

A redescription of *Actinarctus doryphorus* (Tardigrada, Heterotardigrada)

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Abstract: Numerous specimens of adults and juveniles of *Actinarctus doryphorus* have been found in a coarse coralligenous detritus of the Secche della Meloria in the Tyrrhenian Sea. The authors discuss the original description enriching it with some morphological details and give new information about the biology of the species.

Résumé : *Redescription de Actinarctus doryphorus (Tardigrada, Heterotardigrada).*

De nombreux spécimens, adultes et juvéniles de *Actinarctus doryphorus* ont été récoltés dans un sédiment détritique coralligène du banc de la Meloria (Mer Tyrrhénienne). Les auteurs discutent la description originale et ajoutent des données morphologiques et biologiques nouvelles sur cette espèce.

Keywords : *Actinarctus doryphorus*, Tardigrada, Heterotardigrada, Mediterranean Sea.

Introduction

Actinarctus doryphorus was described for the first time in 1935 by Schulz who had found five specimens in an *Amphioxus* sand in Helgoland. Subsequently, Grell (1937) found numerous specimens in a coarse biogenic detritus (*Polygordius* Bruchschill) from Helgoland too, and gave a more detailed description of the species.

Further findings were reported from the same kind of substratum by Swedmark (1956) and Swedmark & Teissier (1967) in France and in Sweden. In 1970 Renaud-Mornant, comparing specimens from Brest and Roscoff, described *A. doryphorus* subsp. *ocellatus*.

In 1996/97 we found in a coarse biogenic sand, collected from a pit bottom of the Secche della Meloria (43° 32' N - 10° 12'E), at 7 m depth, seventy four specimens of *Actinarctus*, whose morphology strongly corresponded to *A. doryphorus*.

The availability of so many specimens, adults and juveniles, gave us the opportunity to discuss the original description, enriching it with some morphological details previously overlooked and to give new informations about the biology of the species.

Materials and methods

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The samples for the study of the Tardigrade fauna were collected monthly from March 1996 to February 1997.

Actinarctus doryphorus was found in the samples from March, April, June, December, January and February with respectively 6, 1, 1, 16, 47 and 3 specimens per 10 cm².

The sediment was a coarse coralligenous detritus (granulometric maximum = 1.4 mm) and the salinity was constantly 38 P.S.U. all the year round.

The water temperature in the sediment varied from 12°C in winter to 24°C in summer.

Sediment cores were collected by scuba diving with a plexiglass corer, 15 cm long and 3.5 cm wide. The specimens were extracted after relaxation in MgCl₂ (7%) and permanent mounts were done in Kaiser's glycerine and Hoyer's medium. Eleven adult females (14.87%), 23 adult males (31.08%), 1 first stage larva (1.35%) and 39 second stage larvae (52.70%) were collected. All specimens were found in the first 5 cm of sediment.

Results

Family Halechiniscidae Thulin, 1928

Diagnosis: Arthrotardigrada without strongly sclerotized dorsal segmental plates. Complete or incomplete set of cephalic appendages: secondary clavae sometimes club-shaped or dome-shaped or indistinguishable. Each adult leg with four digits bearing claws either simple or with accessory hooks or bristles; calcar sometimes present. Peduncles can be present in the digits; seminal receptacles generally present; cirrus E with a basal accordion pleat-shaped articulation.

Subfamily Tanarctinae Renaud-Mornant, 1980

Diagnosis: Halechiniscidae with legs which have lance-like tibia and conic tarsus. Simple claws with a calcar. Dorsal spur sometimes present on the medial claws. Fourth legs' sense organs as long caudal appendages. Epicuticle internally supported by pillars of different length growing from the body centre to the periphery. Dorso-lateral roundish seminal receptacles with spindle-shaped ventral ducts.

Actinarctus Schulz, 1935

Diagnosis: Halechiniscidae with the epicuticle internally supported by very long pillars increasing in length from the body centre to the periphery. Claws with a strong calcar.

Redescription of *Actinarctus doryphorus* Schulz, 1935

Neotype 1: Adult female (slide 3a 16.1.97; Secca Meloria) 122 µm long and 78 µm wide. Dorsal epicuticle supported by pillars increasing in length from the centre to the periphery,

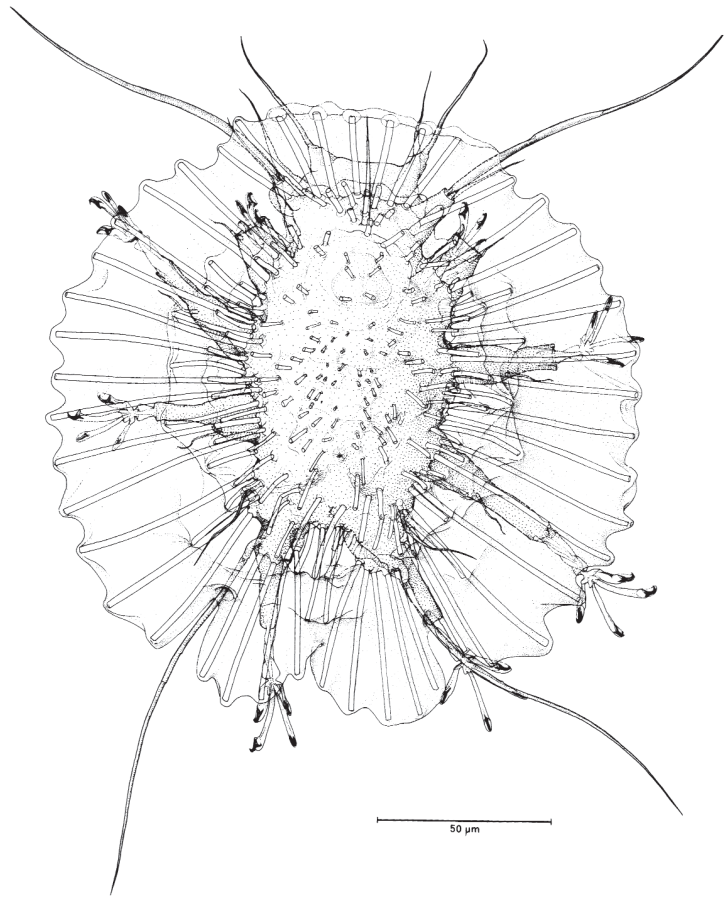


Figure 1. *Actinarctus doryphorus*, adult female (dorsal view).

Figure 1. *Actinarctus doryphorus*, femelle adulte (vue dorsale).

forming a wing all around the body (Tab. 1, Fig. 1).

The pillars on the back of the body are immersed in a jelly matrix absent from the wing, which is therefore transparent. The wing completely surrounds the body and in the caudal region forms two flaps on the fourth legs, separated by a short medial lobe. The wing is supported by about 50 pillars arranged as follows: 10-12 (33 µm long) in the frontal part, 14 pillars 80 µm long in each side, five (55 µm) in each caudal flap and two (35 µm) in the caudal medial lobe (Fig. 3 A).

In the ventral region there is a thin punctuation, whereas very short and thin pillars are arranged as comb-like structures, near the leg bases, close to the ducts of the seminal receptacles and in the anus-gonopore area (Figs. 4A, 5).

All the cephalic cirri consist of a long scapus and a short flagellum and, with the exception of the ventral external ones, all have a strong cirrophorus. The medial cirrus (28 µm) is supported by a 5 µm cirrophorus, the internal cirri (50 µm) have cirrophori (17 µm) interconnected by a membrane 16 µm high. The external cirri are 44 µm long.

Table 1. Measurements (in μm) of adults and larval stages of *Actinarctus doryphorus* Schulz.**Tableau 1.** Dimensions (en μm) des adultes et stades larvaires de *Actinarctus doryphorus* Schulz.

| | Females | | | | Males | | | | 1st stage* | | 2nd stage | | | | |
|-----------------|---------|-----------|-----------|-----------|-----------|----|-----------|-----------|------------|-----------|-----------|-----|----------|-----------|-----|
| | n° | min | max | \bar{X} | neotype 1 | n° | min | max | \bar{X} | neotype 2 | n° | min | max | \bar{X} | |
| L | 10 | 122 (211) | 166 (249) | 164 | 122 (211) | 11 | 111 (175) | 158 (240) | 136 | 147 (234) | 78 | 10 | 89 (127) | 139 (203) | 89 |
| w | 10 | 78 (217) | 110 (238) | 98 | 78 (237) | 11 | 55 (175) | 89 (233) | 747 | 89 (233) | 50 | 10 | 55 (143) | 78 (200) | 65 |
| mC | 1 | 22 | 22 | 22 | 28 | 2 | 20 | 24 | 22 | 20 | | | | | |
| iC | 8 | 41 | 161 | 51 | 50 | 11 | 43 | 67 | 55 | 41 | 17 | 10 | 31 | 74 | 40 |
| eC | 9 | 33 | 41 | 37 | 44 | 10 | 27 | 43 | 33 | 34 | 17 | 9 | 17 | 50 | 29 |
| lC | 7 | 22 | 38 | 33 | 33 | 9 | 23 | 40 | 31 | 33 | | 10 | 17 | 38 | 31 |
| IC ₁ | 9 | 115 | 141 | 132 | 111 | 11 | 100 | 161 | 136 | 110 | 78 | 10 | 89 | 165 | 102 |
| cP | 7 | 19 | 22 | 21 | 22 | 11 | 16 | 33 | 22 | 25 | | 5 | 12 | 22 | 17 |
| CE | 6 | 29 | 41 | 39 | 41 | 9 | 35 | 44 | 34 | 45 | | 3 | 30 | 33 | 32 |
| a ₁ | 10 | 22 | 37 | 33 | 33 | 11 | 24 | 33 | 30 | 33 | | 10 | 11 | 32 | 22 |
| a ₂ | 10 | 63 | 80 | 68 | 80 | 11 | 49 | 77 | 64 | 72 | | 10 | 39 | 66 | 52 |
| a ₃ | 10 | 31 | 44 | 34 | 35 | 10 | 24 | 34 | 26 | 34 | | 9 | 15 | 33 | 23 |
| a ₄ | 9 | 41 | 61 | 51 | 55 | 11 | 40 | 55 | 49 | 53 | | 10 | 28 | 50 | 38 |
| P ₁ | 4 | 19 | 28 | 22 | | 6 | 17 | 31 | 23 | 31 | | 3 | 13 | 22 | 19 |
| P ₂ | | | | | | 6 | 22 | 13 | 17 | 17 | | 2 | 11 | 15 | 13 |
| P ₃ | | | | | | 6 | 14 | 23 | 18 | 18 | | 3 | 11 | 16 | 14 |
| P ₄ | 7 | 103 | 158 | 138 | 111 | 9 | 122 | 155 | 112 | 139 | 100 | 8 | 86 | 138 | 110 |
| A-G | 10 | 17 | 25 | 22 | 22 | 11 | 7 | 13 | 10 | 9 | | | | | |

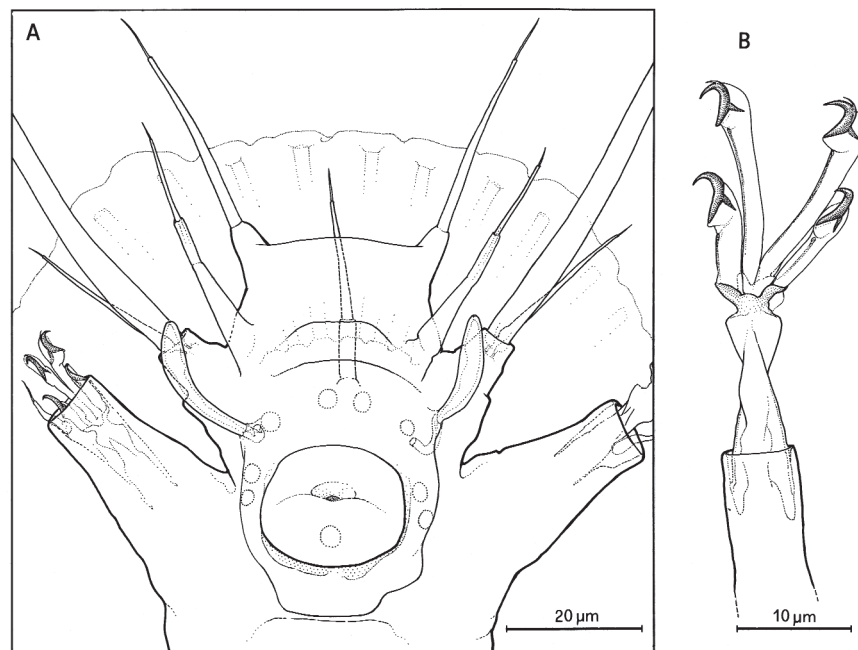
* one specimen

n° number of specimens

In brackets measurements plus wing lengths

Abbreviations:

L: Total body length. W: Body width. mC: medial cirrus. iC: internal cirrus. eC: external cirrus. lC: lateral cirrus. ICl: primary clava. cP: cephalic papilla. CE: Cirrus E. a₁: frontal wing. a₂: lateral wing. a₃: caudal flap. a₄: caudal medial lobe. P₁: 1st leg sense organ. P₂: 2nd leg sense organ. P₃: 3rd leg sense organ. P₄: 4th leg sense organ. A-G: distance anus-gonopore.

**Figure 2.** *Actinarctus doryphorus*, adult female; (A) head, ventral view; (B) 1st leg.**Figure 2.** *Actinarctus doryphorus*, femelle adulte; (A) région céphalique (vue ventrale) ; (B) détail de la patte 1.

Lateral cirri (33 μm) and primary clavae (111 μm) are located on the same cirrophorus 17 μm high (Fig. 2A).

A large sac-like fold partially surrounds the buccal sucker and extends anteriorly up to the basis of two club-shaped processes, the cephalic papillae, 22 μm long, which, as the primary clavae, have a thin terminal pore and a cuticular refracting ring inside their bases.

All around the buccal area there are 9 brown lipid droplets, 5 μm large (Fig. 2). They consist of a dense nucleus, around which numerous lighter layers overlap. They are generally arranged as follows: two anteriorly, three on each side and one posteriorly, but the arrangement is sometimes different.

The mouth opening is at the centre of a large and strong sucker-like ring. The small pharyngeal bulb is not spherical but trilobate, it is 13 μm high and 18 μm wide. The pharyngeal tube is 20 μm long.

The legs have the typical lance like tibia and conical tarsus. The four fingers are: two external shorter, with a basal fold and two medial longer. In all the fingers the ventral part is thick, simulating a peduncle visible only in profile. The thickening is thinner in its proximal part and

becomes stronger near the claw bases (Figs 2B; 4 D, E).

The claws of the internal fingers have an evident dorsal spur. All the claws are retractile and have a strong calcar. Fingers and claws in *A. doryphorus* and *A. lyrophorus* Renaud-Mornant, 1979 are therefore similar.

Sense organs consisting in scapus and flagellum, are present on the first three pairs of legs. These organs are stouter and therefore more easily visible in the male than in the female.

The fourth pair sense organs (111 μm long) are on 11 μm cirrophori. The Van der Land's organ is evident inside their base. They protrude on the surface through an opening in the dorsal cuticle, in the space between the lateral wing and the caudal flap.

Cirrus E emerges from a funnel-shaped groove (8 μm deep) in the dorsal cuticular cover. It consists of an accordion pleat-shaped basal part (8 μm) and a flagellar portion (33 μm).

The rosette-like gonopore is 22 μm from the anus and, laterally to it, the openings of the seminal receptacles ducts are evident. Filiform sperms, with a rod-like head, are numerous in these ducts (Figs. 4 A; 5).

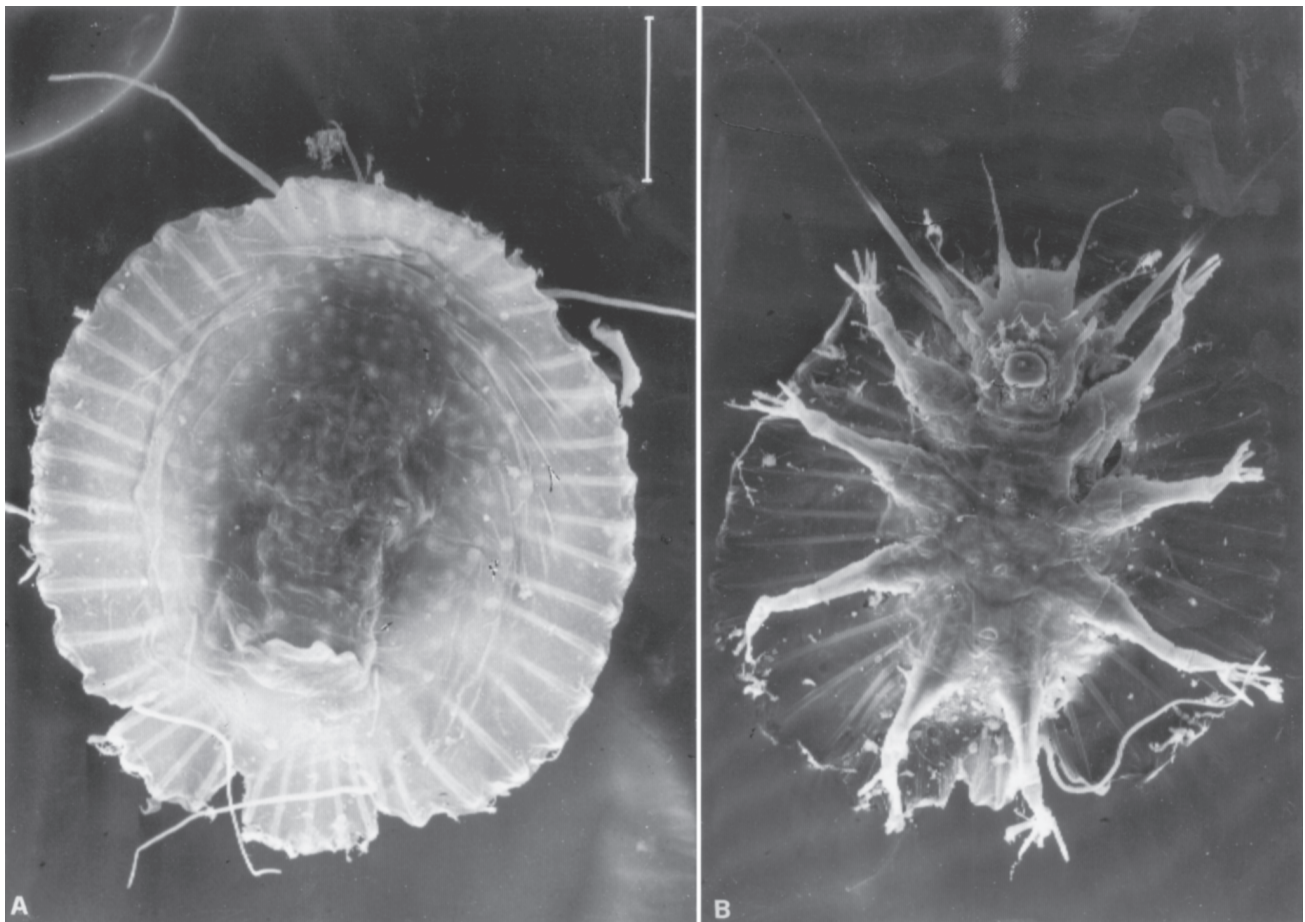


Figure 3. *Actinarctus doryphorus*, (A) adult female (dorsal view); (B) adult male (ventral view); (SEM pictures. Bar = 50 μm).

Figure 3. *Actinarctus doryphorus*, (A) femelle adulte (vue dorsale) ; (B) mâle adulte (vue ventrale) ; (photographie MEB. Echelle = 50 μm).

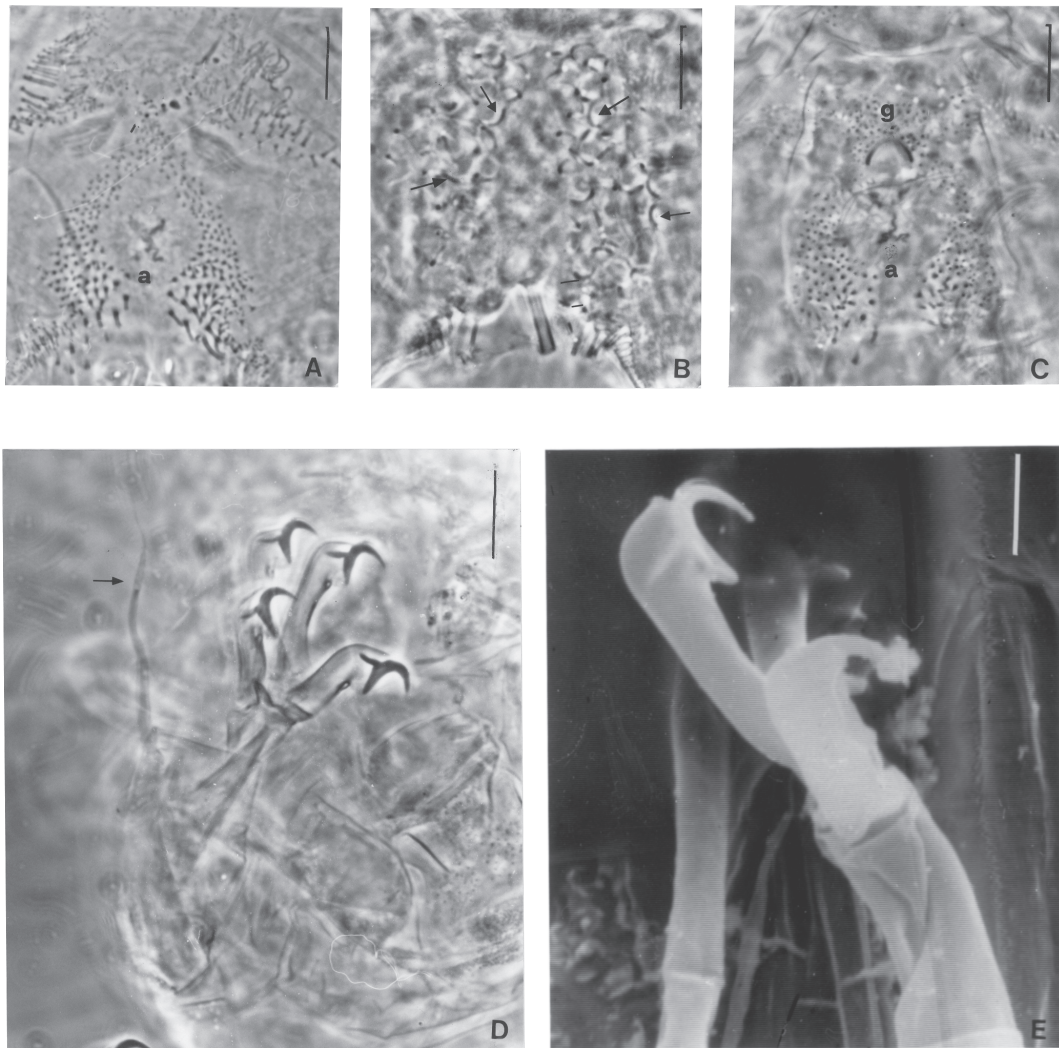


Figure 4. *Actinarctus doryphorus*, (A) female seminal receptacle ducts with sperms; (B) spermiducts with spermatids (arrows); (C) anus (*a*) and male gonopore (*g*); (D) first leg with its sense organ (*arrow*) (Bars A-D = 10 μ m). (E) first leg fingers and claws (SEM picture. Bar = 5 μ m).

Figure 4. *Actinarctus doryphorus*, (A) femelle adulte, conduits des réceptacles séminaux contenant des spermatozoïdes ; (B) mâle adulte, spermiductes avec spermatides (*flèches*) ; (C) anus (*a*) et gonopore du mâle (*g*) ; (D) première patte avec son organe sensoriel (*flèche*) (Echelles A-D = 10 μ m). (E) doigts et griffes de la première patte (photographie MEB. Echelle = 5 μ m).

Neotype 2: Adult male, 147 μ m long (234 μ m with the wing), 89 μ m wide (233 μ m with the wing) (Fig. 3 B). An evident sexual dimorphism does not exist: only the primary clava, mainly in its basal portion, is stouter than in the female. The testis is completely filled with vesiculous spermatids with a peripheric crescent shaped nucleus. Spermatids are present also in the two spermiducts on each side of the gonopore (Fig. 4 B). No sperms are evident. The genital pore is covered with a crescent shaped cuticular fold, 9 μ m from the anus. The anus is covered with two overlapping folds which form an X. Anus and gonopore are

included in a slightly protruded rectangular area characterized by an evident punctuation (Fig. 4 C).

Other neotypes: Only one first stage larva was found. It was covered with detritus and therefore difficult to observe. This larva had only two fingers for each leg, no anus and no gonopore and its dorsal pillars were very short and thin and therefore no wing around the body was formed; the cephalic lipid droplets were absent. It strongly looked like the cyste stadium found by Grell (1937) which however had four digits and therefore is not a first stage larva.

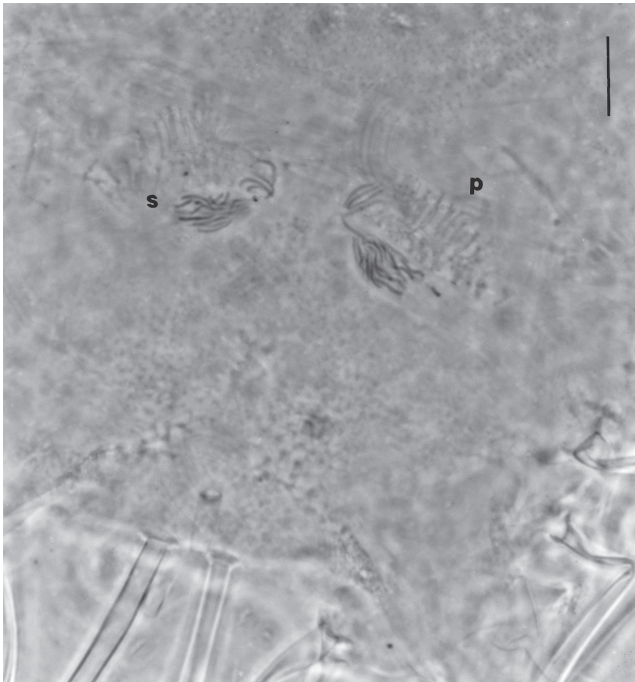


Figure 5. *Actinarctus doryphorus*, adult female: Comb like arrangement of pillars (p) above the seminal receptacle ducts with sperms (s) (Rosa Bengal stained). (Bar =10 μ m).

Figure 5. *Actinarctus doryphorus*, femelle adulte : disposition pectinée des piliers (p) au-dessus des réceptacles séminaux contenant des spermatozoïdes (s) (Coloration au Rose Bengale). (Echelle =10 μ m).

The 39 second stages larvae were similar to the adults, with the exception of the gonopore which was lacking. Two of them were just about to moult and therefore it was possible to observe inside the old cuticle the new legs and the new cuticle with the long pillars regularly arranged as in the Grell's description and drawing.

Biology

A. doryphorus has been collected in the winter months when water temperature was about 12°-13°C. The presence of both adults and juveniles, mainly in December 1996 and January 1997, indicates that the period of the higher reproductive activity is the winter, as in *Batillipes pennaki* Marcus 1946 (Grimaldi de Zio & D'Addabbo Gallo, 1975). In this season, in fact, the male gonad occupies completely the dorsal body region and is filled with spermatids, whereas all the fertilized mature females, have numerous sperms in the seminal receptacles ducts. The presence of sperms only in the females, suggests that the spermiogenesis is completed in the females' seminal receptacles.

Discussion and Conclusion

The species found in the Tyrrhenian Sea widely corresponds to the descriptions of Schulz (1935) and Grell

(1937) and furthermore shows some of the characteristics that allowed Renaud-Mornant (1970) to create the subspecies *A. doryphorus ocellatus*, such as the presence in the head of 7-10 "nodules pigmentés" arranged in a semicircle, the thin ventral punctuation and the sensory setae on the first three pairs of legs.

The lipid droplets which correspond to these "nodules pigmentés" are frequent in Halechiniscidae: we observed them mainly in Tanarctinae such as *Actinarctus neretinus* Grimaldi de Zio et al., 1982 and *A. physophorus* Grimaldi de Zio et al., 1982, but also in Halechiniscinae such as *Halechiniscus greveni* Renaud Mornant & Deroux 1976, *H. remanei* Schulz, 1955 and *Dipodarctus subterraneus* (Renaud-Debyser, 1959). These droplets are often, but not always, present in the specimens from the population of the Meloria Shoals and are variable in form, number and arrangement and therefore they represent a variable character of the species.

The reduction of the dorsal pillars in the ventral region is usual in Arthrotardigrada, as is the presence of sensory setae on all the legs in the Tanarctinae.

The range of variability of the dimensions of the Tyrrhenian population, includes those found in previous studies. The lengths of the body, primary clava and P4 are rather similar and correspond to the Schulz's drawing.

Therefore, on the basis of the present data, the occurrence of these morphological characters in several species and their variability suggest that the subspecies *A. doryphorus ocellatus* should be considered as not valid.

The club shaped ventral cephalic papillae, named clavae (Schulz, 1935; Grell, 1937), or cephalic papillae (Renaud-Mornant, 1970) or secondary clavae (Renaud-Mornant, 1980; Grimaldi de Zio et al., 1982), could be considered owing to their position, as probable tertiary clavae. The secondary clavae, in fact, are generally located between the internal and external cirri. In the present case, on the contrary, they are at the base of the cirrophorus which supports the primary clava and lateral cirrus. Furthermore, SEM pictures show that they could correspond to the protruded anterior part of a wide sensorial area surrounding the mouth, a fact which could exclude their nature as secondary clavae, generally associated with the external cirri. "Buccal clavae" protruding from two sensory areas posterolateral to the mouth opening, are also present in *Paradoxipus orzeliscoides* Kristensen & Higgins, 1989 and, for this species also, the authors postulate that they could be tertiary clavae. Their position corresponds to the sensory areas present in *A. lyrophorus*, in some other Halechiniscidae such as Florarctinae (Kristensen, 1984), Halechiniscinae (Grimaldi de Zio et al., 1990) and in Renaudarctidae (Kristensen & Higgins, 1984).

The study of the Mediterranean specimens of *A. doryphorus*, add new informations to the previous

description of the species by Schulz, Grell and Renaud-Mornant. They are: 1. the sexual dimorphism of the primary clavae, which in the males are more robust than in the females; 2. the morphology of the gonopores which, as in most Halechiniscidae, are crescent shaped in the males and rosette like in the females; 3. the presence of seminal receptacles in the female; 4. Cirri E with an accordion pleat-shaped basal portion as in most of Heterotardigrada; 5. legs, fingers and claws as in *A. lyrophorus*.

In the light of these data, the diagnosis of *A. doryphorus* is: *Actinarctus* without secondary clavae, and with ventral cephalic papillae (tertiary clavae), and long, smooth sensory organs on the fourth legs.

Acknowledgement

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References

- Grimaldi de Zio S. & D'Addabbo Gallo M. 1975.** Reproductive cycle of *Batillipes pennaki* Marcus (Heterotardigrada) and observations on the morphology of the female genital apparatus. *Pubblicazioni della Stazione Zoologica di Napoli*, **4** suppl.: 212-225.
- Grimaldi de Zio S., D'Addabbo Gallo M., Morone De Lucia M.R., Vaccarella R. & Grimaldi P. 1982.** Quattro nuove specie di Halechiniscidae rinvenute in due grotte sottomarine dell'Italia meridionale. (Tardigrada: Heterotardigrada). *Cahiers de Biologie Marine*, **23**: 415-426.
- Grimaldi de Zio S., D'Addabbo Gallo M. & Morone De Lucia M.R. 1990.** Revision of the genus *Halechiniscus*. *Cahiers de Biologie Marine*, **31**: 271-279.
- Grell K. G. von 1937.** Beitrage zur Kenntnis von *Actinarctus doryphorus* E. Schulz nebst Bemerkungen zur Tardigradenfauna des Helgolander Skitt-Gatts. *Zoologischer Anzeiger*, **117**: 143-154.
- Kristensen R. M. 1984.** On the biology of *Wingstrandarcus corallinus* nov. gen. et spec., with notes on the symbiotic bacteria in the subfamily Florarctinae (Arthrotardigrada). *Videnskabelige Meddelelser fra Dansk naturhistorisk Forening i Kjobenhaven*. **145**: 201-218.
- Kristensen R. M. & Higgins R. 1984.** A new family of Arthrotardigrada (Tardigrada: Heterotardigrada) from the Atlantic Coast of Florida U.S.A. *Transactions of the American Microscopical Society*, **103** (3): 295-311.
- Kristensen R. M. & Higgins R. 1989.** Marine Tardigrada from Southeastern United States Coastal Waters. I. *Paradoxipus orzeliscoides* n. gen., n.sp. (Arthrotardigrada: Halechiniscidae). *Transactions of the American Microscopical Society*, **108** (3): 262-282.
- Renaud-Mornant J. 1970.** Campagne d'essais du "Jean Charcot" (3-8 Décembre 1968) 8; Méiobenthos. II. Tardigrades. *Bulletin du Muséum National d'Histoire Naturelle, Paris*, 2^e Série, **42** (5): 957-969.
- Renaud-Mornant J. 1979.** Tardigrades marins de Madagascar. I. Halechiniscidae et Batillipedidae. *Bulletin du Muséum National d'Histoire Naturelle, Paris*, **1** (1): 257-277.
- Renaud-Mornant J. 1980.** Description de trois espèces nouvelles du genre *Tanarctus* Renaud-Debyser, 1959, et création de la sous-famille des Tanarctinae, subfam. nov. (Tardigrada, Heterotardigrada). *Bulletin du Muséum National d'Histoire Naturelle, Paris*, **2** (1): 129-141.
- Schulz E. 1935.** *Actinarctus doryphorus* nov. gen. nov. spec., ein merkwürdiger Tardigrad aus der Nordsee. *Zoologischer Anzeiger*. **111**: 285-288.
- Swedmark B. 1956.** Nouveaux Gastrotriches Macrodasoyides de la région de Roscoff. *Archives de zoologie expérimentale et générale*, **94**: 43-57.
- Swedmark B. & Teissier G. 1967.** Structure et adaptation d'*Halammohydra adherens*. *Cahiers de Biologie Marine*, **8**: 63-74.