

**Marine Tardigrada from the Southeastern  
United States Coastal Waters**

**I. *Paradoxipus orzeliscoides* n. gen., n. sp.  
(Arthrotardigrada: Halechiniscidae)<sup>1</sup>**

REINHARDT MØBJERG KRISTENSEN AND ROBERT P. HIGGINS

Zoological Museum, University of Copenhagen, Universitetsparken 15,  
DK-2100 Copenhagen, Denmark and

Department of Invertebrate Zoology, National Museum of Natural History,  
Smithsonian Institution, Washington, D.C. 20560, U.S.A.

*Abstract.* A new interstitial arthrotardigrade, *Paradoxipus orzeliscoides* n. gen., n. sp., is described from subtidal coarse-sand and shell-hash habitats, at 15 m depth, 8.4 km east of Fort Pierce, Florida, and from medium-sand of the dredged channel, 5 m depth, of the Intracoastal Waterway of the Indian River, north of Fort Pierce. The new species is distinguished by the presence of both suction discs and claws on the toes, two pairs of clavae, and pointed lateral and caudal alae. Interference-contrast optics were used to video-record living specimens of the new species in order to study the functional morphology of the suction discs. Comparisons of the legs of *Orzeliscus* and *Paradoxipus* n. gen. were made. The phylogenetic relationships of the new genus to other genera in Halechiniscidae and Orzeliscidae are discussed. On the basis of characters shared with *Chrysoarctus*, *Paradoxipus* is assigned to the subfamily Halechiniscinae. Complex seminal receptacles and two pairs of clavae in the new genus are considered plesiomorphic characters, and suction discs and transparent alae are considered apomorphic ones.

Since Schulz (1935) discovered the marine tardigrade *Actinarctus doryphorus* from subtidal coarse sand and shelly sediments also known as "Amphioxus-sand," this habitat has been recognized for its rich and diverse tardigrade fauna (Renaud-Mornant, 1971). The surface of the sediment is often covered by or enriched with organogenic detritus consisting of coralline algae, broken shells of mollusks, or fragments of bryozoans. In tropical waters, this type of sediment consists mainly of coarse coralline sand. The lancelet *Branchiostoma lanceolatum* is common in *Amphioxus*-sand at depths of 8–35 m, but disappears as this sediment reaches depths in excess of 400 m off the coast of the Carolinas in the western Atlantic (Higgins & Kristensen, 1986). In the absence of lancelets, this sediment is referred to by several other indicator organism names. Grell

---

<sup>1</sup> The authors gratefully acknowledge the technical assistance of W. Lee, S. Reed, H. Reichardt, and J. Piriano, Smithsonian Marine Station at Link Port, Florida, C. Drew, research assistant to R.P.H., and G. Møberg Kristensen. Our colleagues, J. Renaud-Mornant, Muséum National d'Histoire Naturelle, Paris, S. Grimaldi de Zio, Istituto de Zoologia ed Anatomia Comparata, Bari, Italy, and H. H. Hobbs, Jr., Smithsonian Institution, graciously reviewed this manuscript and offered valuable suggestions. Funds for this study were provided to R.M.K. by the Danish Natural Science Research Council, and to R.P.H. (as Principal Investigator) and R.M.K. by the Smithsonian Marine Station at Link Port (Contribution No. 227). We also thank the Suzanne Liebers Ericksen Danish Exchange Program for its support of this project.



(1937) refers to such sediment as "*Polygordius*-sand" after the polychaete he found commonly associated with this habitat. In the region of Roscoff, France, a similar sand is referred to as "*Dentalium*-sand" after the common scaphopod resident (Kristensen, 1983).

*Polygordius*-sand is extremely clean shell hash, nearly devoid of silt and organogenic detritus. *Dentalium*-sand is more "dirty" or poorly sorted sediment containing varying proportions of finer material. The first semipelagic tardigrade *Tholoarctus natans* was described from the latter sediment off the coast of Roscoff (Kristensen & Renaud-Mornant, 1983), and has been found recently in calcareous sediments of tropical waters.

Recently, Italian investigators have published a series of papers on marine tardigrades of the Amendolara Shoal in the Ionian Sea (D'Addabbo Gallo et al., 1986, 1987; Grimaldi de Zio & D'Addabbo Gallo, 1987; Grimaldi de Zio et al., 1983, 1987). These papers are the first comprehensive systematic and ecological reports on studies of tardigrades from *Amphioxus*-sand and related calcareous sediments in the Mediterranean Sea. They found 35 species of Heterotardigrada in the region of the Amendolara Shoal; this constitutes the highest species diversity known for marine tardigrades. Zoogeographically puzzling was the presence of some tardigrade species originally reported only from the Arctic: *Styraconyx hallasi* Kristensen, 1977; *S. nanoqsunguak* Kristensen & Higgins, 1984; and *S. qivitoq* Kristensen & Higgins, 1984. Also unexpected in coarse sands of the Amendolara Shoal were species previously known only from tropical waters: *S. kristenseni* Renaud-Mornant, 1981; *Wingstrandarctus intermedius* (Renaud-Mornant, 1967); *Florarctus cinctus* Renaud-Mornant, 1976; *Mesostygarctus intermedius* Renaud-Mornant, 1979; and *Parastygarctus stereri* Renaud-Mornant, 1970.

The first studies of tropical marine tardigrades were conducted primarily by Renaud-Mornant from coralline sand (Renaud-Mornant, 1967a, 1982). In their arctic studies, Kristensen & Higgins (1984a) found that the marine subtidal arthrotardigrade fauna was depauperate both in numbers and diversity with the exception of tardigrades from shell-gravel or bryozoa-sand. The suggestion from these studies is that the differences between calcareous and siliceous substrates may be more important than differences in temperature in determining the zoogeographical distribution of marine Heterotardigrada. The best example is demonstrated by the genus *Wingstrandarctus*, a common inhabitant of fine coralline sand in the tropics. Other congeneric members are found in similarly fine calcareous sediments of arctic waters (Kristensen, 1984), but these latter sediments are derived from calcium carbonate-encrusted red algae (*Lithothamnion* sp.).

Along the East Coast of the United States, the subtidal coarse quartz sand habitat with layers of calcareous sand have been reported to have a rich meiofauna (Rieger, 1980; Ruppert, 1982), but little is known of the marine tardigrades frequenting such subtidal sediment. Kristensen & Higgins (1984b) described a new family Renaudarctidae from coarse sediment from intertidal high-energy beaches near Fort Pierce, Florida; however, the same kind of sediment is located subtidally 8.4 km off the coast of Fort Pierce. Here, as in

many places along the entire coast of the southeastern United States (Cory & Pierce, 1967; Futch & Dwinell, 1977), this sediment varies from stratified coarse quartz sand to clean shell hash and commonly is inhabited by the lancelet *Branchiostoma virginiae*. This paper is the first of a series that will describe the tardigrade fauna as well as other meiofaunal taxa of *Amphioxus*-sand.

#### MATERIALS AND METHODS

The specimens upon which this study is based were obtained from two samples of coarse quartz sand mixed with shell hash. One was taken 31 January 1983 at the first station of four 8.4-km interval stations extending eastward along the 27°30.0' meridian near Fort Pierce, Florida. The sample, from a depth of 15 m, was obtained using an anchor dredge designed by the junior author to remove a layer of sediment 45 cm wide and no more than 10 cm deep (about 30 liters volume). Of 275 specimens of tardigrades, only 10, all of which were adult, were members of the new genus. The second sample (about 50 liters volume) was collected 4 February 1988 at 5 m depth from the channel of the Intracoastal Waterway (near marker "175") of the Indian River, about 1.6 km north of Fort Pierce, using the same anchor dredge. Of 173 specimens of tardigrades taken, about 100 belonged to the new genus. Both juveniles and adults were common.

Sand from each sample, in about 1-liter increments, was placed in a bucket of freshwater and agitated for 5–10 sec. This procedure produces osmotic shock, causing the animals to release their grasp on the sand grains, and subsequent suspension of the specimens. The water was decanted through a 63- $\mu$ m mesh net, which was reimmersed in seawater where the sieved meiofauna could regain osmotic equilibrium. Once the entire sample was processed in this manner, the seawater containing the recovering meiofauna was decanted through a 63- $\mu$ m mesh net a second time. The 1983 sample was fixed with 6% buffered formalin, stored in vials, and sorted later with the aid of a stereomicroscope at 50 $\times$ . Using the same extraction procedure, the 1988 sample was not fixed immediately so that live observations could be recorded on video tape using Zeiss interference-contrast optics.

Fixed animals were subjected briefly to ultrasonic vibration to remove detritus, transferred from 6% formalin to a watch glass with 2% glycerin in distilled water, and allowed to evaporate to glycerin over a period of several days. Specimens were transferred to a small drop of glycerin on a microslide, covered with a coverglass, and sealed with Murrayite.

The holotype, allotype, and one paratype were illustrated with aid of camera lucida. Measurements (in  $\mu$ m) were made and the resulting data expressed in a terminology and format used by Kristensen (1984) and Kristensen & Higgins (1984a). The holotype and allotype of the new species were deposited in the Invertebrate Collection of the National Museum of Natural History, Smithsonian Institution, Washington, D.C. The remaining paratypes were deposited at the Zoological Museum of the University of Copenhagen (ZMUC), Denmark.

## SYSTEMATIC ACCOUNT

Order Heterotardigrada Marcus, 1927  
 Suborder Arthrotardigrada Marcus, 1927  
 Family Halechiniscidae Thulin, 1928  
 Subfamily Halechiniscinae (Thulin, 1928)

*Diagnosis.* Halechiniscidae without peduncles on four digits. Only primary hook present on each claw. Claws retractable into membranous sheath. Primary clavae club-shaped, buccal clavae (secondary clavae?) club-shaped or reduced to indistinct flat organ. Seminal receptacles with lateral vesicles and long genital ducts. Dorsal cuticle thinner than ventral cuticle.

*Composition.* The subfamily Halechiniscinae consists of the genera *Halechiniscus* Richters, 1908 (including the genus *Microlyda* Plate, 1851, a juvenile form of *Halechiniscus*), *Chrysoarctus* Renaud-Mornant, 1984, and *Paradoxipus* n. gen.

*Paradoxipus* n. gen.

*Diagnosis.* Halechiniscinae with four similar toes (digits) on each foot. Each toe with proximal suction disc and distal claw. Complete set of cephalic cirri present; primary clavae and lateral cirri arising from distinct lateral extension; buccal clavae, in form of bent sausage-shaped structures situated posterolateral to the mouth opening, extending from flat saccate structures laterally adjacent to mouth cone. Stylet supports, stylets, and placoids present. Paired seminal receptacles with coiled ducts opening lateral to a large female gonopore. Transparent, pointed lateral and caudal alae present.

*Etymology.* The name of this genus is derived from *paradox* (Gr.), "paradox" or "strange," and *pous* (Gr.), "foot," alluding to the foot with toes (digits) having both suction discs and claws.

*Type species.* *Paradoxipus orzeliscoides* n. sp.

*Paradoxipus orzeliscoides* n. sp.  
 (Figs. 1–36)

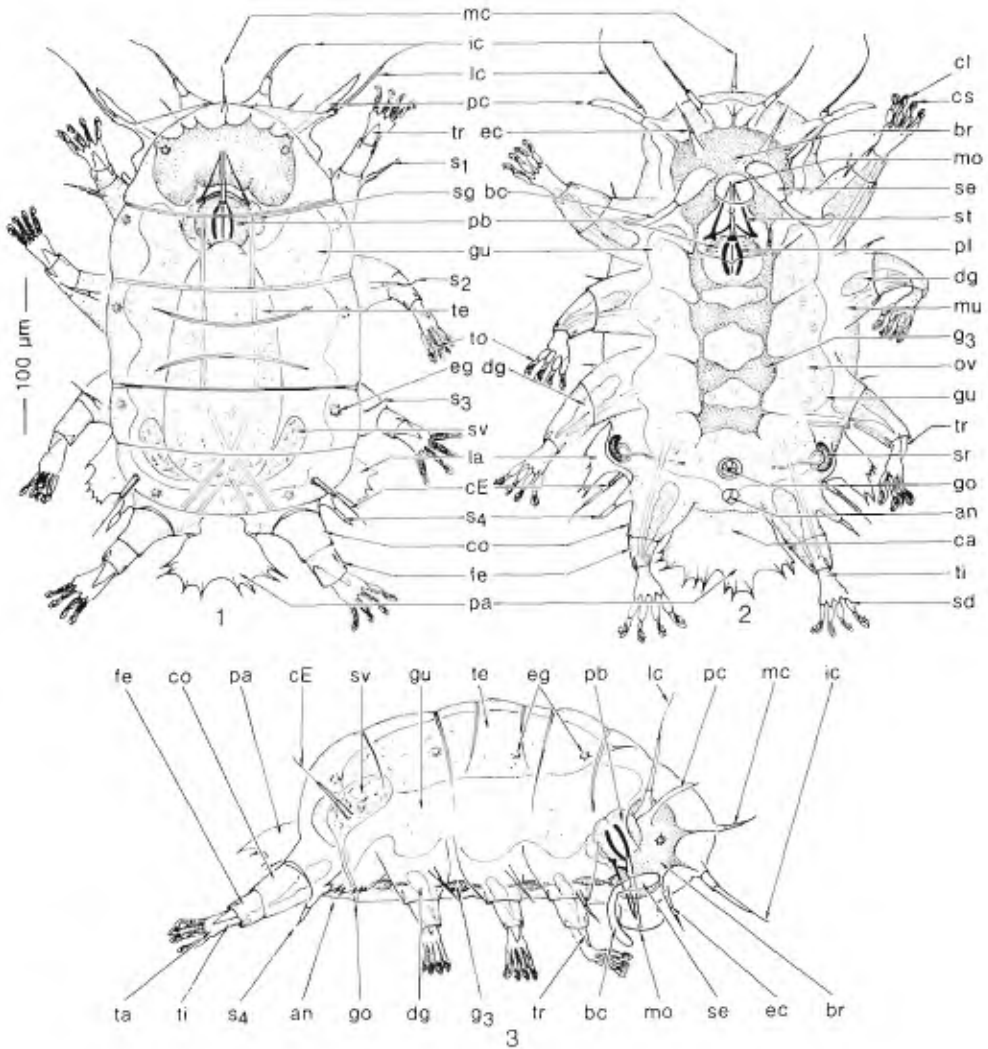
*Diagnosis.* Same as for the genus.

*Etymology.* The name of this species is derived from *Orzeliscus*, referring to its resemblance to *O. belopus* Du Bois-Reymond Marcus, 1952.

*Type specimens.* Holotypic female (USNM 234011), 122  $\mu$ m long, with one large egg, gonopore, and seminal receptacle both evident, collected by R. P. Higgins & R. M. Kristensen, 31 January 1983. Allotypic male (USNM 234012), 125  $\mu$ m long, with mature sperm in the testis and seminal vesicles; other data are the same as for the holotype. Three paratypic females and five paratypic males (RMK 830131.1–RMK 830131.8).

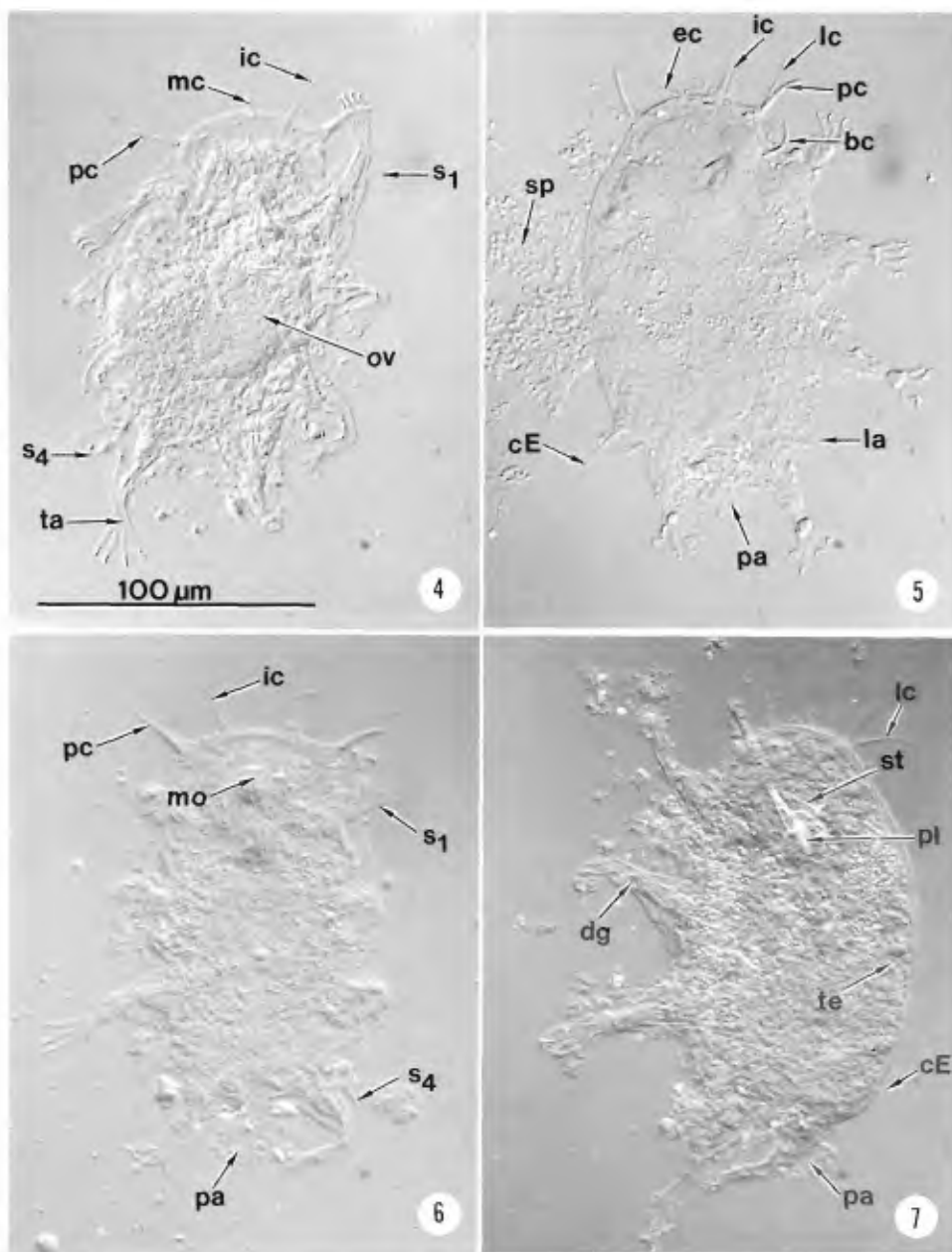
*Type locality.* 8.4 km east of North Hutchinson Island, near Fort Pierce, Florida (27°30.0'N, 80°17.0'W); depth 15 m, in coarse quartz sand mixed with shell hash (*Amphioxus*-sand).

*Additional paratypes.* About 100 specimens were collected by R. P. Higgins



FIGS. 1-3. *Paradoxipus orzeliscoides* n. gen., n. sp. Fig. 1. Allotypic male, dorsal view. Fig. 2. Holotypic female, ventral view. Fig. 3. Paratypic male (RMK 830131.1), lateral view. an, anus; bc, buccal clava; br, brain; ca, caudal caestus; cE, cirrus E; cl, claw; co, coxa; cs, claw sheath; dg, digit gland (claw gland); ec, external cirrus; eg, epidermal gland; fe, femur; g<sub>3</sub>, ventral ganglion 3; go, gonopore; gu, gut; ic, internal cirrus; la, lateral ala; lc, lateral cirrus; mc, median cirrus; mo, mouth opening; mu, muscle; ov, ovary; pa, posterior ala; pb, pharyngeal bulb; pc, primary clava; pl, placoid; s<sub>1</sub>-s<sub>4</sub>, legs I-IV sense organs; sd, suction disc; se, buccal sense organ; sg, stylet gland; sr, seminal receptacle; st, stylet; sv, seminal vesicle; ta, tarsus; te, testis; ti, tibia; to, toe; tr, triquetrum.

and R. M. Kristensen, 4 February 1988, from medium to coarse sand mixed with shell hash, 5 m depth, in the channel of the Intracoastal Waterway in the Indian River near marker "175" about 1.6 km north of Fort Pierce, Florida (27°30'N, 80°20.0'W): Five two-clawed larvae, 10 females, and five males were



FIGS. 4-7. *Paradoxipus orzeliscoides* n. gen., n. sp. (interference-contrast micrographs; all to the same scale). Fig. 4. Holotypic female. Fig. 5. Allotypic male (squashed preparation). Fig. 6. Young paratype, immature female. Fig. 7. Paratype male (same as Fig. 3). bc, buccal clava; cE, cirrus E; dg, digit gland; ec, external cirrus; ic, internal cirrus; la, lateral ala; lc, lateral cirrus; mc, median cirrus; mo, mouth cone; ov, ovary; pa, posterior ala; pc, primary clava; pl, placoid; s<sub>1</sub>, leg I spine; s<sub>4</sub>, leg IV sense organ; sp, spermatoozon; st, stylet; ta, tarsus; te, testis.

mounted for study (RMK 880204.1–RMK 880204.10); additional specimens were fixed for study by transmission electron microscopy (TEM), stored in vials with 2% buffered formalin, and retained in the invertebrate collections of the Zoological Museum of the University of Copenhagen.

### *Description*

*External structures.* The holotypic female (Fig. 2), 122  $\mu\text{m}$  long, has a maximal width of 72  $\mu\text{m}$  between legs III and IV. The outer segmentation in the cuticle is indistinct and can be observed only in dorsal view. Both the dorsal and ventral cuticle are thin and without sclerotized plates. The cuticle is transparent with fine punctation. Each point represents a pillar situated below the epicuticle.

Cuticular expansions (lateral and posterior alae) are thin and easily overlooked. The alae (Figs. 4–6) are always covered by a thick mucous layer with detritus. The ventral procuticle extends into the alae as a proximal support (caestus, Figs. 2, 13) and to the epicuticular elements; the pillars are arranged in a hexagonal pattern. The holotype has a 12-pointed posterior ala and 5-pointed asymmetric lateral alae (Fig. 10). The points of the alae are supported by cuticular ridges.

The ventrally bent head is only indistinctly delimited from the trunk and the mouth opening is located posteroventrally. The opening is surrounded by minute teeth (Fig. 11) and is situated on a large mouth cone. The structures around the mouth opening can telescopically extend and retract slightly (Fig. 12). A complete set of cephalic sense organs is present (Figs. 23–26, 28), and although they are best viewed from the ventral aspect (Figs. 2, 33), they may be difficult to see, especially the buccal clavae. All cephalic cirri have a flagellum consisting of a thick proximal part and a thin thread-like distal part; the flagellum is telescopically inserted in the scapus. Only the internal cirrus has a cirrophore (Fig. 24). The primary clava and the lateral cirrus are mounted on a lateral common pedestal. The two pedestals are connected with a thin epicuticular membrane, which can be observed only in dorsal aspect (Fig. 2). The median cirrus formula is  $8 + 8 + 6 = 22 \mu\text{m}$  (this refers to the lengths of scapus + proximal flagellum + thread-like flagellum = the entire length of the cirrus) and is situated at the anterior margin of the head as in *Florarctus*. The internal cirri ( $12 + 16 + 8 = 36 \mu\text{m}$ ) are robust and slightly ventral. The two internal cirri are connected with a cuticular membrane (Fig. 2). The external cirri ( $8 + 8 + 6 = 22 \mu\text{m}$ ) are more ventral, aligned with and slightly anterior to the internal cirri (Fig. 33). The lateral cirri ( $12 + 20 + 8 = 40 \mu\text{m}$ ) are located dorsally on the base of the primary clavae. The primary clavae (19  $\mu\text{m}$ ) are bent slightly posteriorly and have 3–4 wrinkles in the cuticle. A distinctly refractile structure “van der Land body” (Fig. 35) is located in the pedestal base (6  $\mu\text{m}$ ). The most conspicuous head structures are the buccal clavae (Fig. 21) and buccal sensory plate (Figs. 22, 28). The buccal clavae (20  $\mu\text{m}$ ) are posterolateral to the mouth opening. They are bent anteriorly and protrude from the edge of a large sensory plate (22  $\mu\text{m}$ ). The sensory plates and the

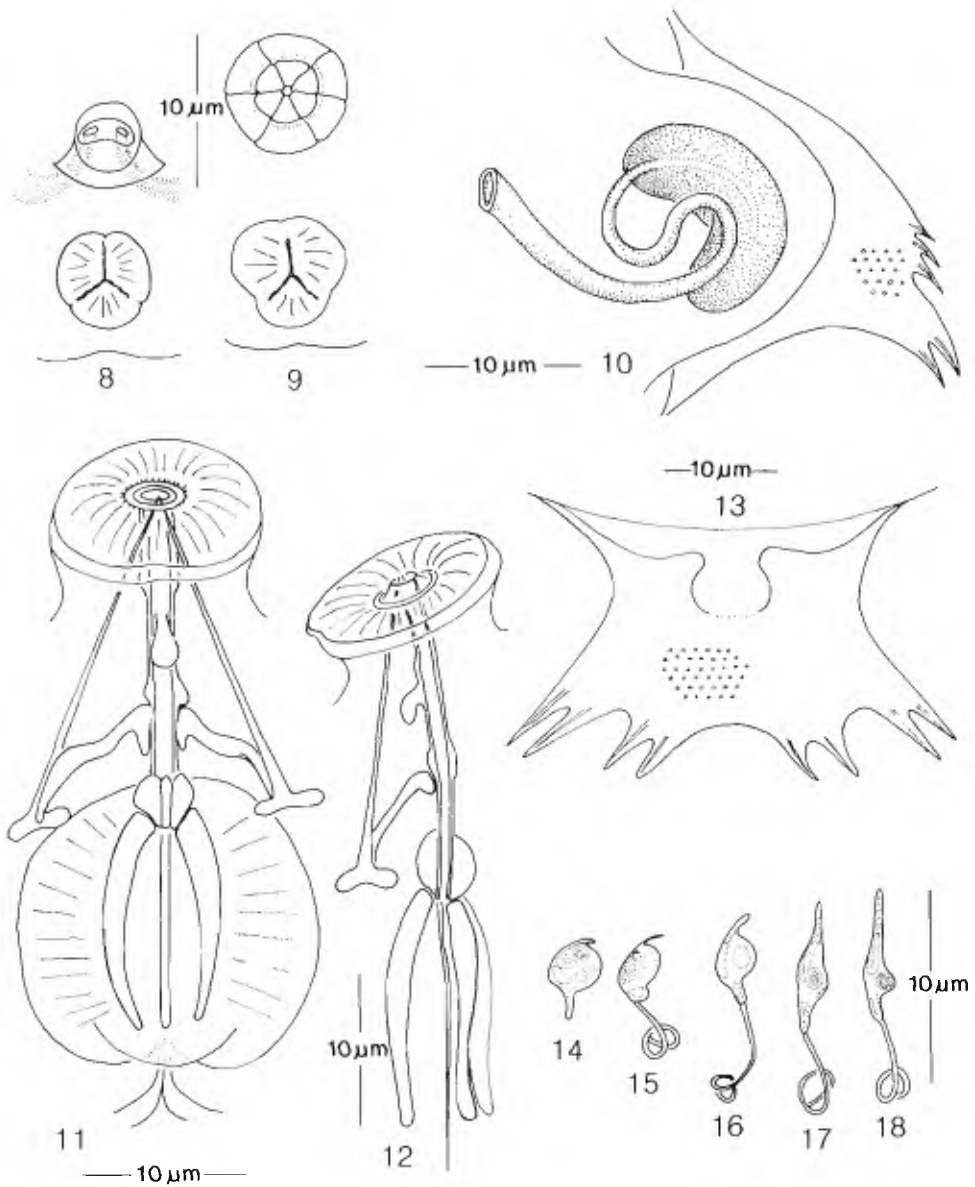


buccal clavae are innervated from separate parts of the brain. Brown globular vesicles were observed in association with the sensory plates.

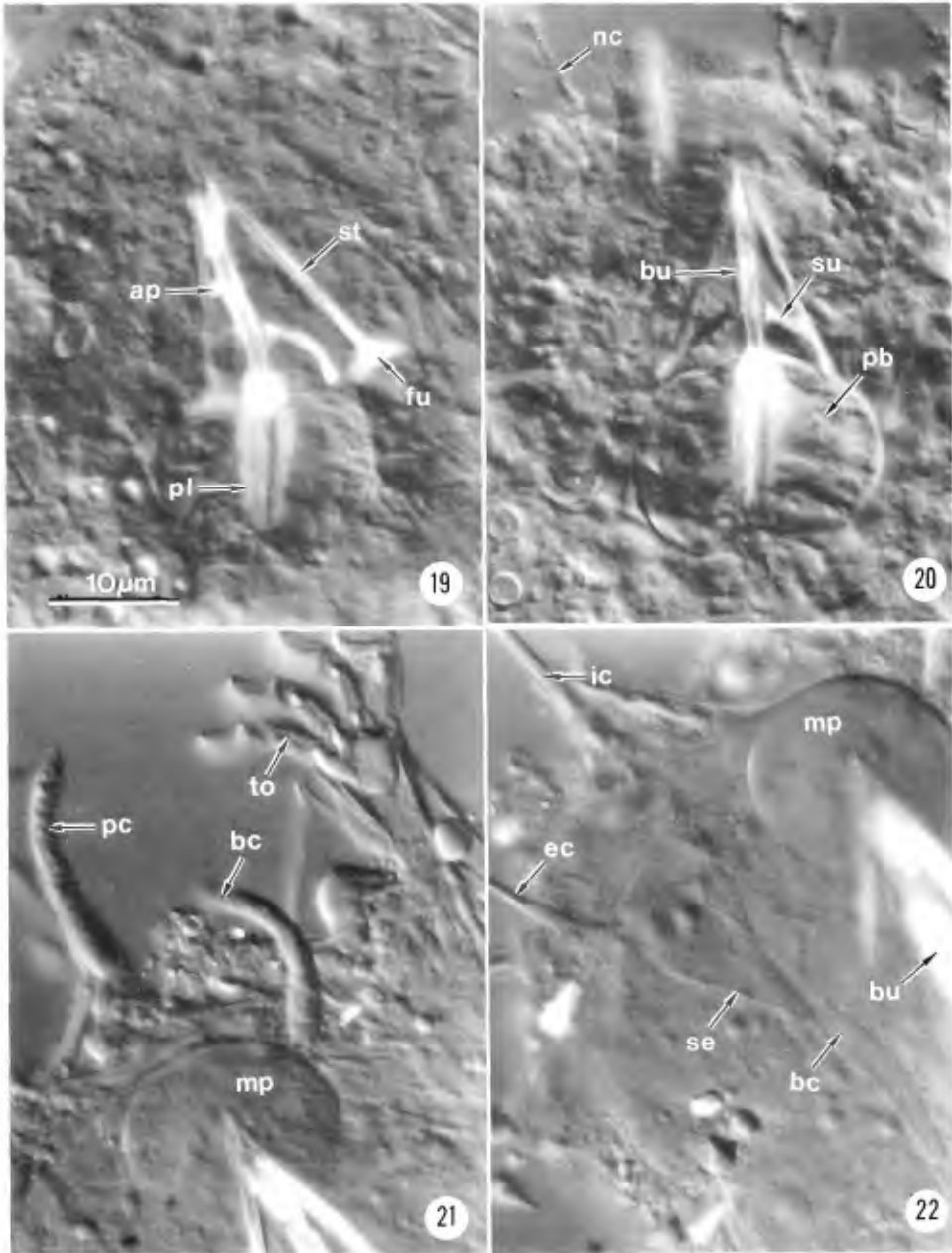
The brain is large and almost entirely fused with the subpharyngeal ganglion. Each sense organ is innervated by a nerve extending from a large ganglion. These ganglia are connected with different parts of the brain, but the exact part is difficult to determine in this case, primarily because the sense organs are located much more ventrally in other tardigrades. The only cirri on the trunk are those designated "E" (30  $\mu\text{m}$ ); each is a complex sensory structure consisting of a bell-shaped cirrophore, a tightly wrinkled scapus, and a three-part flagellum (Fig. 27). The part of the flagellum which can move in and out of the scapus telescopically has a fine accordion-like configuration. The sensory elements of the first three legs each have a scapus and a two-part flagellum (Figs. 29–31, 35). The sense organ of each fourth leg is more complex and consists of a papilla with a small tip and a base. The papilla is clavoid with a refractive van der Land body in the constriction that separates the papilla from the conical base.

Each of the telescopic legs consists of a coxa, femur, tibia, and tarsus of halechiniscid type. Living animals were noted to be able to extend each leg to a length of 50  $\mu\text{m}$  and to retract it to a length of about 20  $\mu\text{m}$  (the tarsus, tibia, and femur were retracted telescopically within the coxa). The coxa cannot be retracted. All sense organs on the legs are located on the coxa. The coxa of the fourth leg has a double cuticular ridge situated dorsally at the level of the sense organ, which is located laterally between the lateral ala and the leg. The rectilinear tibia is relatively short and not separated from the hand-shaped enlarged tarsus. A triangular extension of the cuticle, the triquetrum, is present on the dorsal surface of the tibia. A middorsal leg muscle attaches to the proximal part of the triquetrum. From this muscle attachment, four minute cuticular fibers run through the tibia, tarsus, and out into the four toes where they attach to the claws (Figs. 35, 37–40). The two ventrolateral muscles attach to the articulation between the femur and tibia. From each of these muscle attachments, three cuticular fibers run through the distal part of the leg and attach between the toes. The distal tibia, tarsus, and toes lack cellular elements.

The tarsus bears four toes (digits) of the same length (12–13  $\mu\text{m}$ ). The toes insert distally at the same level on the tarsus. The toes are unique among tardigrades in having both suction discs and claws (hence the genus name *Paradoxipus*). The oval suction disc adheres to the substrate even after death, but no adhesive substance could be detected. In lateral view (Fig. 34), the suction discs might appear as a peduncle, but a peduncle is an internal toe structure. Dorsally, the toes have numerous distinctive wrinkles (Figs. 35, 37, 39, 40). When a claw is retracted within the claw sheath (Fig. 34), the wrinkles become more pronounced than when the claw is extruded (Fig. 40). The retraction of the claw reduces the toe length. Each claw can be retracted independently; thus, toes may appear to have different lengths (Fig. 35). The retraction of the claw in the sheath is a complex movement, and the non-retractile cuticular fiber attaching middorsally to the claw transfers the muscle



FIGS. 8-18. *Paradoxipus orzeliscoides* n. gen., n. sp. Fig. 8. Male gonopore and the trifurcate anus. Fig. 9. Female gonopore and the trifurcate anus. Fig. 10. Ventral view of seminal receptacle with the convoluted genital tube and the lateral ala with five asymmetrical spikes. Fig. 11. Ventral view of the mouth cone and the bucco-pharyngeal apparatus. Fig. 12. Lateral view of mouth cone and bucco-pharyngeal apparatus (pharyngeal bulb is not illustrated). Fig. 13. Eight-pointed posterior ala with caudal caestus. The hexagonal arrangement of the pillars in the epicuticle is indicated. Figs. 14-16. Early to late spermatids from testis. Figs. 17, 18. Mature spermatozoa from seminal vesicle.



FIGS. 19–22. *Paradoxipus orzeliscoides* n. gen., n. sp. (interference-contrast micrographs; all to the same scale). Fig. 19. Optical, lateral view of bucco-pharyngeal apparatus. Fig. 20. Optical view, ventral level of bucco-pharyngeal apparatus. Fig. 21. Ventral view of mouth cone, primary clava and leg I (allotype). Fig. 22. Lateroventral view of mouth cone and cephalic sense organ. Note that the insertion of buccal clava is posterior to the mouth opening. ap, apodeme (ventral) on buccal tube; bc, buccal clava; bu, buccal tube; ec, external cirrus; fu, furca of stylet; ic, internal cirrus; mp, mouth opening on extruded mouth cone; nc, nerve to internal cirrus (sensory dendrite); pb, pharyngeal bulb; pc, primary clava; pl, placoid; se, buccal sensory plate; st, stylet; su, stylet support; to, toe (digit).

contraction in the leg to the toe. The claw (4–5  $\mu\text{m}$  long) is of the "simple type," without accessory hooks or external calcar (Fig. 40). Table I provides measurements of morphological features of the holotype and paratypes.

*Internal structure.* The central nervous system (Fig. 2) consists of a large three-lobed brain totally fused with the large subpharyngeal ganglion, both located in the head, and four large ventral paired ganglia, one pair in each of the four trunk segments. A pair of refractive epidermal glands are present in the head and in each trunk segment.

The reproductive system (Fig. 2) of the holotypic female has a single large ovum in the dorsal ovary (Fig. 4). The female gonopore (Figs. 2, 9, 34) is located caudoventrally, just in front of the fourth pair of legs. The distance (Fig. 9) between the gonopore and the anus is only 6  $\mu\text{m}$ . The gonopore is large and consists of six rosette cells, each with a cuticular valve. Surrounding the gonopore is a ring of sphincter muscles (Fig. 9) which appear to match the six rosette cells when the muscles are contracted. The two seminal receptacles open laterally, about 15  $\mu\text{m}$  from the gonopore. The genital ducts (Fig. 10) are strongly convoluted and extend into each seminal receptacle. Each seminal receptacle is curved, slightly twisted along its long axis, and is located in a lateral projection below the lateral alae.

The mouth cone is located posteroventrally on the head. The buccal tube is short (20  $\mu\text{m}$ ), and its wall is relatively thick with a large midventral drop-shaped apophysis. The wall of the tube is thicker where the stylet supports insert (Figs. 11, 12). The stylets are thin and short (21  $\mu\text{m}$ ). The anterior part of the stylet is hollow (Fig. 19), the posterior part is furcate where the stylet supports insert. The pharyngeal bulb is 16  $\mu\text{m}$  in diameter with three calcareous placoids (Figs. 19, 20). A large pharyngeal apophysis is located rostral of the placoids. A short esophagus leads to the four-lobed midgut. The lobes are distinct and have many white globular vesicles. The lumen of the rectum is tripartite and gives the anal region a three-lobed appearance where it opens near the caudal edge of the trunk.

The allotypic male (Fig. 2), 125  $\mu\text{m}$  long, has a maximum width of 73  $\mu\text{m}$ . No secondary sexual dimorphism exists in *Paradoxipus orzelisoides*, and the males are similar to the female in size. The male gonopore is located caudally about 4  $\mu\text{m}$  from the anus. The gonopore consists of a small papilla with a crescent-shaped opening. Inside the papilla are the openings of the two sperm ducts. The dorsal testis may extend to the region of the pharyngeal bulb; two seminal vesicles are situated laterally. The germ cells in the testis consist of spermatocytes or spermatids (Figs. 14–16). Spermatozoa with rod-shaped heads and coiled tails are present in the seminal vesicles (Figs. 17, 18).

*Juvenile stages.* The "two-clawed" larval stage, the first instar (length 82–87  $\mu\text{m}$ ), is unusually large. It lacks alae, the claws are relatively large (3  $\mu\text{m}$ ), and the suction discs are relatively small (3  $\mu\text{m}$ ) relative to those of the adults. Except for large sausage-shaped buccal clavae, the first instar appears similar to the same stage of *Halechiniscus*.

The juvenile "four-clawed" stage is established with the first molt. The second instar (90–102  $\mu\text{m}$ ) appears similar to the mature adult, except for the lack of

TABLE I  
Measurements (in  $\mu\text{m}$ ) of 12 adults and one larva of *Paradoxipus orzeliscoides*

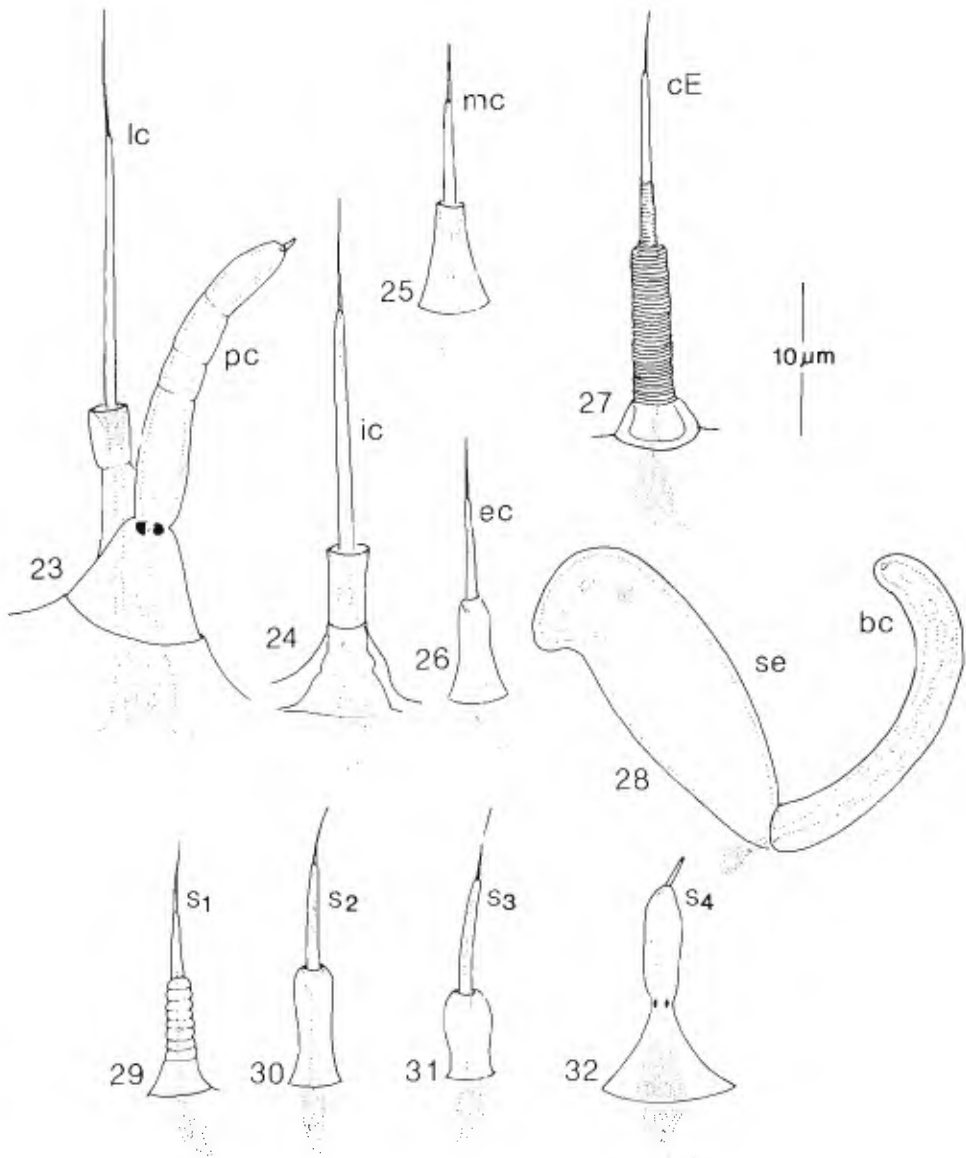
Character	No.	Range	Mean <sup>a</sup>	SD	CV	Holo-type	Allo-type	Larva
Body length	12	100-148	125.8	14.1	11.2	122	125	87
Width between legs III-IV	12	50-79	69.0	8.0	11.6	72	73	43
Buccal canal (length)	10	17-21	19.2	1.1	5.9	20	20	14
Placoid	11	10-14	11.9	1.1	9.5	14	11	8
Pharynx bulb (length)	12	13-16	14.7	1.1	7.2	16	14	11
Stylet (length)	10	17-22	20.3	1.5	7.4	21	20	15
Stylet support (length)	10	5-7	6.4	0.7	11.5	7	6	3
Median cirrus	12	16-22	17.8	2.3	12.8	22	19	15
Internal cirri	12	27-37	33.2	3.0	9.1	36	33	21
External cirri	12	18-24	20.3	2.2	10.5	22	21	12
Lateral cirri	12	31-42	38.0	3.8	9.9	40	39	28
Primary clavae (length)	12	15-20	18.3	1.8	9.7	19	18	13
Buccal clavae (length)	12	13-21	18.3	2.7	14.8	20	16	12
Cirri E	12	23-30	27.2	2.6	9.5	30	26	20
Whole leg IV sense organ	12	11-17	14.8	1.5	10.5	16	14	11
Leg IV papilla	12	6-9	7.9	0.8	10.0	8	8	6
Leg IV papilla basis	12	5-8	6.8	0.8	12.2	8	6	5
Leg III spine	12	12-19	16.1	2.1	13.1	19	15	12
Leg II spine	12	13-19	16.4	1.9	12.0	18	16	13
Leg I spine	12	12-18	15.2	1.6	10.5	17	16	12
External I digits	12	10-15	11.9	1.4	11.9	12	12	10
External I claws	12	4-7	4.8	1.0	19.9	4	4	3
External IV digits	12	10-17	12.8	1.7	13.3	13	12	10
External IV claws	12	4-6	4.8	0.9	18.9	5	5	3
Suction discs IV	12	4-9	6.9	1.2	17.9	7	8	3
Posterior ala (length)	12	18-24	20.6	2.7	13.2	22	20	—
Posterior ala (width)	12	19-44	33.9	8.1	23.8	42	30	—
Lateral alae (length)	12	12-20	18.2	2.6	14.4	20	18	—
Lateral alae (width)	12	10-19	14.3	2.6	18.5	19	14	—

<sup>a</sup> Measurements of the larva are not included in the statistical analysis.

a gonopore. The alae are well developed. We postulate the existence of only two "juvenile" instars.

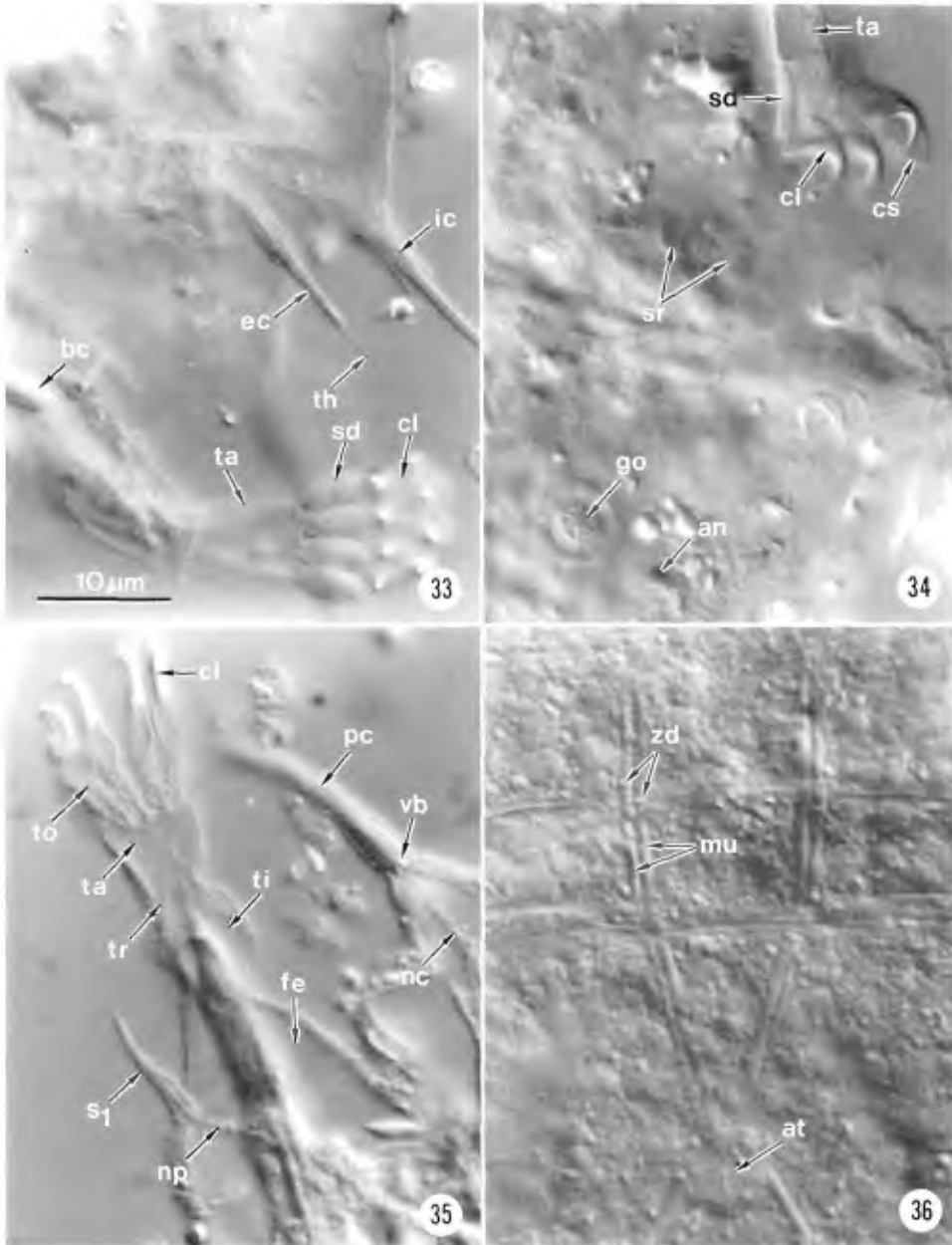
*Additional paratypes.* A paratypic male (RMK 830131.1) was mounted in a lateral position (Figs. 3, 7, 19). This view reveals a compact, nearly box-shaped body profile with a strongly depressed head. Paratypic specimens exhibit considerable variation in the shape and extent of both lateral and posterior alae. Numbers of spikes of posterior alae vary from 6 to 12, and numbers of spikes of lateral alae from 2 to 7. Alae are often asymmetrical and may be broken off or eroded in older animals.

Two paratypes were treated with  $\text{OsO}_4$  vapor (Figs. 6, 35, 36). Both the leg and trunk muscles were remarkably well preserved. The leg has at least eight muscles, but only three of them (Figs. 37-40) appear to be involved in the function of suction discs and claws. All muscles are cross-striated, including the minute double muscles situated dorsally in the trunk (Fig. 36).



FIGS. 23-32. Sense organs of *Paradoxipus orzeliscoides* n. gen., n. sp. Fig. 23. Common pedestal (socket) for lateral cirrus (lc) and primary clava (pc). Fig. 24. Internal cirrus (ic). Fig. 25. Median cirrus (mc). Fig. 26. External cirrus (ec). Fig. 27. Cirrus E (cE). Fig. 28. Buccal clava (bc) and buccal sensory plate (se). Figs. 29-32. Sense organs ( $s_1$ - $s_4$ ) of legs I-IV.

FIGS. 33-36. *Paradoxipus orzeliscoides* n. gen., n. sp. (interference-contrast micrographs; all to the same scale). Fig. 33. Ventral view of cephalic sense organs and leg I. Note the suction disc adhering to the coverglass (holotype). Fig. 34. Ventral view of the female gonopore, seminal



receptacle, anus and lateral view of leg III with toes (holotype). Fig. 35. Primary clava and leg I (holotype). Fig. 36. Optical view, dorsal level of trunk muscles with oval muscle attachment. an, anus; at, muscle attachment; bc, buccal clava; cl, claw; cs, claw sheath; ec, external cirrus; fe, femur; go, female gonopore; ic, internal cirrus; mu, dorsal trunk muscle; nc, nerve to primary clava; np, nerve to sense organ of leg I; pc, primary clava; s<sub>1</sub>, sense organ of leg I; sd, suction disc; sr, seminal receptacle; ta, tarsus; th, thread-like part of external cirrus; ti, tibia; to, toe (digit); tr, triquetrum; vb, van der Land body; zd, z-disc of muscle.

## FUNCTIONAL MORPHOLOGY OF THE LEGS

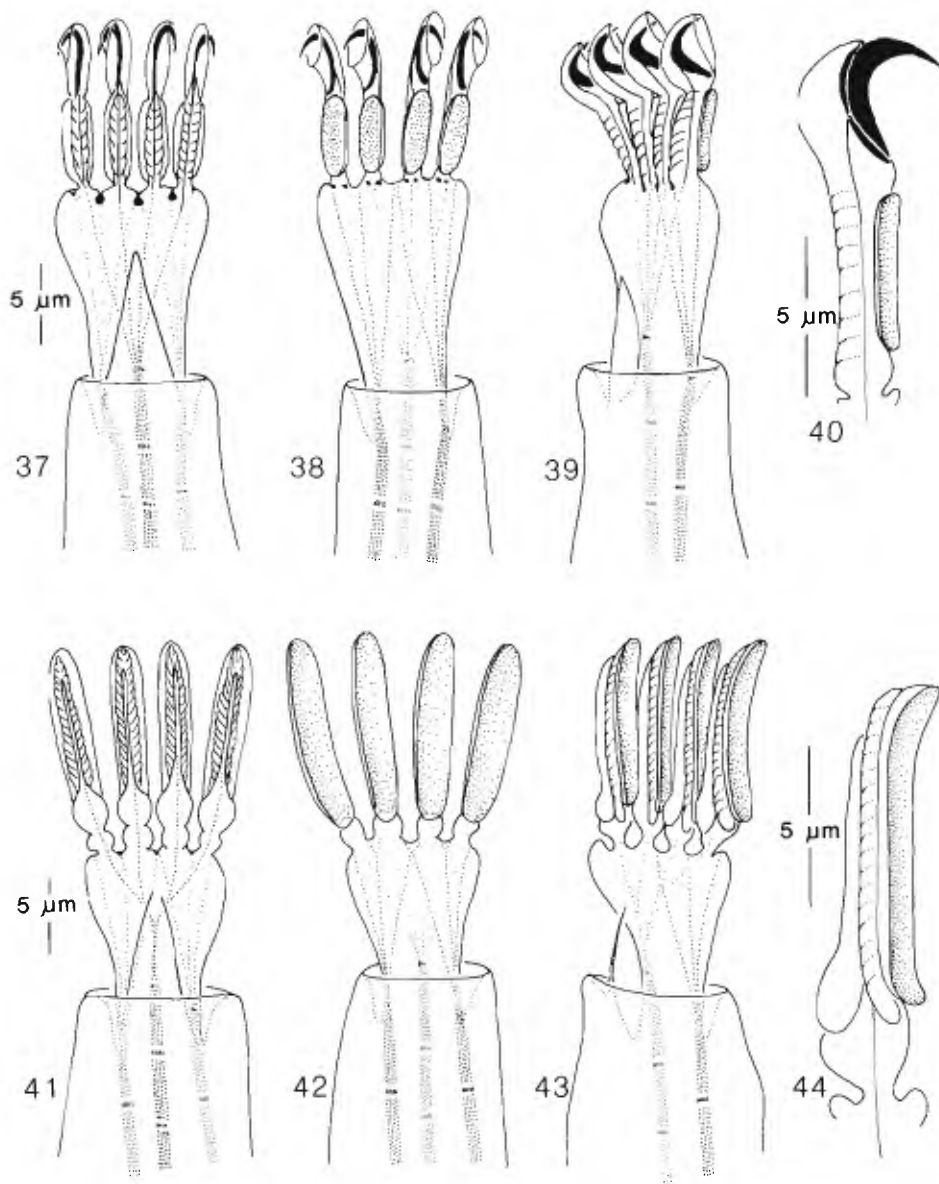
We suggest that the development of telescopic legs with both claws and suction discs on the toes are adaptations for living in both the upper detritus layer and in the interstitial water between coarse sediment particles. Strongly telescopic legs and toes with claws are common in the family Halechiniscidae. In the two samples containing *Paradoxipus orzeliscoides*, representatives of five other halechiniscid genera were present: *Florarctus*, *Wingstrandarctus*, *Raiarctus*, *Tholoarctus*, and *Tanarctus*. Members of these genera normally are associated with the detritus layer, and all are well adapted to a semi-planktonic life (Grimaldi de Zio et al., 1983) and to passive transportation by strong water currents. These adaptations are manifested in the development of long appendages, e.g., clavae and cirri (*Tanarctus* and *Florarctus*); modifications of the cuticle, e.g., long lateral pillars (*Raiarctus*), bell-shaped epicuticle (*Tholoarctus*), or wing-shaped alae (*Wingstrandarctus*, *Florarctus*). Video recordings of these genera show that body movement is slow and awkward when the animals are in contact with detritus or algae, but when suspended, even momentarily, they are able to extend and withdraw the telescopic legs rapidly and with great agility. Only cross-striated muscles were seen in the legs of the above genera.

The family Stygarctidae was represented in the samples only by members of the genus *Parastylarctus*. We assume that these animals were associated also with the detritus layer inasmuch as they have some adaptive and behavioral features similar to those of the Halechiniscidae. Members of the genus *Parastylarctus* have a strongly dorsoventrally flattened body (Renaud-Mornant & Anselme-Moizan, 1969), the feet lack toes, but the internal claws have extremely long accessory spines (hooks). Often, the entire body is covered with a detrital coat (Renaud-Mornant, 1967b) and the animal moves sluggishly.

The family Orzeliscidae was represented in the samples by *Orzeliscus belopus*. Our specimens of *O. belopus* probably occurred much deeper in the sediment, because members of this species are known to live interstitially. The compact body shape and short cephalic and trunk appendages adapt *O. belopus* well for an interstitial existence. This species had a typical, slow bear-like gait when it was removed from a sand grain. Individuals did not adhere to the detritus, but exhibited a pronounced stereotaxis to both quartz and coralline sand grains. The legs are less telescopic than in representatives of other genera and the four large suction discs cannot be fully retracted into the leg.

The family Batillipedidae was represented in the samples by three undescribed species of the genus *Batillipes*. Two of them have a robust, box-shaped body, and the third species has a ventrally flattened body with relatively long appendages; these are characteristics we have come to associate with the quartz-sand biotope. The movement of the trunk and the legs is extremely fast when they lose contact with the respective substrata (sand grains), and individuals run more rapidly across the bottom of a Petri dish or a glass microslide about 100  $\mu\text{m}$  per second. Being able to move relatively rapidly may be an important





FIGS. 37-44. Comparative anatomy of leg I. Figs. 37-40. *Paradoxipus orzeliscoides* n. gen., n. sp. Figs. 41-44. *Orzeliscus belopus*. Figs. 37, 41. Dorsal view. Figs. 38, 42. Ventral view. Figs. 39, 43. Lateral view. Figs. 40, 44. Detail of external toe (digit). Note that the claw is extended in Fig. 40. Three leg muscles are illustrated, each with three or four thread-like fibers.

adaptation for living in the upper layers of coarse sand. These members of *Batillipes* do not appear to attach to the detritus. The morphology of the legs of representatives of this genus has been studied extensively by McKirdy (1975) and Kristensen (1976). The adults have six toes, of different lengths and of a noticeably different structure from that of the toes of *Orzeliscus* (see Pollock, 1982).

When first seen alive under the stereomicroscope, specimens of *Paradoxipus* were thought to be juveniles of *Batillipes* because of their four-toed configuration and the rapidity of movement from sand grain to the Petri dish bottom. This latter feature (rapid locomotion) was until now manifested only in the family Batillipedidae. We noted that in at least one instance, a specimen encountering a piece of green algae unhesitatingly associated with it rather than with nearby sand grains.

During this series of observations, we noted that there seemed to be no discernible behavioral or morphological differences among specimens flushed from their habitat with or without prior exposure to fresh-water (osmotic) shock. Of those subjected to fresh-water treatment, the paratypic male (RMK 830131.1) still was adhering to a round piece of green algae with its extruded claws; three other paratypes were still attached to sand grains using only the suction discs as an anchoring mechanism, while three remaining specimens had dissociated from whatever substrate they adhered to before treatment.

Until now, only a few species of the family Halechiniscidae, those belonging to the genus *Styraconyx*, have been described as possessing adhesive, heart-shaped ventral discs, and these only on the internal toes (Kristensen, 1977). As noted in the description of the new genus *Paradoxipus*, the presence of both four elongated suction discs and claws is unique. These structures represent an adaptation that appears to facilitate living in a broader range of microhabitats, namely in the interstices of the sediment particles and in association with the organogenic debris at the water-sediment interface. Evidence of this is borne out by the morphology of the leg and trunk muscles. The double middorsal muscles (Fig. 36), as well as the leg muscles (Figs. 37-39), are striated. Kristensen (1978) reported the same type of muscle in *Batillipes noerrevangi* and postulated that it increased speed and synchrony of movement of legs. A video recording of *Paradoxipus* indicates that *P. orzeliscoides* uses the suction disc on hard substrate and the claws on the soft organogenic sediment. Furthermore, all four pairs of legs are used telescopically when the animal is climbing. When the animal is running, the first three pair of legs are moved in a characteristic ambling pace without using the claws. The fourth pair of legs are not used in this rhythmic movement; they remain relaxed and free from the substrate as in *Batillipes* (see Marcus, 1929: 154). If the animal is disturbed while running, the fourth pair of legs are activated immediately and enable the animal to develop maximum attachment to the substrate. The fourth legs also are used when the animal is moving backwards. The dorsal trunk muscles have a broad attachment zone between the third and fourth segments. In dorsal view (Fig. 1), the caudal part of this muscle system appears to diverge (Fig. 36). A pair

of muscles for the retraction of the fourth legs are located more dorsally than the crossed or divergent muscle configuration.

All of the distal region of the foot (toes, tarsus, triquetrum, and the distal part of tibia) consists of cuticular material only; no cellular elements are visible. The retraction of the claw into the claw sheath, the independent movement of the four toes, and operation of the suction discs all are operated by a complex system of cuticular muscle attachment fibers. These fibers develop from two lateral apodemes located in the junction between the femur and tibia, and a large apodeme inside the triquetrum. Lateral apodemes were described first by Kristensen (1977) for *Styraconyx hallasi*. The dorsal apodeme inside the triquetrum has been observed only in *Paradoxipus* and *Orzeliscus*, but it also may be present in *Chrysoarctus*. The four cuticular fibers from a middorsal apodeme retract the four claws (Fig. 39) and press the suction disc strongly to the surface. The six cuticular fibers from the lateral apodemes (Fig. 38) operate antagonistically to the four toe fibers, and, moreover, can move each toe separately.

#### *Comparison of the Legs of Orzeliscus with Paradoxipus*

The genus *Paradoxipus* provides an important link in the postulated evolutionary relationships between *Chrysoarctus* and *Orzeliscus*. The unique toe character of *Paradoxipus* (Figs. 37–40) also supports Pollock's (1982) contention that the development of suction discs in Batillipedidae and Orzeliscidae are analogous. The suction discs in Halechiniscidae and Orzeliscidae likely are homologous structures. A closer examination of the leg of *Orzeliscus belopus* (Figs. 41–44) indicated that the suction disc may have developed directly from the type of disc seen in *Paradoxipus*. The total reduction of claws may be postulated to be autapomorphy for the family Orzeliscidae. The foot of *Orzeliscus* has the same fundamental structure as that of *Paradoxipus*. In both genera, the two lateral muscles attach to the articulation between the femur and tibia; the middorsal muscle attaches within the triquetrum. The four cuticular fibers from the muscle attachment in the triquetrum attach to rod-like structures inside each toe, and when this muscle is contracted, the four suction discs are lifted from the substratum. The two lateral muscles are operated antagonistically as in *Paradoxipus*. The six cuticular fibers from the two lateral apodemes attach between the four proximal round swellings of each toe. When the lateral muscles retract, the four toes are pressed to the substratum.

The fine structure of the toe of *Orzeliscus* is unique and complex (Fig. 44). It consists of six parts. The proximal part is a (1) small stalk inserting directly on the tarsus and is continuous with a (2) round swelling best observed from ventral view. Dorsally, a (3) second swelling is continuous with a (4) middorsal ridge of sclerotized cuticle that supports the long, ventral, and oval (5) suction disc. Between the suction disc and the middorsal ridge a (6) third layer is present. This layer consists of lamellate flexible cuticle, the proximal end of which is attached to the cuticular fiber from the muscle. The three different

layers in the distal part of the toe have been documented clearly by the scanning electron microscopical studies of Pollock (1982: fig. 2D).

#### PHYLOGENETIC RELATIONSHIPS

The presence of strongly telescoping legs with toes and suction discs in the Halechiniscidae, Orzeliscidae, and Batillipedidae are significant apomorphic characters within the Arthrotardigrada (Kristensen & Higgins, 1984b). The toes appear to have developed secondarily from a foot with claws directly inserted on the tarsus as in the Stygarctidae. The four toes have lost their claws in the Orzeliscidae, and most of the ventral region has been modified as an elongated suction organ. In the Batillipedidae, only the most distal part of each of the six toes has been modified as a round or nearly square-shaped suction disc; the proximal portion of each toe is narrowly elongate, thin, and of variable length.

The new genus *Paradoxipus* suggests that the family Orzeliscidae is a sister group to the Halechiniscidae and not to the Batillipedidae. The similarities between the feet of *Paradoxipus* and *Orzeliscus* support the statement of Pollock (1982) that the family Orzeliscidae is related to the Halechiniscidae and perhaps should be included in this family as Renaud-Mornant (1984) has indicated. The new genus *Paradoxipus* clearly is closely related to the genus *Chrysoarctus* (see Renaud-Mornant, 1984, *C. briandi* from the Mozambique Channel). More recently, D'Addabbo Gallo et al. (1986) redescribed *Chrysoarctus flabellatus* (= *Halechiniscus flabellatus* Grimaldi de Zio et al., 1983). This species, originally described from a marine cave in the Tyrrhenian Sea, has some conspicuous structures in common with *Paradoxipus*, especially the caudal and lateral expansions (alae). Grimaldi de Zio et al. (1983) also noted the absence of appendicular peduncles and the presence of a ventral thickening that might be homologous with the suction disc. The same structure is illustrated by Renaud-Mornant (1984: fig. 6B) for *Chrysoarctus briandi*. Both *C. briandi* and *C. flabellatus* have pointed external calcarae on each simple claw; these are absent in *Paradoxipus*. The two pairs of cephalic clavae might be a plesiomorphic character despite the absence of buccal clavae in the subfamily Halechiniscinae; the latter organs might have been overlooked in previous studies. The location of the buccal clavae behind the mouth makes little sense with respect to the secondary clavae such as are found in the family Stygarctidae. Even more puzzling is the presence of buccal sensory plates in front of the buccal clavae. Similar dome-shaped areas are described by Noda (1985) in *Angursa biscupis clavifera* from Japan, and by Kristensen & Higgins (1984b) in *Renaudarctus psammocryptus* from Florida, U.S.A. These sensory structures may represent the postulated, but otherwise missing, tertiary clavae.

#### LITERATURE CITED

- CORY, R. L. & PIERCE, E. L. 1967. Distribution and ecology of lancelets (order Amphioxi) over the continental shelf of the southeastern United States. *Limnol. Oceanogr.*, 12: 650-656.
- D'ADDABBO GALLO, M., GRIMALDI DE ZIO, S. & MORONE DE LUCIA, M. R. 1986. Description of two species of Halechiniscidae belonging to the subfamilies Halechiniscinae and Stygarctynxinae (Tardigrada: Heterotardigrada). *Oebalia*, 13: 69-75.
- D'ADDABBO GALLO, M., MORONE DE LUCIA, M. R. & GRIMALDI DE ZIO, S. 1987. Heterotardigrada of the Amendolara Shoal, High Ionian Sea. In Bertolani, R., ed., *Biology of Tardigrades. Sel. Sym. Mon. U.Z.I.*, Mucchi, Modena, 1: 103-110.

- FUTCH, C. R. & DWINELL, S. E. 1977. Nearshore marine ecology at Hutchinson Island, Florida: 1971-1974. IV. Lancelets and fishes. *Fl. Mar. Res. Pubs.*, 24: 1-24.
- GRELL, K. G. 1937. Beiträge zur Kenntnis von *Actinarctus doryphorus* E. Schulz nebst Bemerkungen zur Tardigradenfauna des Helgoländer Skitt-Gatts. *Zool. Anz.*, 117: 143-154.
- GRIMALDI DE ZIO, S. & D'ADDABBO GALLO, M. 1987. *Archechiniscus minutus* n. sp. and its systematic position within Arthrotardigrada (Tardigrada: Heterotardigrada). In Bertolani, R., ed., *Biology of Tardigrades. Sel. Sym. Mon. U.Z.I.*, Mucchi, Modena, 1: 253-260.
- GRIMALDI DE ZIO, S., D'ADDABBO GALLO, M. & MORONE DE LUCIA, M. R. 1983. Marine tardigrades ecology. *Oebalia*, 9: 15-31.
- HIGGINS, R. P. & KRISTENSEN, R. M. 1986. New Loricifera from southeastern United States coastal waters. *Smithson. Contrib. Zool.*, 438: 1-70.
- KRISTENSEN, R. M. 1976. On the fine structure of *Batillipes noerrevangi* Kristensen, 1976. 1. Tegument and moulting cycle. *Zool. Anz.*, 197: 129-150.
1977. On the marine genus *Styraconyx* (Tardigrada, Heterotardigrada, Halechiniscidae) with description of a new species from a warm spring on Disko Island, West Greenland. *Astarte*, 10: 87-91.
1978. On the structure of *Batillipes noerrevangi* Kristensen, 1978. 2. The muscle-attachments and true cross-striated muscles. *Zool. Anz.*, 200: 173-184.
1983. Loricifera, a new phylum with Aschelminthes characters from the meiobenthos. *Z. Zool. Syst. Evol.-Forsh.*, 21: 163-180.
1984. On the biology of *Wingstrandarctus corallinus* nov. gen. et spec., with notes on symbiotic bacteria in the subfamily Florarctinae (Arthrotardigrada). *Vidensk. Meddr. Dansk. Naturh. Foren.*, 145: 201-218.
- KRISTENSEN, R. M. & HIGGINS, R. P. 1984a. Revision of *Styraconyx* (Tardigrada: Halechiniscidae) with description of two new species from Disko Bay, West Greenland. *Smithson. Contrib. Zool.*, 391: 1-40.
- 1984b. A new family of Arthrotardigrada (Tardigrada: Heterotardigrada) from the Atlantic coast of Florida, U.S.A. *Trans. Am. Microsc. Soc.*, 103: 295-311.
- KRISTENSEN, R. M. & RENAUD-MORNANT, J. 1983. Existence d'Arthrotardigrada semi-benthiques de genres nouveaux de la sous-famille des Styraconyxinae subfam. nov. *Cah. Biol. Mar.*, 24: 337-353.
- MARCUS, E. 1929. Tardigrada. In Bronn, H. G., ed., *Klassen und Ordnungen des Tierreichs, Akademische Verlagsgesellschaft, Leipzig*, 5(Sec. 4, Book 3): 1-608.
- MCKIRDY, D. J. 1975. *Batillipes* (Heterotardigrada): comparison of six species from Florida (U.S.A.) and a discussion of taxonomic characters within the genus. In Higgins, R. P., ed., *Int. Symp. Tardigrades, Pallanza, Italy, June 17-19, 1974. Mem. Ist. Ital. Idrobiol.*, 32 Suppl.: 177-223.
- NODA, H. 1985. Description of a new subspecies of *Angursa biscopis* Pollock (Heterotardigrada, Halechiniscidae) from Tanabe Bay, Japan. *Publ. Seto Mar. Biol. Lab.*, 30: 269-276.
- POLLOCK, L. W. 1982. A closer look at some marine Heterotardigrada. I. The morphology and taxonomy of *Orzeliscus*. In Nelson, D., ed., *Proc. Third Int. Symp. Tardigrada. Johnson City, Tennessee, U.S.A. 1980*, East Tennessee State University Press, pp. 193-202.
- RENAUD-MORNANT, J. 1967a. Tardigrades de la Saint-Vincent, Nouvelle Calédonie. In *Exp. Fr. Recif. Coral. Nouvelle Calédonie. Ed. Fondation Singer-Polignac.*, 12: 103-118.
- 1967b. *Parastygarctus higginsi* Renaud-Debyser, 1965 sur la côte orientale de Malaisie. Description de la femelle (Tardigrada). *Bull. Mus. Natl. Hist. Nat. Paris*, 2nd series, 39: 205-208.
1971. Campagne d'essais du "Jean Charcot" (3-8 Décembre 1968) 8. Meiobenthos. II Tardigrades. *Bull. Mus. Natl. Hist. Nat.*, 2nd series, 42: 957-969.
1982. Species diversity in marine Tardigrada. In Nelson, D., ed., *Proc. Third Int. Symp. Tardigrada. Johnson City, Tennessee, U.S.A. 1980*, East Tennessee State University Press, pp. 149-178.
1984. Halechiniscidae (Heterotardigrada) de la campagne Benthedi, canal du Mozambique. *Bull. Mus. Natl. Hist. Nat.*, 4th series, 6: 67-88.
- RENAUD-MORNANT, J. & ANSELME-MOIZAN, M.-N. 1969. Stades larvaires du tardigrade marin *Stygarctus bradyus* Schulz et position systématique des Stygarctidae. *Bull. Mus. Natl. Hist. Nat. Paris*, 2nd series, 41: 883-893.

- RIEGER, R. M. 1980. A new group of interstitial worms, Lobatocerebridae nov. fam. (Annelida) and its significance for metazoan phylogeny. *Zoomorphologie*, 95: 41-84.
- RUPPERT, E. E. 1982. Comparative ultrastructure of the gastrotrich pharynx and the evolution of myoepithelial foreguts in Aschelminthes. *Zoomorphology*, 99: 181-220.
- SCHULZ, E. 1935. *Actinarctus doryphorus* nov. gen., nov. spec., ein merkwürdiger Tardigrad aus der Nordsee. *Zool. Anz.*, 111: 285-288.