

Life-history traits and description of the new gonochoric amphimictic *Mesobiotus joenssoni* (Eutardigrada: Macrobiotidae) from the island of Elba, Italy

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Comparative analyses of life-history theory studies are based on the characteristics of the life cycles of different species. For tardigrades, life-history traits are available only from laboratory cultures, most of which have involved parthenogenetic species. The discovery of a new gonochoristic bisexual *Mesobiotus* species in a moss collected on the island of Elba (Italy) provides us with the opportunity to describe *Mesobiotus joenssoni* sp. nov. and to collect data on the life-history traits of cultured specimens to increase our knowledge of the life-history strategies present in tardigrades. This new species is differentiated from all other species of the genus by the presence of granules (~1 µm in diameter) on the dorsal cuticle of the last two body segments, two large bulges (gibbositities) on the hindlegs and long, conical egg processes. The species exhibits sexual dimorphism in body length, with females being longer than males of the same age. The mean lifespan of specimens was 86 days, with a maximum of 150 days. The mean age at first oviposition was 19.8 days and the mean egg hatching time 15.4 days. The life-cycle traits correspond to those collected for the only other two macrobiotid species with gonochoric amphimictic reproduction examined so far.

ADDITIONAL KEYWORDS: amphimictic – life cycle – life-history theory – *Mesobiotus arguei* – spermatozoa – Tardigrada.

INTRODUCTION

Tardigrades or water bears are able to colonize many different habitats in marine, freshwater and terrestrial environments, owing to their capacity to use several dormancy strategies (for reviews, see Guidetti *et al.*, 2011; Møbjerg *et al.*, 2011; Welnicz *et al.*, 2011). Dormancy strategies, in particular anhydrobiosis, certainly have an impact on the life cycle of species, although they cannot affect some life-history traits, such as longevity (Hengherr *et al.*, 2008). To understand adaptations, biological trade-offs and reproductive

costs of tardigrade life cycles for more precise life-history theory studies in this phylum, life-history strategies must be identified. Life-history strategies in tardigrades are influenced by their environments and, for the species able to undertake dormancy, by their capacity to suspend their metabolism to withstand adverse environmental conditions. Several tardigrade species have already been shown to have adaptations to specific environmental conditions in their life cycles. The freshwater populations of *Acutuncus antarcticus* (Richters, 1904) have a life-history strategy (e.g. parthenogenetic reproduction, short life cycle, fast embryonic development) that allow them to take advantage of the short period of favourable conditions present in Antarctica during the summer season (Altiero *et al.*, 2015). Marine species of the genus

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Halobiotus and moss-dwelling species of the genus *Bertolanius* have evolved different life cycles involving diapause stages to withstand low temperatures or periods of oxygen depletion and heat stress (Møbjerg *et al.*, 2007; Guidetti *et al.*, 2008; Guidetti & Møbjerg, 2018).

Life-history traits of tardigrade species from birth to death are available only from laboratory culture studies (for a review, see Altiero *et al.*, 2018). Most life-cycle studies were conducted on eutardigrades and, in particular, on parthenogenetic species, which are easier to culture, and only two studies have been performed on gonochoristic amphimictic tardigrades (e.g. Lemloh *et al.*, 2011; Bingemer *et al.*, 2016).

The discovery of a new gonochoristic bisexual species in the genus *Mesobiotus* Vecchi *et al.*, 2016 (Macrobiozoa: Macrobiotidae) gave us the opportunity to describe this taxon and to culture it, thus collecting data on life-history traits to increase our knowledge of life-history strategies in tardigrades. *Mesobiotus* is a recently described genus characterized by both morphological and molecular characters (Vecchi *et al.*, 2016), in which 60 species have been described (Guidetti & Bertolani, 2005; Degma & Guidetti, 2007; Degma *et al.*, 2018). The nominal species of the taxon, *Mesobiotus harmsworthi* (Murray, 1907), has recently been redescribed from new material collected at the type locality (Kaczmarek *et al.*, 2018). Most of the species of this genus have similar morphology of the adult animals, and the different species are identified mainly based on egg morphology and other morphometric characters. For this reason, the taxonomic description, which combines light microscopy (LM) and scanning electron microscopy (SEM) observations, has been completed to characterize this new species.

MATERIAL AND METHODS

SAMPLING AND CULTURE PROTOCOL

Specimens were originally extracted from a dry moss collected on rock from Elba Island (Fig. 1) close to Porto Azzurro, Reale Bay, in Italy (42°46'36.2"N, 10°24'51.8"E; 45 m a.s.l.). In order to extract tardigrades, moss fragments were placed in and maintained in distilled water for ~30 min. After soaking, the sample was sieved (using 500 µm and 38 µm mesh sieves) to separate tardigrades and their eggs from the substrate. Animals and eggs were later isolated using a needle and a glass pipette under a stereomicroscope (Olympus, SHZ 10).

A laboratory culture of the new *Mesobiotus* species was established by rearing animals in three groups of ten animals each (mixing males and females), and scaled up for growth in plastic culture dishes (closed with a cover) with 4 mm layers of 3% agar

(peqGOLDUniversal agarose; peqLAB, Erlangen, Germany), covered with a thin layer of Volvic water (Danone Waters, Wiesbaden, Germany) at a room temperature of 20 °C and with a 12 h–12 h light–dark cycle. The green algae *Chlorogonium elongatum* Dang. (Haematococcaceae) was provided as food *ad libitum*, the algae produced a constant layer on the agarose. Culture dishes were checked daily under the stereomicroscope, and the medium was changed if necessary.

COLLECTION OF LIFE-HISTORY TRAITS

Experimental set-ups for studying life-cycle traits varied according to the life-history data to be examined. After deposition, 360 eggs were collected. These eggs were divided into groups (formed by eggs of the same age) and placed in 24-well plates in order to determine the time of development from deposition until hatching (hatching time) and their hatching success. The hatched tardigrades were separated and kept in culture individually to determine longevity ($N = 209$) or pairwise (two animals of known or unknown sex) to determine the age of first oviposition ($N = 15$), according to the protocol of Lemloh *et al.* (2011).

MORPHOLOGICAL ANALYSES

Specimens were mounted on slides in Hoyer's medium for observations with LM. Observations and measurements were carried out with LM under both phase contrast and differential interference contrast up to the maximal magnification ($\times 100$ oil objective), with a Leica DM RB microscope equipped with a Nikon DS-Fi1 digital camera. The measurements of the lengths of the animals and of their cuticular parts (i.e. claws, feeding apparatus) were made according to Kaczmarek & Michalczyk (2017). The *pt* indexes of the structures (the percent length of a structure with respect to the buccal tube length; Pilato, 1981) were compared between species. For the eggs, the process height and the internal diameter of the process base were measured. The processes that had at least half of the base diameter present in a hemisphere were counted. The number of processes in an optical section was not considered, because only a few of the processes (which have a large base) were present in a single optical section. To determine differences between sexes, measurements of the body length were collected from 40-day-old females ($N = 10$) and males ($N = 10$). Body lengths were measured during the most elongated state of the animals during walking, using the distance from the tip of the head to the junction of the fourth pair of legs. They were measured according to the method described by Suzuki (2003), and successfully used by Lemloh *et al.* (2011) for other



Figure 1. Sampling site of *Mesobiotus joenssoni* and type locality of *Mesobiotus arguei*.

Macrobiotus species in culture, using a digital camera (Nikon Ds Fi1; Nikon Instruments Europe) attached on a stereo microscope (Olympus SHZ 10). Given that the data did not have a normal distribution (Levene test), statistical analysis was conducted with the non-parametric Mann–Whitney *U*-test, using SPSS v.25 (IBM) software.

Measurements were carried out by analysing time-lapse movies (recorded with the software NIS Elements v.3.0, NIKON) of the tardigrades whilst walking. Figures were prepared using NIS Elements v.3.0 (NIKON) and the Picolay free software (www.picolay.de) to combine pictures with different focal planes.

Additional specimens were prepared for SEM observations by fixing them in boiling absolute ethanol for a few minutes. They were then rinsed three times in

absolute ethanol, desiccated by evaporation, mounted on stubs and sputter coated with a thin layer of gold. Observations with SEM were carried out with a Nova Nano SEM 450 (FEI company), available at the ‘Centro Interdipartimentale Grandi Strumenti’ (CIGS) at the University of Modena and Reggio Emilia (Italy).

RESULTS

TAXONOMIC DESCRIPTION

MESOBLOTUS JOENSSONI GUIDETTI,
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(MACROBIOTIDAE)

Zoobank registration: E2ACD822-E39B-42C1-9993-C434D87F9527

Type locality

Type material: ITALY. **Livorno:** Reale Bay close to Porto Azzurro at the south-eastern side of Elba Island (Fig. 1), 42°46'36.2"N, 10°24'51.8"E (45 m a.s.l.), from a moss sample on rock. Collected 19 August 2016, leg. R. Schill, holotype (slide C4035-13b) and paratypes (32 animals and eight eggs are on slides, five animals and three eggs are on stub for SEM analysis).

Type repositories: Holotype, 26 paratypes and six eggs are in the Bertolani collection at the Department of Life Sciences, University of Modena and Reggio Emilia (Italy), three paratypes in the Natural History Museum of Verona (Italy), and three paratypes in the Binda & Pilato collection at the Department of Biological, Geological and Environmental Sciences of the University of Catania (Italy).

Description

Morphometric data are presented in Table 1. Body is whitish. Eye spots are present (but not visible in most specimens mounted on permanent slides). Cuticle has scattered granules ($\geq 1 \mu\text{m}$ in diameter) clearly visible on the dorsolateral surface of the last two body segments. Other granules (not always visible with LM) may be present on the posterior portion of the third leg pair and on the dorsal surface of the cuticle of the second body segment (Figs 2, 3). Some granules are visible with SEM (but not always visible with LM) on the lateral cuticle at the level of the second pair of legs. A fine granulation of small granules ($\leq 0.5 \mu\text{m}$) is present only on the external surface of the second and third legs (close to the claws) and on the dorsal and medial parts of the hindlegs (Figs 2–4). There are a large and flat bulge (defined as a pulvinus-like bulge by Stec *et al.*, 2018) on the internal surface of the first three pair of legs (Figs 3, 4), and large, evident bulges on the dorsal surface of the hindlegs (Fig. 2) in both sexes. The mouth is surrounded by large and square-shaped peribuccal lamellae (Fig. 4). Buccal armature can be seen in the oral cavity (visible with LM): the anterior band at the beginning of the buccal ring (at the base of the peribuccal lamellae) has five or six rows of many small, round teeth of different sizes (Figs 3, 4); the posterior band at the beginning of the buccal tube has an anterior crown of long, triangular, strong teeth (ventrally followed by one or two lines of small, rounded teeth in larger specimens; Figs 3, 4). Transverse crests are present: dorsally, three large evident crests; ventrally, two lateral crests and a generally triangular median crest (in some specimens, rounded or separated into two or three teeth, or fused with the lateral crests; Figs 3, 4). The buccal tube has an evident ventral lamina (Fig. 3).

Stylet supports are long and thick (Fig. 3). Stylet furca are well developed, with large condyles and small apophyses on their branches (Fig. 3). A slightly oval pharyngeal bulb contains large, triangular apophyses, three macroplacoids (the first being the shortest, and the third equal to or larger than the second) arranged along a curved line, and a microplacoid close (less than its length) to the third macroplacoid (Fig. 3). The first macroplacoid is arrowhead shaped (in smaller specimens) or trapezoidal (in larger specimens), the second rounded and the third quadrangular, with an evident subterminal constriction (Fig. 3). Claws are of the *Mesoblotus* type (see Vecchi *et al.*, 2016): Y-type double-claws, with a wide common tract characterized by an internal septum defining a distal part (Figs 3, 4). Evident accessory points are found on the main branches of all claws. Smooth lunules are present on all claws. Lunules are small on the first three pairs of legs, and larger on the hindlegs (posterior claws with larger lunula than the anterior ones; Figs 3, 4).

It is a gonochoric amphimictic species, with males [mean length 240.1 μm (SD 39.1 μm)] statistically smaller ($F_{2,18} = 5.357$, $P = 0.033$; Mann–Whitney $U = 91.00$, $P = 0.001$; $N = 20$) than females [mean length 301.1 μm (SD 20.2 μm)] of the same age. The mature male has small gonad full of spermatozoa. Spermatozoa inside the testis are filiform, with a long head that appears helicoidal (Fig. 5).

Eggs (Fig. 5) are ornamented, 69–77 μm in diameter excluding processes, and are laid freely. Egg processes are like ‘elf hats’: long and conical, with a circular thickness at their base not in contact with the egg surface, and long, convoluted/crumpled tips. Processes are 27–36 μm high, with an inner diameter at their base of 15–19 μm . The number of processes per hemisphere ranges from 11 to 13. With LM, the process wall appears reticulated (owing to inner trabecular structures), with meshes of irregular size and shape. With SEM, the surface of the processes appears to possess small hollows of irregular size (Fig. 5). Between the processes, the eggshell is sculptured by fine meshes interspersed with some thicker areas with irregular margins, sometimes forming round, wide areas (Fig. 5).

Etymology: This species is dedicated to our friend and colleague Professor Ingemar K. Jönsson (Kristianstad University, Sweden).

Differential diagnosis

Mesoblotus joenssoni is most similar to *Mesoblotus arguei* (Pilato & Sperlinga, 1975), but differs by the wider cuticular area covered with granules and by the shape of the egg processes (larger and longer; in *M. arguei*, the maximal height of processes is 19 μm ;

Table 1. Morphometric data of type specimens of *Mesobiotus joenssoni*

Specimen (N = 21)	bol	btl	ssi	pt ssi	btd	pt btd	mpri	pt mpri	I mp	pt I mp	II mp	pt II mp	III mp	pt III mp	I cl	pt I cl	III cl	pt III cl	IV cl	pt IV cl
C4035-18b	201.69	28.71	20.79	72.41	3.47	12.07	12.87	44.83	3.47	12.07	1.98	6.90	2.97	10.34	7.43	25.86	7.92	27.59	8.91	31.03
C4035-10a*	244.02	32.67	24.75	75.76	4.95	15.15	10.89	33.33	3.47	10.61	2.97	9.09	3.47	10.61	8.42	25.76	8.91	27.27	9.90	30.30
C4035-10b*	241.53	33.17	24.75	74.63	4.95	14.93	11.39	34.33	3.96	11.94	2.97	8.96	4.46	13.43	8.42	25.37	8.91	26.87	10.40	31.34
C4035-18c	251.49	33.66	24.75	73.53	3.96	11.76	11.88	35.29	3.96	11.76	2.28	6.76	3.96	11.76	7.43	22.06	8.42	25.00	8.91	26.47
C4035-10c*	313.74	38.12	28.71	75.32	5.45	14.29	13.37	35.06	3.96	10.39	2.97	7.79	3.96	10.39	11.39	29.87	11.88	31.17	12.87	33.77
C4035-16a	318.72	38.61	29.70	76.92	4.75	12.31	13.86	35.90	3.96	10.26	2.97	7.69	3.96	10.26	9.90	25.64	10.89	28.21	12.87	33.33
C4035-17c*	369.00	39.11	29.21	74.68	4.95	12.66	14.36	36.71	4.95	12.66	3.76	9.62	4.46	11.39	11.88	30.38	12.87	32.91	13.86	35.44
C4035-17a*	343.62	40.10	30.69	76.54	4.95	12.35	14.85	37.04	4.95	12.35	3.47	8.64	4.46	11.11	10.40	25.93	11.88	29.63	13.86	34.57
C4035-13a	353.58	41.58	31.68	76.19	4.46	10.71	14.85	35.71	4.95	11.90	3.47	8.33	4.95	11.90	11.39	27.38	12.87	30.95	13.86	33.33
C4035-12a	358.56	42.57	32.67	76.74	5.94	13.95	15.84	37.21	4.95	11.63	3.96	9.30	4.95	11.63	11.88	27.91	13.37	31.40	14.85	34.88
C4035-17b*	368.52	44.55	32.67	73.33	5.94	13.33	15.84	35.56	4.95	11.11	3.96	8.89	4.95	11.11	11.39	25.56	13.86	31.11	14.85	33.33
C4035-13b	483.06	50.00	37.62	75.25	5.94	11.88	18.81	37.62	5.94	11.88	4.46	8.91	6.44	12.87	12.38	24.75	13.86	27.72	14.85	29.70
C4035-9	487.55	52.47	40.59	77.36	6.93	13.21	19.80	37.74	6.44	12.26	4.46	8.49	6.93	13.21	12.87	24.53	13.86	26.42	14.85	28.30
C4035-14b	487.55	52.47	39.60	75.47	6.93	13.21	19.80	37.74	6.44	12.26	4.95	9.43	6.93	13.21	11.88	22.64	13.37	25.47	14.85	28.30
C4035-14c	488.05	52.47	40.10	76.42	6.93	13.21	19.80	37.74	6.44	12.26	4.95	9.43	6.93	13.21	12.47	23.77	13.86	26.42	15.44	29.43
C4035-8	507.45	54.45	45.54	83.64	6.93	12.73	21.78	40.00	6.44	11.82	4.95	9.09	6.93	12.73	13.86	25.45	14.85	27.27	15.84	29.09
C4035-14a	477.60	54.45	42.57	78.18	7.92	14.55	20.79	38.18	7.43	13.64	4.95	9.09	6.93	12.73	12.87	23.64	13.86	25.45	15.84	29.09
C4035-11b	646.75	56.43	43.56	77.19	7.92	14.04	19.80	35.09	5.94	10.53	5.45	9.65	7.43	13.16	14.85	26.32	15.84	28.07	18.32	32.46
C4035-12b	467.65	56.43	42.57	75.44	7.43	13.16	20.79	36.84	6.44	11.40	4.95	8.77	7.43	13.16	12.87	22.81	14.36	25.44	15.35	27.19
C4035-12c	537.30	58.41	43.56	74.58	6.93	11.86	19.31	33.05	5.94	10.17	4.46	7.63	7.43	12.71	13.86	23.73	15.84	27.12	17.82	30.51
C4035-11a	597.00	62.37	46.53	74.60	7.43	11.90	21.29	34.13	5.94	9.52	4.95	7.94	8.42	13.49	13.86	22.22	14.85	23.81	16.83	26.98
Maximum	646.75	62.37	46.53	83.64	7.92	15.15	21.78	44.83	7.43	13.64	5.45	9.65	8.42	13.49	14.85	30.38	15.84	32.91	18.32	35.44
Minimum	201.69	28.71	20.79	72.41	3.47	10.71	10.89	33.05	3.47	9.52	1.98	6.76	2.97	10.26	7.43	22.06	7.92	23.81	8.91	26.47
Mean	406.88	45.85	34.89	75.91	5.95	13.01	16.76	36.62	5.28	11.54	3.96	8.59	5.63	12.11	11.51	25.31	12.68	27.87	14.05	30.90
SD	123.20	9.87	7.90	2.27	1.32	1.16	3.62	2.55	1.16	0.99	1.00	0.85	1.62	1.14	2.14	2.25	2.39	2.46	2.64	2.75

Measurements are given in micrometres. Holotype measurements are in bold.

Abbreviations: bol, body length; btd, buccal tube diameter; btl, buccal tube length; I cl, first claw length; III cl, third claw length; IV cl, fourth claw length; I mp, first macroplacoid length; II mp, second macroplacoid length; III mp, third macroplacoid length; mpri, macroplacoid row length; pt, ratio between a selected structure and the length of the buccal tube multiplied by 100; ssi, stylet support insertion on the buccal tube.

*Male specimens.

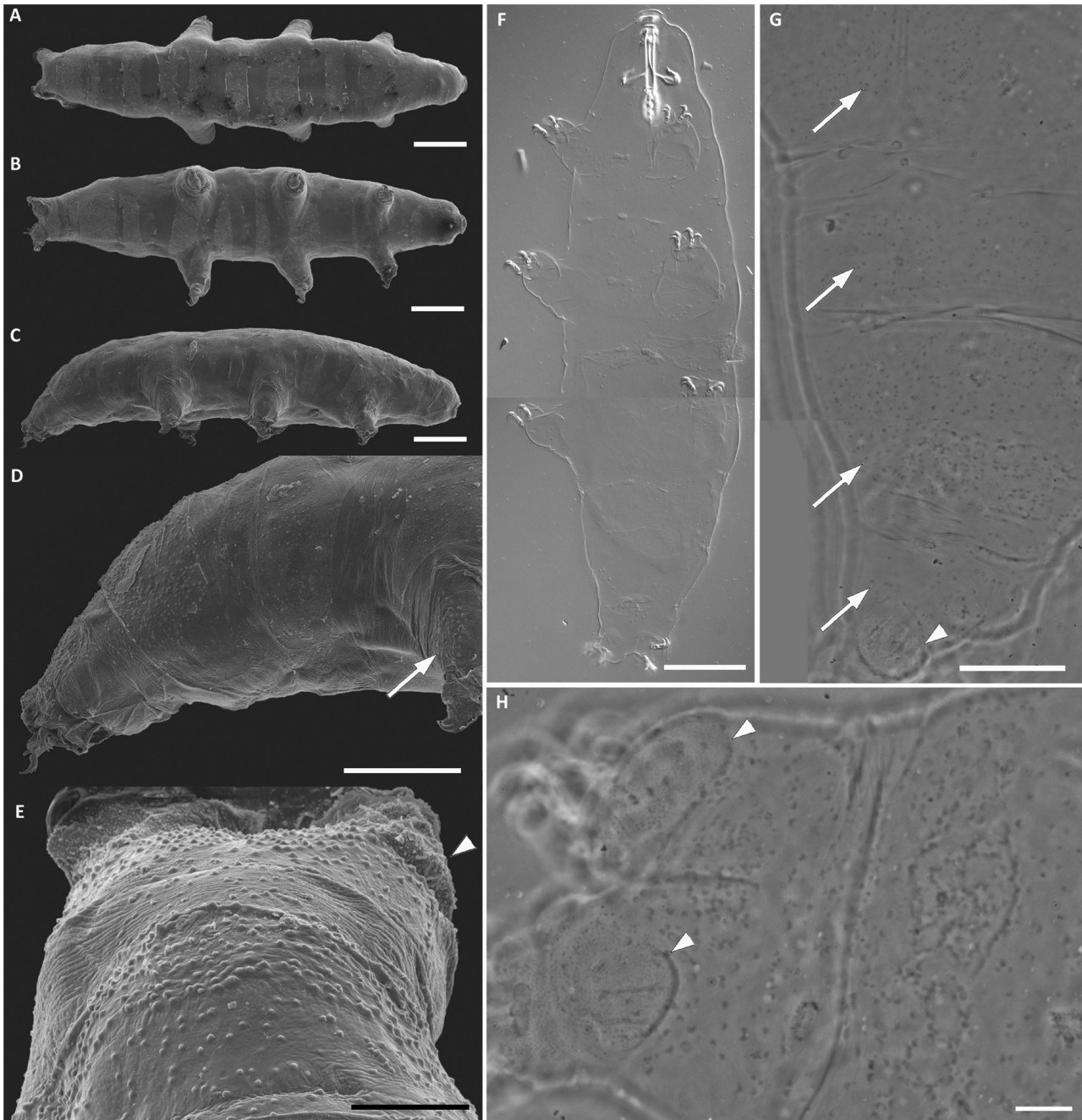


Figure 2. *Mesobiotus joenssoni* A, animal *in toto* (dorsal view). B, animal *in toto* (ventral view). C, animal *in toto* (lateral view). D, granules on cuticle of posterior segments (lateral view). Arrow indicates granules on third leg. E, granules on cuticle of posterior segments (dorsal view). Arrowhead indicates bulge on a hindleg. F, holotype *in toto*. G, granules on dorsal cuticle of the two more posterior segments. Arrows indicate granules, and arrowhead indicates bulge on a hindleg. H, granules on dorsal cuticle of the posterior segment (arrowheads indicate bulges on hindlegs). A–E, scanning electron microscopy; F–H, light microscopy, with phase contrast. Scale bars: 50 µm in A–C, F; 10 µm in D, E, G, H.

Pilato & Sperlinga, 1975; Fig. 5). Specifically, in *M. joenssoni*, granules are present from the level of the third legs to the posterior end of the animal (in most specimens, they are also visible on the third legs), whereas in *M. arguei*, granules are present only on the

caudal extremity of the body. Moreover, *M. joenssoni* has a more anterior stylet support insertion point (SSI) on the buccal tube and longer claws compared with *M. arguei* (*pt* SSI = 85.4, *pt* claw IV = 26.0; Pilato & Sperlinga, 1975).

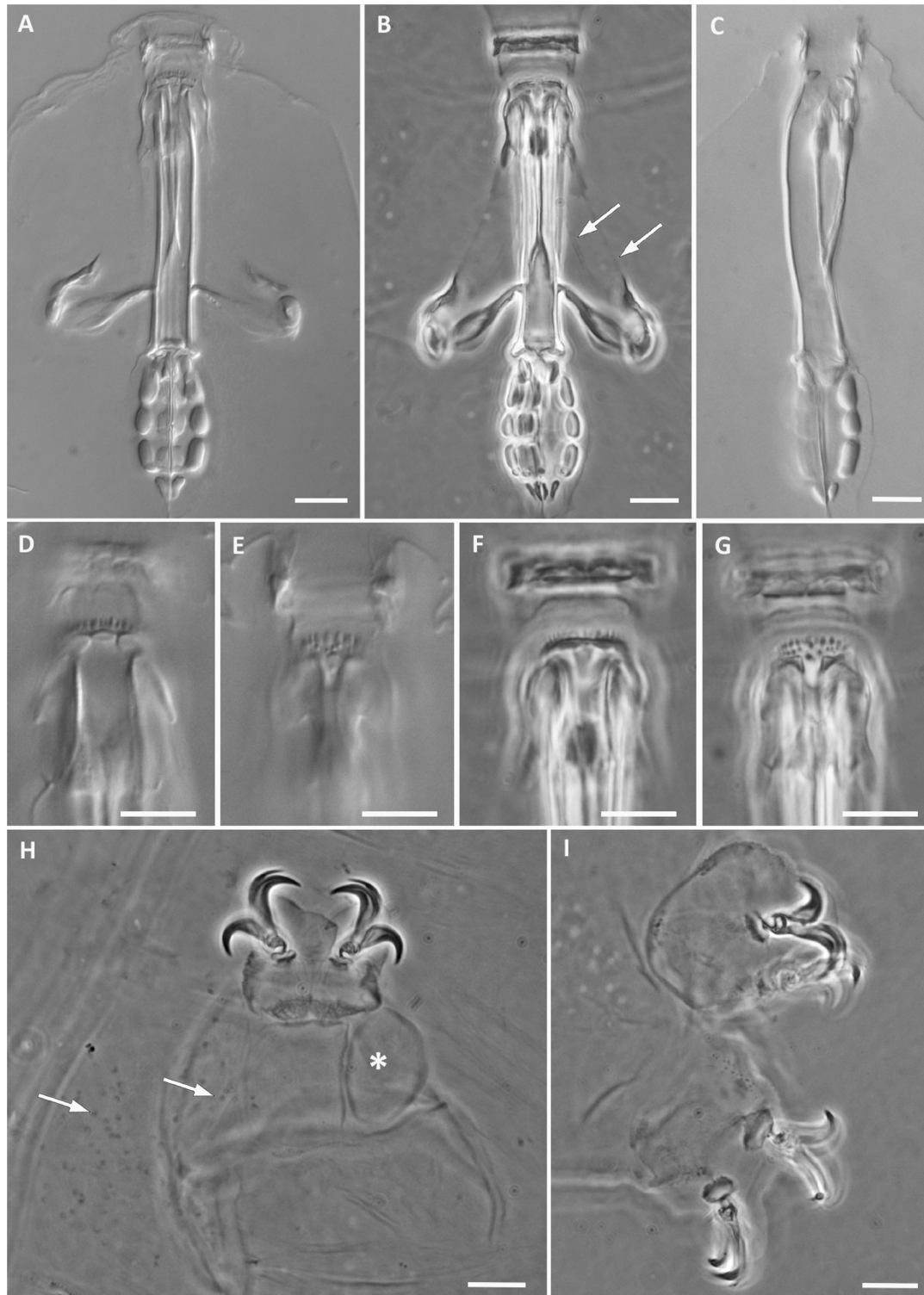


Figure 3. *Mesobiotus joenssoni* A, bucco-pharyngeal apparatus (holotype; merging of eight pictures). B, bucco-pharyngeal apparatus (merging of two pictures). Arrows indicate traces of the stylet coats (see Guidetti *et al.*, 2013). C, bucco-pharyngeal apparatus (lateral view). D, buccal armature (dorsal view; holotype). E, buccal armature (ventral view; holotype). F, buccal armature with fused transverse crests (dorsal view). G, buccal armature (ventral view of the paratype in F). H, leg of the third pair. Arrows indicate cuticular granules. *Bulge on the internal side of the leg. I, legs of the fourth pair (ventral view). A, C–E, light microscopy, with differential interference contrast. B, F–I, light microscopy, with phase contrast. Scale bars: 10 μ m.

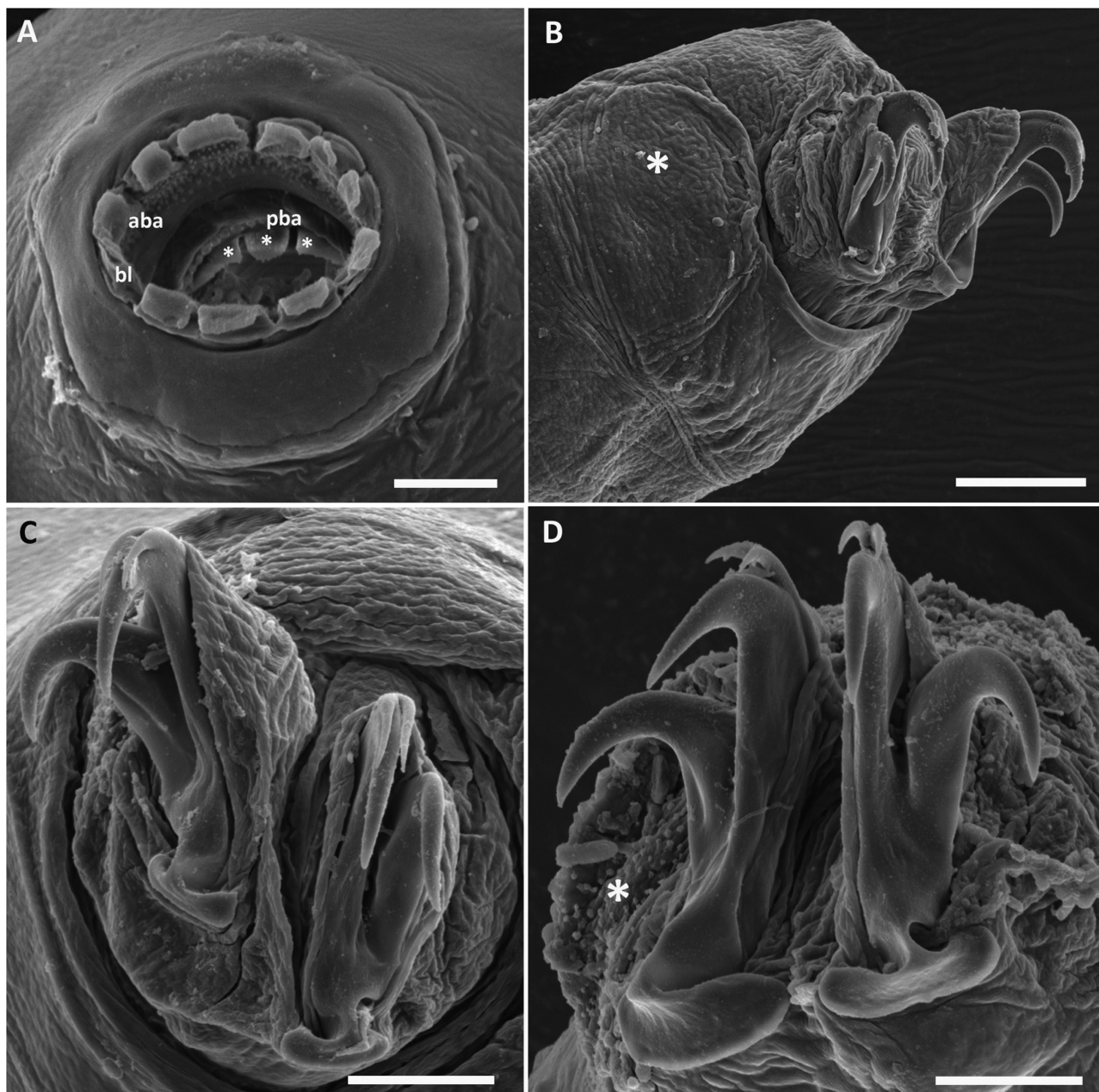


Figure 4. *Mesobiotus joenssoni* A, mouth opening with buccal lamellae (bl) and buccal armature (aba, anterior band; pba, posterior band; *transverse crests). B, leg of the second pair. *Bulge on internal side. C, claws of a second pair of leg. D, claws of a hindleg. *Fine granulation on the cuticle. A–D, scanning electron microscopy. Scale bars: 5 μm in A, C, D; 10 μm in B.

No other *Mesobiotus* species have granules as large as $\sim 1 \mu\text{m}$ in diameter on the cuticle surface (see Kaczmarek *et al.*, 2011). The tiny tubercles or granules present on the cuticle surface of *Mesobiotus contii* (Pilato & Lisi, 2006), *Mesobiotus perfidus* (Pilato & Lisi, 2009), *Mesobiotus pseudocoronatus* (Pilato *et al.*, 2006), *Mesobiotus pseudonuragicus* (Pilato *et al.*, 2004) and *Mesobiotus radiatus* (Pilato *et al.*, 1991) are difficult to see. Moreover, *M. joenssoni* differs from: *M. contii* by larger egg processes, with reticulated surface and in

lower numbers per hemisphere (8.0–8.8 μm in diameter and 82 processes per hemisphere in *M. contii*; Pilato & Lisi, 2006) and by shorter posterior claws on the hindlegs ($pt = 26.0$ in *M. contii*); *M. perfidus* by the presence of the first band of teeth in the buccal armature and the granulation on the third pair of legs, by egg processes without digitations forming a ring of stripes at their base and in lower number per hemisphere (23–29 per hemisphere in *M. perfidus*) and by shorter macroplacoids (pt of macroplacoids: first = 13.3–17.2,

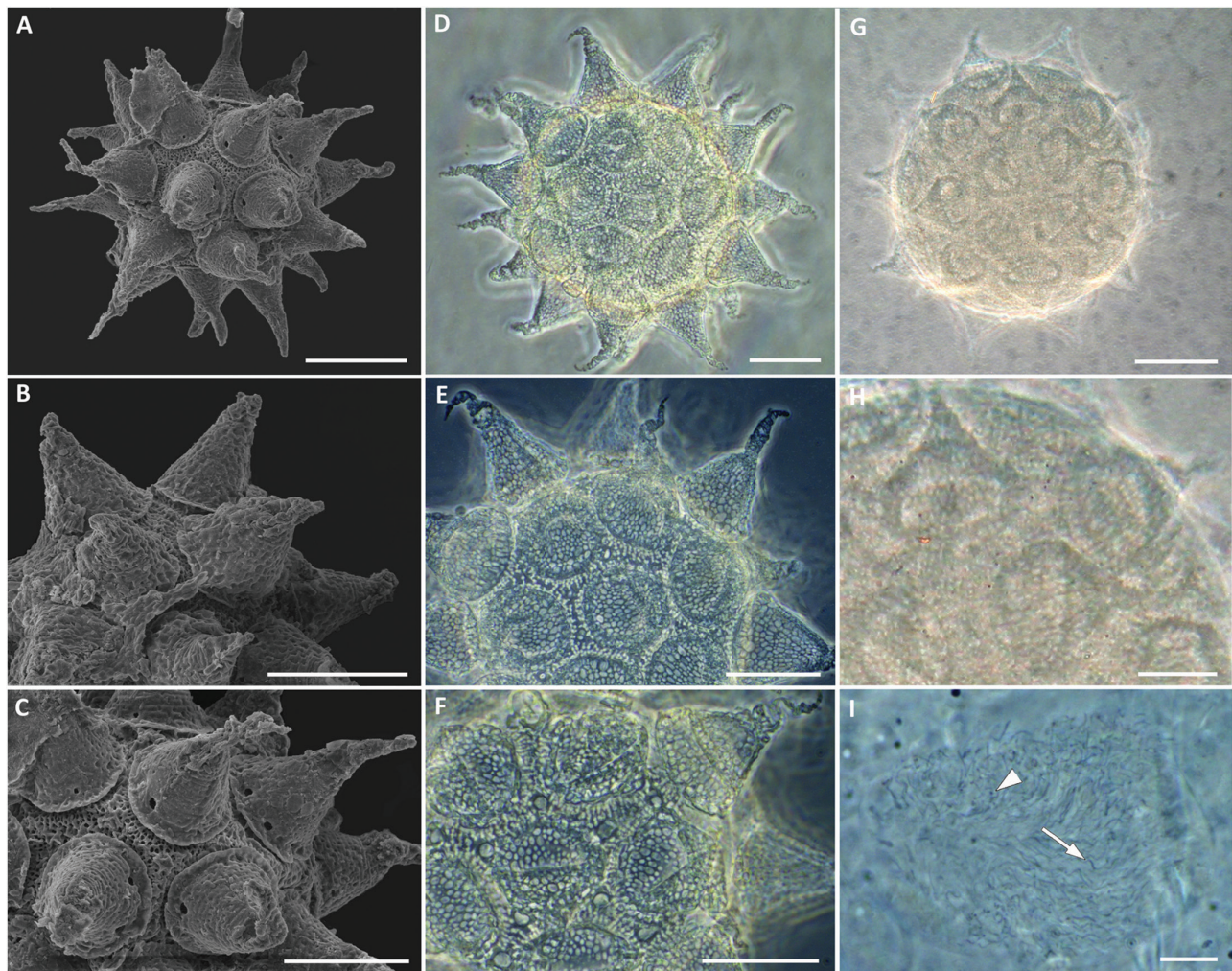


Figure 5. *Mesobiotus joenssoni* eggs (A–F) and male gonad (I). *Mesobiotus arguei* egg (G, H). A, D, egg *in toto*. B, egg processes. C, E, F, egg processes and egg surface. G, egg *in toto* of *M. arguei* (type population). H, egg processes and egg surface of *M. arguei* (type population). I, male gonad with spermatozoa (arrow) and spermatids (arrowhead). A–C, scanning electron microscopy. D–I, light microscopy, with phase contrast. Scale bars: 25 µm in A–G; 10 µm in H, I.

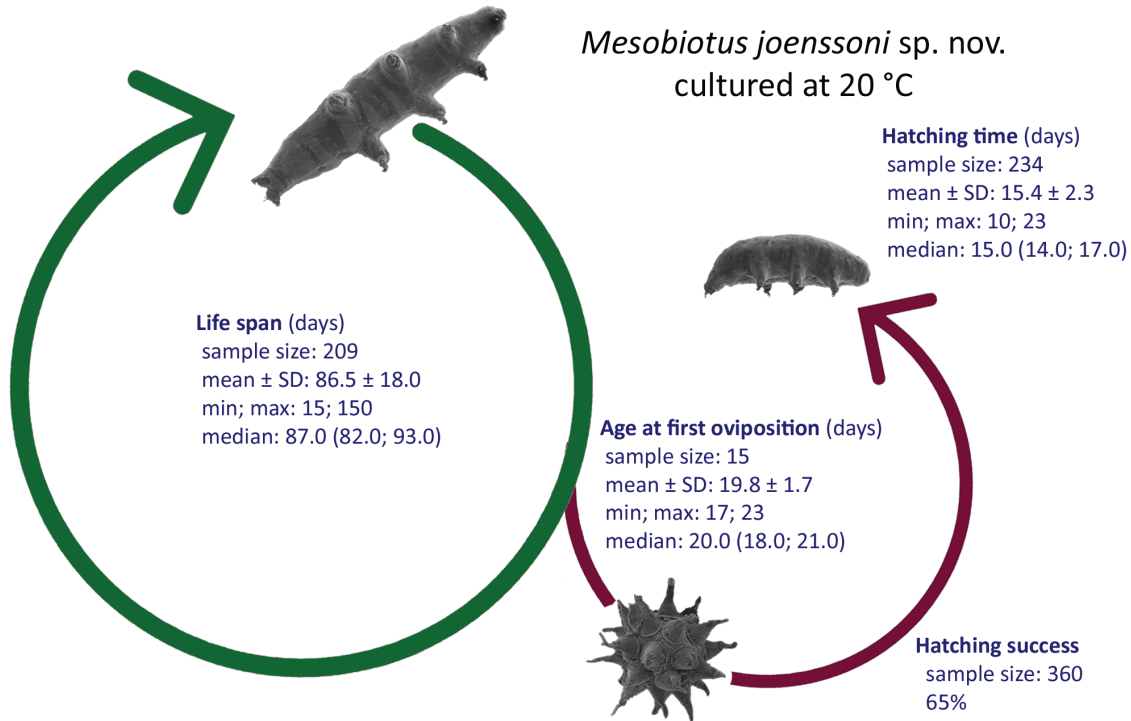
second = 9.6–14.5 in *M. perfidus*; Pilato & Lisi, 2009); *M. pseudocoronatus* by the presence of smooth lunules on the hindlegs, by longer egg processes (without a ring of dots at their base) and in lower numbers per hemisphere (10.9–12.7 µm high and 33–35 per hemisphere in *M. pseudocoronatus*), by shorter macroplacoids (*pt* of macroplacoids: first = 13.9–15.8, second = 11.5–13.6, third = 14.7–18.4 in *M. pseudocoronatus*; Pilato *et al.*, 2006); *M. pseudonuragicus* by the presence of egg processes without areolae around their base, and by shorter macroplacoids (*pt* of macroplacoids: first = 15.5–15.6, second = 10.9–12.0, third = 13.1–14.5, in *M. pseudonuragicus*; Pilato *et al.*, 2004); and *M. radiatus* according to the description of Stec *et al.* (2018), by the presence of smooth lunules in the hindlegs, and by egg processes with reticulated surface and without terminal flexible filaments.

DESCRIPTION OF LIFE CYCLE

The life-history traits of *M. joenssoni*, such as lifespan, age at first oviposition, egg hatching time and egg hatching success, are presented in Figure 6. The active lifespan (i.e. time spent in the active stage) of *M. joenssoni* reached a maximal value of 150 days. In general, adult females laid eggs for the first time at a minimal age of 17 days. The laid eggs all had a similar morphology, and 65% of them hatched after a minimum of 10 days (Fig. 6).

DISCUSSION

The new species is similar to *M. arguei*. In particular, both species share the unique character of having relatively large granules (~1 µm in diameter) on the



Life history traits of cultured tardigrades. Data are shown as either average \pm SD, range, or simple available data.

Species	Life span (days)	Age at first oviposition (days)	Hatching success (%)	Hatching time (days)	Temp. (°C)	Reference
Amphimictic species						
<i>Isohypsibius dastychi</i>	-	44.9 \pm 6.5	80.0	13.1 \pm 1.5	12	Bingemer <i>et al.</i> , 2016
<i>Isohypsibius dastychi</i>	-	28.2 \pm 4.6	66.0	7.6 \pm 1.1	20	Bingemer <i>et al.</i> , 2016
<i>Macrobotus sapiens</i>	83.0 \pm 33.5	16.5 \pm 3.8	78.0	11.9 \pm 2.7	20	Lemloh <i>et al.</i> , 2011
<i>Paramacrobotus tonollii</i>	69.0 \pm 45.1	24.4 \pm 4.4	82.2	16.7 \pm 6.7	20	Lemloh <i>et al.</i> , 2011
<i>Mesoblotus joenssoni</i>	86.5 \pm 18.0	19.8 \pm 1.7	65.0	15.4 \pm 2.3	20	Present study
Parthenogenetic species						
<i>Macrobotus hufelandii</i>	84 max	31	-	26-31	20	Baumann, 1970
<i>Paramacrobotus kenianus</i>	125 \pm 35	10	33.0	7	20	Schill, 2013
<i>Paramacrobotus kenianus</i>	141 \pm 54	10	51.0	8	20	Schill, 2013
<i>Paramacrobotus palaui</i>	97 \pm 31	10	54.0	9	20	Schill, 2013
<i>Paramacrobotus cf richtersi</i>	-	64.2 \pm 1.7	100	28.1 \pm 1.2	16	Hohberg, 2006
<i>Paramacrobotus fairbanksi</i> ^{1*}	194.9 \pm 164.4	76.9 \pm 16.4	83.1 \pm 12.7	41.0 \pm 9.5	14	Altiero <i>et al.</i> , 2006, 2010
<i>Paramacrobotus fairbanksi</i> ^{2*}	137.3 \pm 136.4	70.7 \pm 19.4	51.0 \pm 36.0	60.0 \pm 16.7	14	Altiero <i>et al.</i> , 2006, 2010

¹clone named CDMr01; ²clone named CDMr02. * this species was reported as *Paramacrobotus richtersi* in Altiero *et al.* (2006, 2010) but according to Guidetti *et al.* (2019) it is *P. fairbanksi*.

Figure 6. Life-history traits of *Mesoblotus joenssoni* compared with those of other gonochoric amphimictic tardigrade species or parthenogenetic macrobotids. Numbers in parentheses are the 25th and 75th percentiles. Data are shown as the mean \pm SD, the maximal value (max) or simply available data. Temp. is the temperature at which the species were cultured. The data reported refer to Altiero *et al.*, (2006, 2010), Baumann (1970), Bingemer *et al.* (2016), Guidetti *et al.* (2019), Hohberg (2006), Lemloh *et al.* (2011), and Schil (2013).

dorsolateral surface of the last body segments; these granules are absent in all other species of the genus and even of the family. These two species certainly exhibit a

close phylogenetic relationship and probably represent sister taxa (unfortunately, no genetic data are available for *M. arguei*). *Mesoblotus arguei* was found on Sardinia,

which is 180 km south-west of Elba Island, where *M. joenssoni* was found (Fig. 1). Elba is a relatively young island (~7 Myr old; Bortolotti *et al.*, 2001) and was not in contact with Sardinia in the past. Therefore, it is possible to hypothesize a two-step scenario for the origin of the two species: a colonization of one of the islands (Elba or Sardinia), followed by relatively recent colonization of the other island that induced a speciation event owing to geographical isolation (allopatric speciation).

Mesobiotus joenssoni is a dimorphic species, with males that are morphologically different from females, i.e. males are smaller than females. Secondary sex characters have been observed in many heterotardigrade species and in a few eutardigrade taxa. For example, in both classes, males are generally smaller than females, although the body size is not a useful criterion to distinguish sex in tardigrades, because mature males may be larger than immature females (Bertolani, 1992; Rebecchi & Nelson, 1998). Bulges (gibbositities) are reported on the hindlegs of males of some *Ramazottius* and *Macrobiotus* species (Baumann, 1970; Bertolani, 1992), but in *M. joenssoni*, bulges on the hindlegs are present in both sexes.

Mesobiotus joenssoni is the first species in the genus *Mesobiotus* for which the life cycle has been analysed. For this reason, it is not possible to compare its life-history traits with other species of the same genus, but only with other phylogenetically related species belonging to Macrobiotidae. Life-history data are available for only two gonochoric amphimictic species of macrobiotids: *Macrobiotus sapiens* Binda & Pilato, 1984 and *Paramacrobiotus tonollii* (Ramazzotti, 1956). *Mesobiotus joenssoni* has a mean lifespan similar to that of *M. sapiens* (83 days; Fig. 6), which lives for a maximum of 145 days (Lemloh *et al.*, 2011), whereas *P. tonollii* has a lower mean lifespan (69 days; Fig. 6), but it reaches a higher maximal age (237 days; Lemloh *et al.*, 2011). The age of first oviposition and the egg hatching time appear similar among all three species. Specifically, for all three species, the age of first oviposition is 17–24 days and the hatching time of eggs 10–23 days (Fig. 6). The only difference is in the hatching success, which is clearly lower in *M. joenssoni*.

The other Macrobiotidae species for which the life-history traits data are available reproduce by parthenogenesis, i.e. *Macrobiotus hufelandi* C. A. S. Schultze, 1834, *Paramacrobiotus kenianus* (Schill *et al.*, 2010), *Paramacrobiotus palaui* (Schill *et al.*, 2010), *Paramacrobiotus fairbanksi* (Schill *et al.*, 2010) and *Paramacrobiotus cf. richtersi* (Fig. 6). Although comparisons among these species are possible, some of them were cultured at lower temperatures, which reduced the metabolic and developmental rates (e.g. Bingemer *et al.*, 2016; Vasanthan *et al.*, 2019). The cultured species in the genus *Paramacrobiotus* had a mean lifespan longer (96–194 days) than *M. joenssoni*,

whereas the only representative of the genus *Macrobiotus* (*M. hufelandi*) lived for a shorter time than *M. joenssoni* [84 vs 150 days as a maximum, respectively; the mean lifespan was not reported by Baumann (1970); Fig. 6]. Adult females of *M. joenssoni* reached sexual maturity earlier than females of *P. fairbanksi* and *P. cf. richtersi* (64–77 days). The laid eggs of *M. joenssoni* hatched earlier (20–60 days) and with a lower hatching success than the eggs of *P. fairbanksi* and *P. cf. richtersi* (83–100%), with only one exception for the eggs of the *P. fairbanksi* (clone CDMr02; Altiero *et al.*, 2006; Fig. 6). The other two parthenogenetic Macrobiotidae (i.e. *P. kenianus* and *P. palaui*) reached sexual maturity earlier (10 days), had a lower percentage of hatched eggs (33–54%) and took less time to hatch (7–9 days) than *M. joenssoni* (Fig. 6). Finally, females of *M. joenssoni* reached sexual maturity earlier than those of *M. hufelandi* (31 days) and laid eggs that took less time to hatch (26–31 days; Fig. 6).

These data show that different adaptive strategies are present in Macrobiotidae. Although considerable variations in life-history traits may occur within populations of the same species and among different species (Altiero *et al.*, 2018), based on the minimal data available, it seems that the macrobiotid species with amphimictic reproduction have more uniform life-history traits than the macrobiotid parthenogenetic species, independently of the genus to which they belong.

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