.6 / 39.6 • 17.5
WT fore claw base height	19.22 ± 2.81 (11.5-24.5) [29] * 14.6 / 56.0 • 24.5

2) *PUI* (the **p**osterior **t**ube unit length **i**ndices):

PU mouth cavity (ext.) width	21.46 ± 2.95 (15.4-31.9) [49] * 13.6 / 49.2 • 19.7
PU buccal tube width (ext.)	15.01 ± 1.79 (11.8-20.0) [51] * 11.9 / 43.8 • 15.4
PU buccal tube width (int.)	7.13 ± 0.81 (5.1-9.7) [51] * 11.4 /54.1 • 7.7
PU macroplacoid row length	45.42 ± 3.91 (35.3-51.9) [52] * 8.6 / 69.9 • 46.2
PU macroplacoid 1 length	21.25 ± 2.51 (15.3-27.5) [52] * 11.8 / 52.4 • 23.1
PU macroplacoid 2 length	17.33 ± 2.41 (13.3-23.1) [52] * 13.9 / 50.6 • 19.7
PU claw 1 (ext.) length	162.20 ± 16.69 (132.8-192.3) [42] * 10.29 / 71.1 • 138.5
PU claw 1 (ext.) base height	63.67 ± 8.49 (46.2-78.6) [49] * 13.3 /57.1 • 53.8
PU claw 1 (ext.) main branch length	103.10 ± (76.9-133.3) [47] * 13.7 / 49.5 • 107.7
PU hind claw length	202.80 ± 19.45 (153.8-246.2) [45] * 9.6 / 72.9 • 192.3
PU hind claw base height	76.56 ± 7.94 (60.0-92.9) [51] * 10.4 / 68.6 • 84.6
PU hind claw main branch length	126.30 ± 13.51 (92.3-153.8) [50] * 10.7 / 65.1 • 123.1
PU hind claw main branch *LRU* length	39.29 ± 4.67 (30.8-50.0) [46] * 11.9 /46.9 • 38.5
PU hind claw secondary branch length	51.43 ± 6.77 (30.5-66.7) [34] * 13.2 / 45.9 • 53.8
PU fore claw length	72.68 ± 11.4 (45.5-100.0) [48] * 16.0 / 49.9 • 84.6
PU fore claw base height	44.77 ± 6.56 (27.3-58.3) [34] * 14.7 / 60.9 • 53.8
PU fore claw main branch length	46.53 ± 7.80 (23.1-66.7) [33] * 16.8 / 36.4 • 38.5

Figs 28-36. *Ramazzottius agannae* sp. nov., **28-34**: variability of egg processes, **35**. fragment of testis with spermatogonia and spermatids, **37**. fragment of testis with spermatids and spermatozoa. (Scale bars: 10 μm).


37
10 µm
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2 µm
39
2 µm
40
10 µm
41
10 µm

3) Other indices:

Macroplacoid index (*MPLI*)	81.73 ± 9.02 (66.7-100.0) [56] * 11.0 / 64.2 • 85.2
External claws index (*ECI*)	80.96 ± 7.24 (70.8-100.0) [43] * 8.9 / 81.0 • 72.0
Claw main branch index (*MBI*)	82.74 ± 9.42 (63.7-109.0) [52] * 11.4 / 66.9 • 87.5
Hind claw base index (*HBI*)	60.70 ± 6.61 (42.9-76.9) [58] * 10.9 / 67.5 • 68.8
Hind claw LRU index (*HLRUI*)	30.29 ± 2.93 (25.0-37.5) [55] * 9.7 / 75.2 • 31.0
Hind claw secondary branch index (*HSBI*)	66.65 ± 6.6 (55.6-83.3) [37] * 9.2 / 71.3 • 63.6

Eggs (*n* = 9)

Diameter 60-84 µm (without processes)
Non-filamentous processes 2.7-13.0 µm long
Filamentous (thread-like) processes up to 20 µm in length
Basal width of processes 1.5-3.6 µm
Distance between processes 1.0-10.0 µm.

VARIABILITY: Individuals of *R. agannae* sp. nov. show distinct intra-specific variability in their body sculpturing (granulation), on the head region and the fourth pair of legs. In these areas the granulation can be very poorly developed. Moreover, morphometric values of the fore claw and the second macroplacoid indices (see morphometric data) proved to vary.

The variation coefficient (*V*) for *WT SSA* (= *pt ss*) and *WT SSB* indices is very low (4.1 and 4.9 %) and these indices have a very high degree of correlation of their variables, with the *r* squared equalling 91.2 and 90.3 %, respectively. The values have a wide range (between *c*. 50 and 60 % and *c*. 37 and 47 %, respectively), and should not be used for discrimination, when only a few individuals are available for study. A well discernible and discriminating feature of the new species, the long light-refracting unit (*LRU*) of the main branch of external claws (Fig. 22), is characterized by the index *HLRUI*. The value of its variation coefficient is low (9.7 %; *r* squared = 75.2 %), but the range of the index values is relatively wide, between *c*. 25 and 37 %.

The eggshell structures of the new species, i.e. the shape and size of the egg processes, their density/distance and distribution (e.g. Figs 33, 37) are sometimes extraordinarily variable. Most eggs have similarly shaped processes rather homogenously distributed (Fig. 28). In some egg areas the shape of the processes can be extremely different, even within the same hemisphere. For example, in one egg the largest area was covered with bullet-like and the remaining area with thread-like processes (Fig. 34).

DIFFERENTIAL DIAGNOSIS: The very long light-refracting unit of the main branch of external claws (*LRU*) (Fig. 22) readily separates *R. agannae* sp. nov. from most congeners, as the majority of *Ramazzottius* ssp. have short *LRU*. *R. cataphractus, R. nivalis* and, to a lesser degree, *R. ljudmilae* have a light-refracting unit similar to that of *R. agannae* sp. nov. The new species differs clearly from these three taxa by its strikingly large cuticular granulation.

Figs 37-41. *Ramazzottius agannae* sp. nov., variability of egg processes.

In addition, *R. agannae* sp. nov. can be separated from *R. ljudmilae* by the formers longer *LRU* [the *HLRUI* index c. 25-38 % (*n* = 55) vs. *c*. 16 and 17 % in holotype and paratype of *R. ljudmilae*: see Biserov 1997/98 and Dastych 2006], wider mouth cavity [*WT* (= *pt*) index, $\bar{x}$ = 9.23 (*n* = 46), vs. 6.3 (*n* = 5) in the latter species], shorter hind claws [*WT*, $\bar{x}$ = 84.8 (*n* = 40) vs. 119.2 (*n* = 11)], and longer secondary branch of hind claws [HSBI, $\bar{x}$ = 66.6 (*n* = 37) vs. 52.6 (*n* = 5)]. Moreover, the stylet supports of *R. agannae* sp. nov. are more anteriorly located [*WT SSA* (= *pt ss*), $\bar{x}$ = 54.5, range *c*. 50-60 % *n* = 47), holotype 52.5] than in *R. ljudmilae* [range 64.2-67.1 %, holotype 67.6 % (*n* = 11) (The mean value ($\bar{x}$) cannot be calculated from data by Biserov *l.c.*)]. The processes of eggshell are rather similar in *R. agannae* sp. nov. and *R. ljudmilae*, though in the former species are mostly sharply tipped and there are thread-like structures present whereas in *R. ljudmilae* the processes are stumpy and more truncate and thread–like structures are lacking.

The new species differs from *R. cataphractus* by the presence of accessory spines on the main branches, these spines are absent in the latter taxon. Wide cuticular bars transversely placed under claws' bases on legs I to III are not present in *R. agannae* sp. nov. but well developed in R. *cataphractus*. Moreover, the shape of the dorsal and ventral anterior buccal appophyses (for the attachment of stylets muscles) is different in both species. In *R. agannae* sp. nov. the dorsal and ventral apophyses are of different shape, as they "typically" are in *Ramazzottius* (see Binda & Pilato 1986). In *R. cataphractus* (and *R.* cf. *cataphractus* from the Antarctic: Alexander Island, examined here) the apophyses are almost identical in their shape, resembling in this aspect those of *Hypsibius* Ehrenberg, 1848 (comp. Fig. 8 and 9, respectively). The difference in the shape relation hints at a presence of two separate phyletic lineages within the genus. Other discriminating characters are narrower mouth cavity in *R. agannae* sp. nov. [*WT*, $\bar{x}$ = 9.2 (*n* = 46) vs. $\bar{x}$ = 13.7 (n = 7) in *R. cataphractus*], distinctly shorter external claws [*WT* of hind claws, $\bar{x}$ = 84.8 (*n* = 40) vs. 131.4 (one paratype of *R. cataphractus*)] and a longer *LRU* of the claws' main branches [the values of *HLRUI* c. 25-38 % vs. *c*. 21 and 22 % (two paratypes)]. The egg processes of *R. agannae* sp. nov. and *R. cataphractus* are similar in their shape and, to some degree, also in their distribution on the eggshell. However, the processes in the new species are more homogenously distributed and mostly spear-like, in *R. cataphractus* more irregularly located and thorn- or spine-like. The eggs of *R. cataphractus* are known from a single record (West Spitsbergen: Dastych 1985) and were not reported in the original description (Maucci 1984).

R. agannae sp. nov. is most similar to *R. nivalis*, and both species are described from the nival zone in the Alps. In contrast to R. nivalis, the cuticular sculpturing is coarser, the stylet supports are more anteriorly inserted [*WT SSA*, $\bar{x}$ = 54.5 (*n* = 47) vs. 62.8 (*n* = 9)], the mouth tube slightly wider [*WT*, $\bar{x}$ = 6.4 (*n* = 47, vs $\bar{x}$ = 9.4 (*n* = 9)], the macroplacoids shorter and round [(as indicated by shorter macroplacoid row: *WT*, $\bar{x}$ = 19.1 (*n* = 47), vs. $\bar{x}$ = 23.2 (*n* = 9) and lower values of the macroplacoid index: *MPLI*, $\bar{x}$ = 81.7 (*n* = 65) vs. $\bar{x}$ = 91.1 (*n* = 9)]. Compared to the claws of *R. nivalis* (see Dastych 2006) those of *R. agannae* sp. nov. are slightly shorter (lower values of claw indices in the latter taxon). The egg processes in *R. nivalis* are extremely long and thread-like, in *R. agannae* sp. nov. thread-like processes may be present, but they are decidedly shorter and intermingled with other, differently shaped processes.

Amongst all *Ramazzottius* spp., *R. agannae* sp. nov. and *R. baumanni* are the species most similar in respect to of their body sculpturing. The shape and size of the cuticular knobs are almost the same, as judged from the examination of five, partly deteriorated syntypes of the former taxon. These tubercles are up to 7.2 µm in diameter, i.e., only slightly smaller than mentioned in the original description ("8-9 µ": Ramazzotti 1962) and the tubercles of *R. agannae* sp. nov. However, that comparison of size is based on examined recently syntypes no more than 283 µm in length. A difference among the two taxa is ornamentation between the pseudo-segments. In *R. agannae* sp. nov. there are small granules (Fig. 17, arrow), and these are absent in *R. baumanni*. The most important criterion to separate both species is the length of the light-refracting unit (*LRU*). It is very long in *R. agannae* sp. nov. (*HLRUI* index c. 25-38 %) but short in *R. baumanni* (*HLURI* equals 10.8 %: the only available measurement within five syntypes examined). Other differences are, the internal claws, in *R. baumanni* more stumpy than in *R. agannae* sp. nov., the accessory spines on the claws' of external claws' main branches tiny or barely visible in *R. baumanni*, but decidedly larger in *R. agannae* sp. nov., and the position of the stylet supports, posteriorly in *R. baumanni* (*WT SSA* range: 61.3-66.5, n = 3), but anteriorly in *R. agannae* sp. nov. (the *WT SSA* index ranged 50.0-59.8 %, n = 47).

REPRODUCTION MODE: Orcein-stained individuals of *R. agannae* sp. nov. from Mt. Nörderkogel (5) and Mt. Lünsenser Spitze (43 animals) have been examined for gender and ploidy. More than a half of examined individuals had distinctly formed gonads with germ cells. In the testes of 13 males occurred spermatogonia, spermatids and/or spermatozoa (Figs 35, 36). Males are also present in the slide-mounted type series, i.e., two males from Mt. Nörderkogel and Mt. Lüsenser Spitze, respectively. These individuals have spermatids and spermatozoa strongly faded by the mounting medium, but still definable for sex determination. The sex dimorphism in *R. agannae* sp. nov. indicates bisexual (amphimictic) mode of reproduction. The ploidy in the new taxon is unknown, as no chromosomes have been found in the stained animals.

BIOLOGY AND DISTRIBUTION: All *R. agannae* sp. nov. are from the nival zone of two neighbouring mountain ranges, the Ötztal- and Stubai Alps, all sites located at more than 3000 m a.s.l. According to these though scanty data, this is a species of high altitudes. The animals and eggs have been found in soil, on mosses or lichens collected from silicate bedrock. These habitats suggest affiliation of the new taxon to "non-carbonate association", the latter defined in Dastych 1985, 1988.

REMARKS: In two of three localities both *R. agannae* sp. nov. and similar-looking *Ramazzottius* individuals were found, on Mt. Nörderkogel and

Mt. Lünsener Spitze, one and 37 individuals, in the latter locality they even outnumbered the new species (28 individuals). The animals differed from *R. agannae* sp. nov. by their cuticular ornamentation, mostly weakly developed, and barely visible or absent on the head and fourth pair of legs.

Other characters of these sympatric tardigrades were very similar to those of *R. agannae* sp. nov. Two animals represented males, with well formed spermatozoa. These individuals slightly different from *R. agannae* sp. nov., have not been included into the latter's type-series and the taxonomic status needs further studies. Momently it is difficult to say if the belong to *R. agannae* sp. nov. (and then extend the variability range of the latter taxon) or represent a new cryptic species within *ljudmilae-nivalis* group.

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