

Three New Tardigrade Species Associated with Barnacles from the Thai Coast of Andaman Sea

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Three new marine heterotardigrades, *Archechiniscus symbalanus*, and *Styraconyx craticuliformis* of the family Halechiniscidae and *Echiniscoides andamanensis* of the family Echiniscoididae, are reported on the basis of the specimens sieved from intertidal barnacles in the Thai coast of the Andaman Sea. *Archechiniscus symbalanus* n. sp. is related to *Archechiniscus minutus* Grimaldi De Zio and D'Addabbo Gallo, but discernible from it by possessing median cirrus, stylet sheath, sensory spine of leg I, prominent basal processes on all leg pairs, and S-shaped seminal receptacles. *Styraconyx craticuliformis* n. sp. is similar to *S. craticulus* (Pollock) in bearing the grid-like dorsal cuticle, but distinguished from it by the shape of claws and cirrophore of primary clava. *Echiniscoides andamanensis* n. sp. is related to *Echiniscoides pollocki* Hallas and Kristensen and *E. sigismundi sigismundi* (M. Schultze) in sharing the same patterns of sensory leg appendages and the claw configuration of 8-8-8-7, but characterized by the shape of cirri, pharyngeal apparatus and female gonopore.

Reports on the marine tardigrades associated with other invertebrates, most of which concerned the barnacles, are still rare. Until now, only eight marine tardigrade species or subspecies were known as the barnacle-associates: *Archechiniscus marci* Schulz, 1953, *Styraconyx craticulus* (Pollock, 1983), *Echiniscoides sigismundi sigismundi* (M. Schultze, 1865), *E. s. galliensis* Kristensen and Hallas, 1980, *E. s. groenlandicus* Kristensen and Hallas, 1980, *E. s. hispaniensis* Kristensen and Hallas, 1980, *E. hoepneri* Kristensen and Hallas, 1980, *E. s. mediterranicus* Kristensen and Hallas, 1980.

As a result of examining our collection sieved from barnacles of *Balanus variegatus* Darwin, 1854 at a rocky shore in the eastern coast of the Andaman Sea, near Ao Nang beach, Krabi province, Thailand, three new species were recognized. This report contains the description of the new species with illustrations and scanning electron microscope (SEM) photographs. This is the first report of marine tardigrades from Thailand.

Materials and Methods

Materials were obtained by scraping the barnacles of *B. variegatus* at the upper intertidal zone of rocky shore. Samples were filtered in the field through with a nylon net (64 µm in pore diameter) after rinsing with freshwater for less than a minute for osmotic shock

(Kristensen, 1989), to be fixed with 5% formalin.

Specimens were drawn and measured in lactic acid on Cobb's aluminum hole slide, and also observed under a differential interference contrast microscope. After examination, specimens were mounted in glycerine and sealed with glyceel. All drawings were prepared using a camera lucida. Specimens for SEM examination were treated with hot (about 96°C) ethanol immediately after extraction, and fixed again overnight at 4°C in a 2.5% buffered glutaraldehyde, then followed by postfixation with 1% cold buffered osmium tetroxide. After dehydration through a graded ethanol series (50%, 60%, 70%, 80%, 90%, 100%, 100%) for 30 min each, the material was dried at the critical point, and coated with gold-palladium in a high evaporator, and then examined in a Hitachi S-520 scanning electron microscope operated at 20 KV.

Systematic Accounts

Order Heterotardigrada
Suborder Arthrotardigrada
Family Halechiniscidae Thulin, 1928
Subfamily Archechiniscinae Grimaldi De Zio and
D'Addabbo Gallo, 1987
Genus *Archechiniscus* Schulz, 1953

Archechiniscus symbalanus n. sp.
(Fig. 1, 4A-D)

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Material examined: 25 females, 13 males, 12 juveniles from *Balanus variegatus*, Ao Nang, Krabi, Thailand (08° 02' N, 98° 50' E), 29 January 1998, C. Y. Chang

and H. S. Rho. 25 specimens mounted, 12 specimens gold-coated on the aluminium stub for SEM preparation, and 13 specimens stored in 5% buffered formalin. Holotype female and three paratypes (2 males and 1 juvenile) will be deposited in the National Museum of Natural History, Smithsonian Institution. Other paratypes are kept in the collection of the authors.

Diagnosis: Cuticle finely pointed; median cirrus and stylet sheath present; seminal receptacles S-shaped; with sensory spine on leg I and prominent basal processes on all legs.

Holotype: Body conically tapering toward mouth end. Trunk cylindrical. Distal margin of caudal portion round. Length 209.2 μm , measured from mouth opening to caudal extreme region, and 63.8 μm wide, 30.5 U (percentage unit of the ratio to body length) between third pair of legs. Not bearing any particular cephalic, somatic (between leg pairs) or caudal projections. Eye spots present, colored black in lactic acid mounting.

Head somewhat protruding anteriorly, but not as long as wide, 30.8 μm long (14.7 U) from foremost part of mouth to the line connecting lateral cirri when conical projection of mouth protruded; 41.7 μm wide between lateral cirri; anterior margin of head without any notch between internal and external cephalic cirri. Cephalic appendages with complete set of relatively reduced sensory cirri and clava. Unpaired median cephalic cirrus very small, 2.3 μm long (1.1 U), consisting of distinct pedunculate base and sharp spine arising mid-dorsally, locating 13.5 μm posterior to mouth opening. Paired internal cirri 4.6 μm long (2.2 U) including thickened peduncle; distance between internal cirri 16.5 μm , without any papilla between them; 11.9 μm posterolaterally apart from mouth opening. Paired external cirri 5 μm long (2.4 U), locating posterolaterally to mouth opening and internal cirri, arising from prominent pedestals. Paired lateral cirri 5.8 μm long (2.8 U), without cirrophores and annulated pedestal, situated just near eye spots. Primary clava 3.8 μm long (1.8 U) and 3.2 μm wide, emerging together with lateral cirri, without cirrophore. Secondary clava slightly protruded, 5.8 μm wide and 3.1 μm thick, attached directly to head, situated just on base of external cirri. Each median, internal or external cirri consisting of thick peduncle and relatively sharp spine.

Mouth protrusion 7.7 μm long, 7.7 μm wide, locating subterminally to anterior margin of head, telescopically divided into three portions under observation of differential interference contrast microscope and scanning electron microscope. Mouth opening smooth, without any ornamentation. Buccal tube 38.1 μm long (18.2 U) and 2.3 μm wide, ended to pharyngeal bulb. Pharyngeal bulb relatively small and spherical, 12.3 μm long (5.9 U) and 15 μm wide, with notch in anterior and posterior region, furnishing 3 short placoids (8.5 μm , 8.8 μm , and 8.5 μm in lengths from left to right, fused

anteriorly and connected to buccal tube), situated at same level of first leg pair. Piercing stylets smooth, 28.5 μm long (13.6 U), tapering anteriorly with sharp apical point and wide basal portion, but without any stylet supports; stylet sheath present, 17.7 μm in length.

Cuticle transparent or yellowish, armed with fine processes, bearing somatic sensory spines (paired cirri E) and an ovoid papilla on dorsolateral surface of caudal region near base of leg IV. Cirrus E, locating just above base of leg IV, relatively long, 7.3 μm (3.5 U), tapering posteriorly, bare and not equipped with any basal peduncle or accordion-shaped basal portion.

Legs similar to those of *Echiniscoides* species. Stubby legs nearly same in length, and not telescopic. Sensory spine minute (2.9 μm long), present only on first leg pair, without annulation. Each leg prominently swollen into process at distal part. In all leg pairs, internal two claws longer than external two, external ones directly ended in its base, without toes, while internal ones are separated from the base by prominent pedestal toes, ending with the unci. Unci of internal claws provided with an accessory dorsal point. External claws bearing stout basal spur in proximal region with large unci. Two conspicuous peduncles (3.5 μm long and 1.9 μm wide in leg IV) situated at bases of external claws. All claws without claw sheath. In leg IV, internal toes 10.4 μm long (5.0 U) and slightly wrinkled with parallel folds.

Paired seminal receptacles (28.5 μm long and 3.1 μm wide at opening) S-shaped, with simple genital duct whose opening lateral to gonopore. Female gonopore just ahead of leg IV, relatively large, 7.3 μm in diameter, surrounded with 6 rosettes of a small cuticular membrane. Distance between gonopore and anus 4.6 μm . Anus surrounded with numerous wrinkles.

Variability: No sexual dimorphism could be detected except the shape of gonopore consisting of six rosettes of cuticular plates in females, whilst circular in male, and the relative distance between gonopore and anus (much farther in female) as shown in Fig. 1A, C. Furthermore, female and juvenile specimens do not bear claw sheath, whereas males do.

Juveniles (Fig. 1B) showed the remarkable morphological differences from adults: (1) possessing only two claws (internal claws) on all legs, (2) eye spots situating posteriorly compared with adults, (3) basal processes indistinct on all legs, (4) large peduncles of external claws absent, (5) position of pharyngeal apparatus a little posterior to that of adult, (6) gonopore and anus not observed, (7) all sensory cirri, spines, and pharyngeal apparatus much developed in consideration to their small body sizes.

Etymology: The proposed specific name, *symbalanus*, is from the Greek sym- (together) and balanos (barnacle). It alludes to "living together with barnacles".

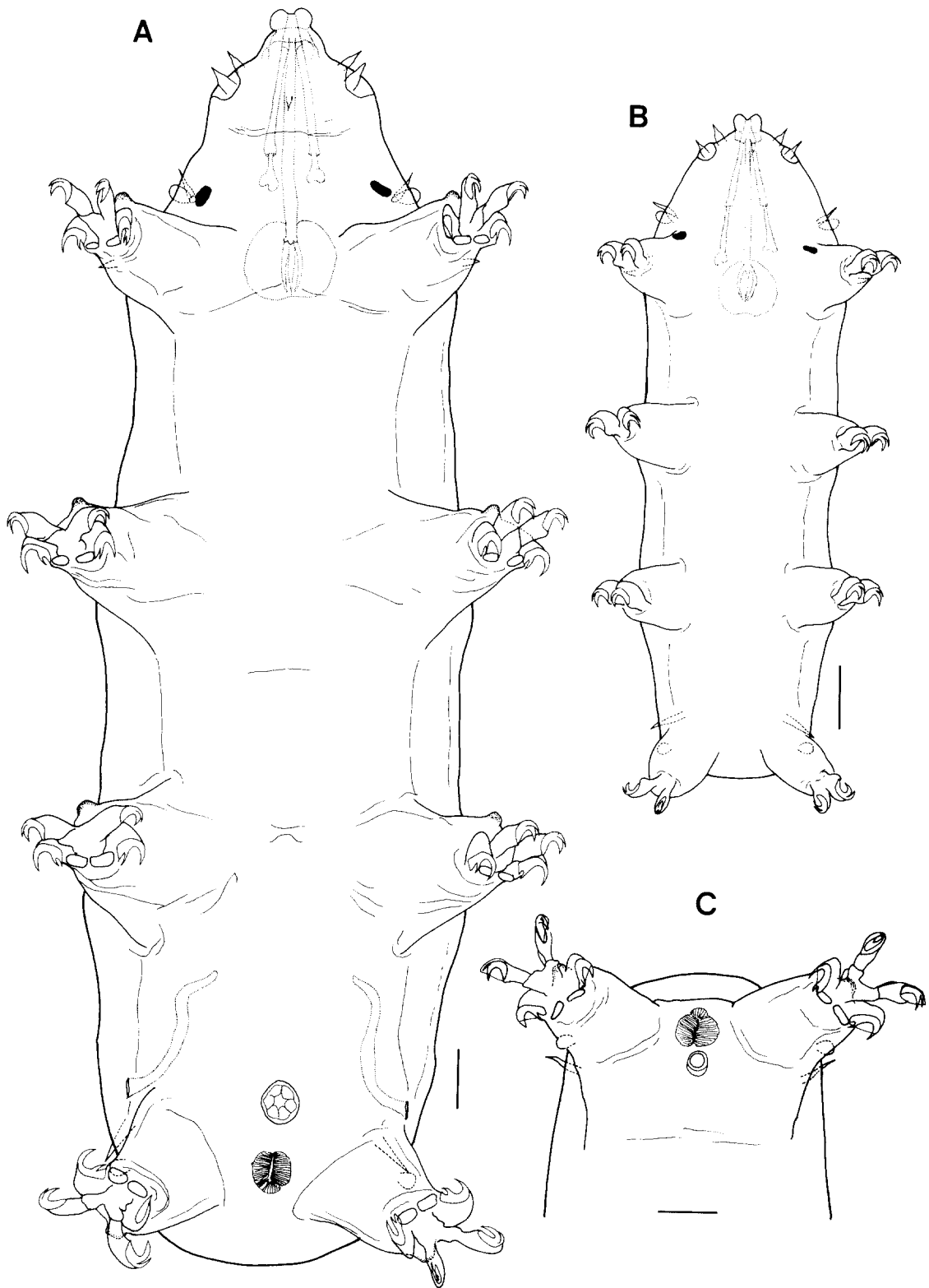


Fig. 1. *Archechiniscus symbalanus* n. sp. A, Habitus, holotype female (ventral view). B, Habitus, juvenile (ventral view). C, Caudal portion, allotype (ventral view). Scale bars=10 μ m.

Remarks: Until now, only two species have been recorded in the genus *Archechiniscus*, *A. marci* Schulz, 1953 and *A. minutus* Grimaldi De Zio and D'Addabbo Gallo, 1987. This rarely known halechiniscid genus is mainly characterized by the morphology of its unique claw shape, that is, two external claws directly inserted on the tarsus and internal claws supported by toes (Grimaldi De Zio and D'Addabbo Gallo, 1987). Of the preceding species, the new species apparently greatly resembles *A. minutus* in having the finely pointed cuticles, smooth stylet and large peduncles of external claws in the terminal portion of each leg pair, but clearly differs from it in having the following combination of characteristics: (1) median cirrus, stylet sheath, tiny leg sensory spine I, and prominent basal processes on all legs present, (2) secondary clava protruded, (3) papilla situated not on leg IV but on dorsolateral surface of caudal region near base of leg IV, (4) seminal receptacles S-shaped, (5) claw sheath absent in females and juveniles, (6) transversal metameric dorsal cuticular folds absent.

Subfamily Styraconyxinae Kristensen and
Renaud-Mornant, 1983

Genus *Styraconyx* Thulin, 1942

Styraconyx craticuliformis n. sp.
(Fig. 2)

Material examined: 2 adult females, collection data same as in the preceding species. Holotype will be deposited in the National Museum of Natural History, Smithsonian Institution, and paratype is kept in a collection by the authors.

Diagnosis: Presence of grid-like dorsal cuticle with fine punctuation; with 4 peduncles in each leg; primary clava and lateral cirrus surrounded basally by common cirrophore.

Holotype: Body not protruded in frontal region. Trunk cylindrical and protruding between each leg pair. Distal margin of caudal portion somewhat flattened. Body length 131.9 μm excluding cirri, width 45.6 μm (34.1 U) between leg pair II, in lactic acid mounting.

Dorsal cuticle transparent or brownish, armed with 22 grid-shaped structure arranged in a column and 6 ones in a row. Ventral cuticle regularly punctuated. Not bearing any particular cephalic, somatic or caudal projections. Eye spots absent.

Head width 31.9 μm between bases of lateral cephalic cirri. Anterior margin of head without any notch between internal and external cephalic cirri. Cephalic appendages with complete set of relatively well developed sensory cirri and clavae. Median cirrus very large, 7.8 μm long (5.9 U), consisting of distinct pedunculate scapus and flagellum, 2.8 μm apart from anterior margin of head. Paired internal cirri com-

paratively long 10 μm (7.6 U), also divided into two parts including thickened scapus and sharp flagellum; distance between internal cirri 15 μm , without any papilla between them. Paired external cirri 8.6 μm long (6.5 U), not segmented, locating posterolaterally to mouth opening, arising from smooth pedestals. Lateral cirrus, 21.3 μm long (16.1 U), not segmented, emerging together with primary clava (8.8 μm long and 3.8 μm wide) from small pedestal, both of them surrounded basally by common cirrophore. Cirrophore, 3.8 μm long (2.9 U) and 5.6 μm wide. Van der Land's body not observed. Secondary clava indistinct.

Large mouth protrusion not telescopic, 10 μm wide, locating subterminally. Mouth opening smooth, without any ornamentation. Buccal tube 16.3 μm long (12.4 U) and 0.9 μm wide, ended to pharyngeal bulb. Pharyngeal bulb very large and spherical, 15 μm long (11.4 U), 16.3 μm wide, without notch in anterior and posterior region. Three long placoids (17.5 μm , 15.3 μm , and 17.5 μm long from left to right) fused anteriorly and connected to buccal tube. Piercing stylets smooth, 15 μm long (11.4 U), tapering with wide basal portion to sharp apical point; with stylet supports; sheath present, 11.6 μm long.

Cirrus E located just above base of leg IV, relatively long, 20.6 μm (15.6 U), equipped with prominent basal cirrophore, but without accordion-shaped basal portion.

Each leg composed of coxa, femur, tibia and tarsus of styraconyxid type. Coxa of legs I-III each bearing a spinous sensory appendage. Each sensory spine without annulation. Sensory spine on leg I of two portion, scapus (4.4 μm long) and flagellum (2.5 μm long), but the spines on leg II-III not segmented and antero-dorsally curved. Appendage on leg IV consisting of papilla (5 μm long) and long spine (4.4 μm long) as shown in Fig. 2F. Tarsus of legs I through IV bearing 4 digits of different size; external digit shorter than internal digit (external digit 10.2 μm long; internal one 12.9 μm , in leg IV). Peduncles of external digits hook-shaped, and those of internal ones simple rod-shaped on heart-shaped proximal pads. Claws three-pointed with accessory, primary and secondary hooks, of which accessory or secondary ones reduced, while the primary one is well-developed. Claw sheath covering accessory and secondary hooks, but tip of primary hook free from it.

Seminal receptacles not observed. Female gonopore relatively large, 6.3 μm in diameter, surrounded with 6 rosettes of small cuticular membrane. Distance between gonopore and anus 5.5 μm . Anus consists of 2 large lateral plates.

Etymology. The specific name *craticuliformis* is referring to the similarity of the new species with *S. craticulus* (Pollock, 1983), especially in possessing the grid-like pattern of the dorsal cuticle (craticulus, L.: lattice comprised of an open structure of crossed strips; formis, L.: suffix denoting 'resembling' or 'having the form of').

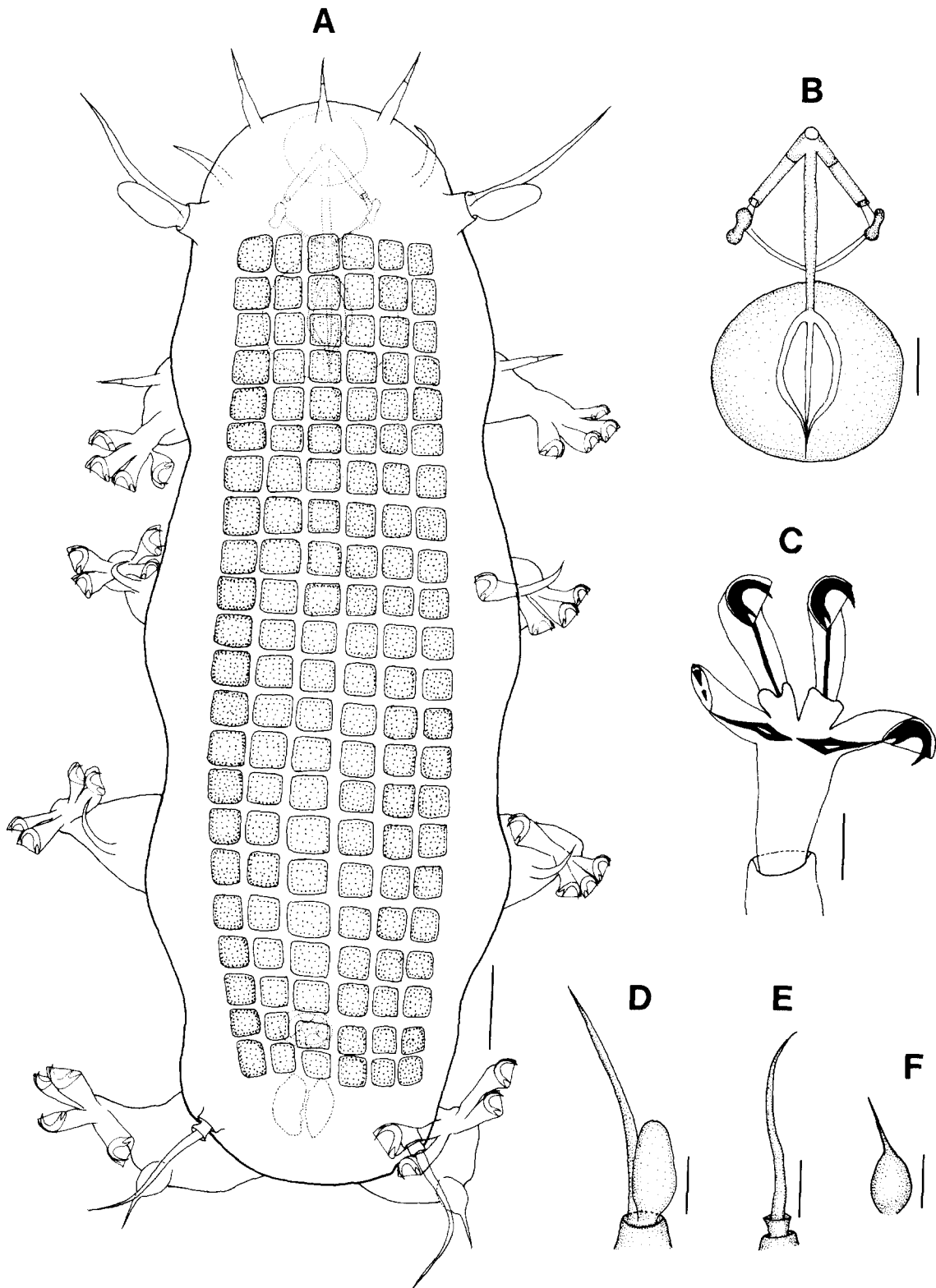


Fig. 2. *Styraconyx craticuliformis* n. sp. A, Habitus, female (dorsal view). B, Pharyngeal apparatus (dorsal view). C, Internal claw of leg IV (ventral view). D, Primary clava and lateral cirrus (dorsal view). E, Cirrus E. F, Papilla on leg IV. Scale bars=10 μ m (A) and 5 μ m (B-F).

Remarks: A total of eleven species are currently recorded in the genus *Styraconyx* Thulin, 1942, the classification of which depends on the character combinations such as the shape of head and somatic appendages, the general morphology of claws, and the number of peduncles (e.g. two or four), and the genus is generally divided into two major groups, that is, the *S. hallasi* group and the *S. sargassi* group. *Styraconyx hallasi* group bears two peduncles and three-pointed claws of different size, while *S. sargassi* group bearing four peduncles and three-pointed claws of almost identical size (Kristensen and Renaud-Mornant, 1983; Kristensen and Higgins, 1984; D'Addabbo Gallo et al., 1989). The new species possesses the cross (mosaic) character combination of four peduncles and claws of different size, sharing the former characteristics with the *S. sargassi* group, whereas the latter with the *S. hallasi* group. Consequently, it cannot be decided which group the new species is to be allocated to for the present.

Styraconyx craticuliformis n. sp. appears to be most similar to *S. craticulus* (Pollock, 1983) in having the grid-like dorsal cuticle, the decisive characteristic differentiating these two species from the other congeners. However, it shows a few discrepancies from *S. craticulus*: (1) primary clava surrounded only basally by cirrophore in *S. craticuliformis*, whereas enveloped in *S. craticulus*; (2) sensory appendage on leg IV nearly smooth, whereas it is covered with tubercles in *S. craticulus*; (3) three-pointed claws progressively longer in the series of accessory-secondary-primary, while accessory-primary-secondary in *S. craticulus*; (4) anus composed of two lateral cuticular folds only, whilst with a small triangular flap additionally in *S. craticulus*.

Styraconyx craticuliformis also shows some resemblance to *S. tyrrenus* D'Addabbo Gallo et al., 1989 in the overall shape of sensory appendage of leg IV and having the pointed claws of different size, but discernible from it in the shape of cirrus E (not annulated in *S. craticuliformis*, against annulated proximally in *S. tyrrenus*).

Suborder Echiniscoidea Marcus, 1927

Family Echiniscoididae Kristensen and Hallas, 1980

Genus *Echiniscoides* Plate, 1889

Echiniscoides andamanensis n. sp.
(Fig. 3, 4E-I)

Material examined: 15 females, 15 males, 10 juveniles, collection data same as in the preceding species. 20 specimens mounted, 12 specimens gold-coated for SEM preparation, and 8 specimens stored in 5% buffered formalin. Holotype female and three paratypes (2 males and 1 juvenile) will be deposited in the National Museum of Natural History, Smithsonian Institution. Other paratypes are kept in the collection by the authors.

Diagnosis: Cuticle smooth; cirrus E annulated; stylet sheath present; stylet flexible; both leg I and leg II bearing dome-shaped appendage; with 8 claws on legs I-III and 7 on leg IV; female gonopore surrounded with 6 rosettes, forming a protrusion.

Holotype: Body contour smooth and conically tapering toward mouth end. Trunk cylindrical. Distal margin of caudal portion flattened. Length 231 μm , measured from mouth opening to caudal extreme region, and width 84.8 μm (36.7 U), between third pairs of legs, in lactic acid mounting. Eye spots present, colored black. Cuticle transparent or yellowish, smooth without any ornamentation except on the cirri E and sensory appendages of legs.

Head broader than long, 31 μm long (13.4 U) from foremost part of mouth to the line connecting lateral cirri when conical projection of mouth protruded, and 48.6 μm wide; without any notch between internal and external cephalic cirri. Cephalic appendages with abnormal morphs are relatively reduced. Median cirrus absent. Paired internal cirri 5.2 μm long (2.3 U) including thickened peduncle; 11.4 μm posterolaterally apart from mouth opening; distance between internal cirri 18.1 μm . Paired external cirri 4.3 μm long (1.9 U), smaller than internal ones, arising from prominent pedestals. Paired lateral cirri 7.1 μm long (3.1 U), with weak annulations proximally, without cirrophores, adjoining primary clavae. Primary clava papilla-shaped, 4.3 μm long (1.9 U) and 2.9 μm wide. Secondary clava dome-shaped, situated between internal and external cirri, 9.5 μm wide and 4.3 μm thick, attached directly to head.

Large mouth protrusion not telescopic, 10.5 μm wide, located subterminally. Mouth opening smooth. Buccal tube 38.6 μm long (16.7 U) and 1.9 μm wide, ended to pharyngeal bulb. Pharyngeal bulb laterally oblong (17.1 μm long, 21.9 μm wide) from dorsal or ventral view, but ovoid from ventrolateral view, with notch in anterior and posterior region; furnished with 3 short placoids (11.9 μm , 11 μm and 11.9 μm in lengths from left to right, fused anteriorly and connected to buccal tube), situated between first leg pair. Piercing stylets smooth and flexible, 35.6 μm long (15.4 U), tapering with wide basal portion to sharp apical point; equipped with stylet supports and prominent stylet sheath (27.8 μm long).

Cirrus E rather short, 9.3 μm (4 U), with weak annulations, but not equipped with basal peduncles.

Stubby legs nearly same in length, not telescopic. Sensory appendages present on all legs, as shown in Fig. 3F: small dome-shaped papilla each on legs I and II (2.4 μm and 1.5 μm in diameter, respectively); a setal appendage thickened at its proximal part (6.7 μm long) on leg III; a papilla (3.3 μm long and 2.7 μm wide) on leg IV. Appendage on leg I situated more distally as compared to those of other legs. Each leg pair directly ended with claws. Legs I-III with 8 claws, and legs IV

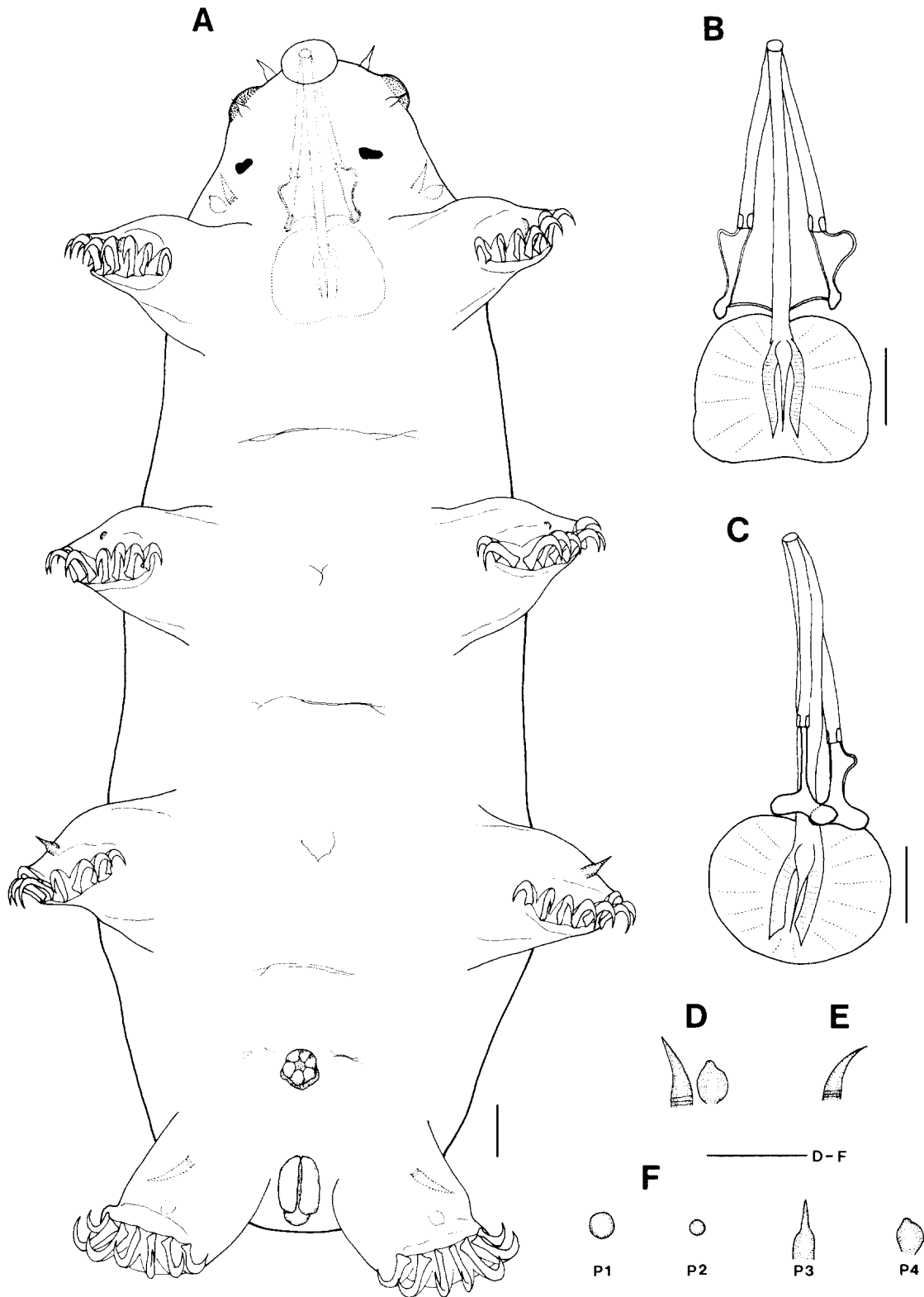


Fig. 3. *Echiniscoides andamanensis* n. sp. A, Habitus, female (ventral view). B, Pharyngeal apparatus (dorsal view). C, Pharyngeal apparatus (ventrolateral view). D, Primary clava and lateral cirrus (dorsal view). E, Cirrus E (dorsal view). F, Leg appendages (lateral view). Scale bars=10 μ m.

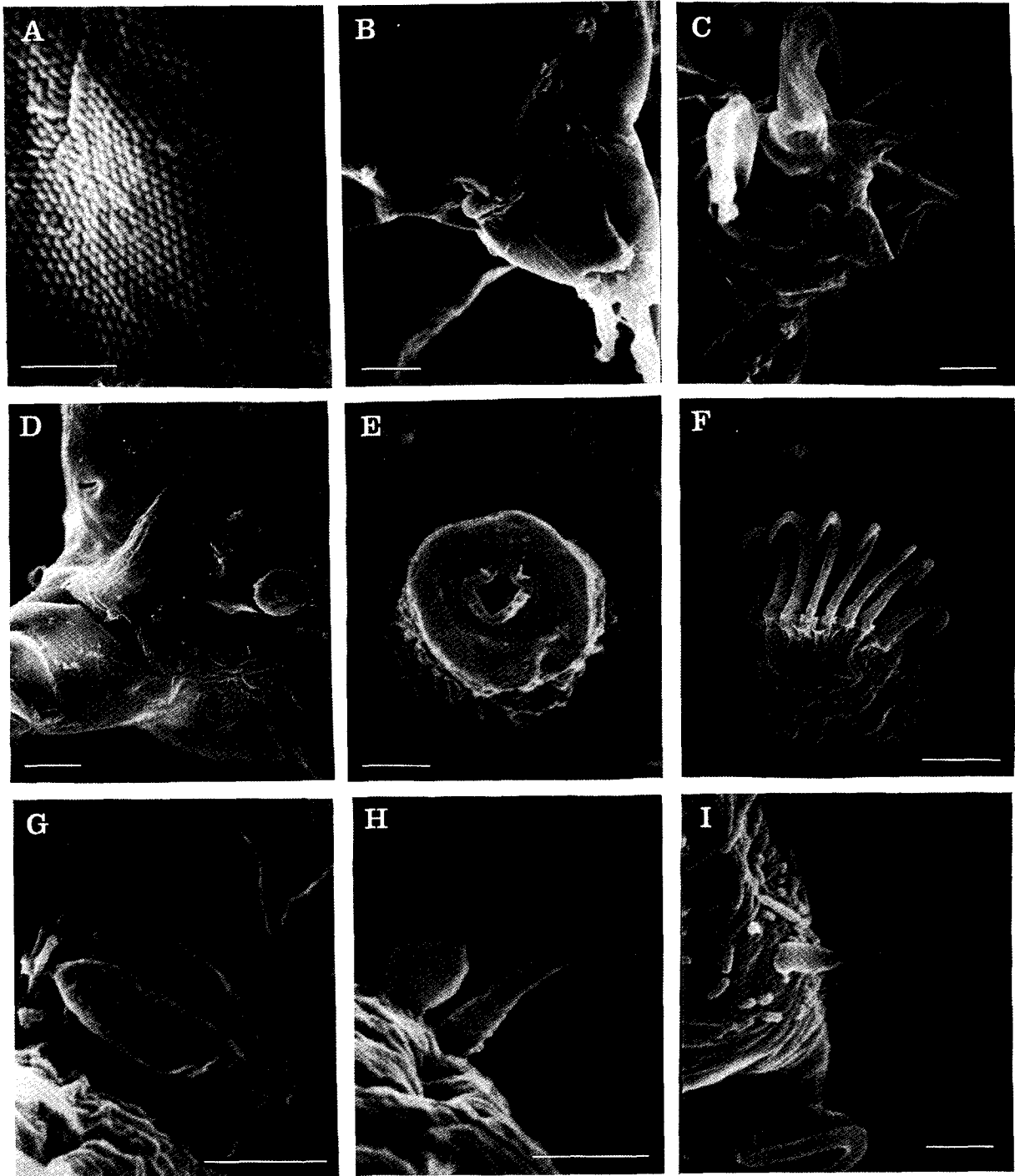


Fig. 4. *Archechiniscus symbalanus* n. sp.: A, Dorsal cuticle. B, Leg IV and its basal process. C, Claws of leg IV. D, Genital region of female. *Echiniscoides andamanensis* n. sp.: E, Mouth opening. F, Claws of leg I. G, Anus. H, Clava and lateral cirrus. I, Sensory spine of leg III. Scale bars=2 μm (A, C, E, I) and 5 μm (B, D, F-H).

with 7 claws, claw configuration of 8-8-8-7, without exception in adult specimens examined. External claws relatively shorter than internal ones. All claws not bearing accessory points and basal spurs. Basal

membrane connecting each claw to foot.

Female gonopore relatively large, 7.3 μm in diameter, surrounded with 6 rosettes of small cuticular membrane, forming a protrusion. Anal opening covered by

3 plates, consisting of 2 large lateral plates and a smaller hind one. Distance between gonopore and anus 13.3 μm .

Etymology: The specific name is taken from the Andaman Sea, the type locality.

Remarks: Genus *Echiniscoides* Plate, 1889 currently comprises of six species including the polytypic species, *E. sigismundi*. Judging from the criteria adopted by Kristensen and Hallas (1980) and Hallas and Kristensen (1982), who made an outstanding revision of the genus with the erection of the family Echiniscoididae and the description of several new taxa, *E. andamanensis* n. sp. is apparently related to *E. sigismundi sigismundi* (M. Schultze) and *E. pollocki* Hallas and Kristensen. The new species supposedly rather resembles *E. pollocki* than *E. sigismundi sigismundi*, considering they share the consistent occurrences of dome-shaped sensory appendages on both leg I and leg II, the claw configuration of 8-8-8-7, and the annulation of cirrus E. Nevertheless, the new species differs also from *E. pollocki* by the relatively short cirri E, smooth cuticle, the presence of stylet sheath, and the absence of anal cuticular flaps.

The retention of stylet sheath as well as the annulation of cirrus E makes *E. andamanensis* n. sp. analogous to *E. bruni* D'Addabbo Gallo, Grimaldi De Zio, Morone De Lucia, and Troccoli, 1992. However, they do not appear to be related, for the characteristics above are regarded as plesiomorphic, and they are clearly distinguished from each other by the pattern of sensory leg appendages (setal form on leg I and absent on leg II in *E. bruni*) and different claw configuration (10-10-10-10 in *E. bruni*).

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