

## New description of *Neostygarctus acanthophorus* (Tardigrada, Arthrotardigrada)

Susanna Grimaldi de Zio<sup>1</sup>, Maria D'Addabbo Gallo<sup>1</sup>, M. Rosaria Morone De Lucia<sup>1</sup>  
and Alberto Troccoli<sup>2</sup>

1) Istituto di Zoologia ed Anatomia Comparata.

Università di Bari, via Amendola 165/A, 70126 Bari (Italy)

2) Laboratorio di Nematologia del CNR via Amendola 165/A, 70126 Bari (Italy)

**Résumé :** *Neostygarctus acanthophorus* est décrit de nouveau par les Auteurs, avec une analyse critique et comparative des trois familles Stygarctidae, Neostygarctidae et Renaudarctidae.

**Abstract :** A new description of *Neostygarctus acanthophorus* is given and a critical and comparative analysis of the three families Stygarctidae, Neostygarctidae and Renaudarctidae is done.

### INTRODUCTION

*Neostygarctus*, the only monospecific genus of the family Neostygarctidae (Grimaldi de Zio et al., 1982), is very interesting for its evident and close relationship with the family Stygarctidae ; it is related in particular to the genus *Parastygarctus* which is close to the origin of the two orders, Arthrotardigrada and Echiniscoidea, of the Heterotardigrada class.

Furthermore the peculiar shape of the claws of *Neostygarctus*, which resemble the articulated chetae of some Annelida, is a very important taxonomic characteristic giving further evidence of the annelidean origin of tardigrades.

The new findings of some specimens of *N. acanthophorus* : a female in the Tirrhenian Sea (Orosei Gulf, Sardinia 40°09'00"N - 9°39'19"E) in a coarse detritus at 20 m depth, and a male in the Adriatic Sea (Torre Guaceto, Brindisi, 40°42'49"N - 17°47'58"E) in the sand of a *Posidonia* meadow at 20 m depth and of three preadults, one in the Adriatic Sea near Otranto (40°12'18"N - 18°27'28"E) in the sand of a *Posidonia* meadow at 20 m depth and two in Ionian Sea (Armeleia Shoal 40°29'00"N - 17°03'25"E) in a coarse coralligenous detritus at 35 m depth, give us a good opportunity to present a more detailed and correct description of it.

### Taxonomic observations

Family Neostygarctidae Grimaldi de Zio, D'Addabbo Gallo, Morone De Lucia, 1987.

Diagnosis : Arthrotardigrad furnished with legs having four tubular claws ending in triangular hooks, the two medial of which are provided with a dorsal bristle ; dorsal plates with thick mid dorsal and marginal spines ; a complete set of cephalic sense organs ; kidney-shaped brown eyes in the cephalic plate.

Type genus and species : *Neostygarctus acanthophorus* Grimaldi de Zio, D'Addabbo Gallo, Morone De Lucia, 1982.

### Description of the male

The one male found is 182  $\mu\text{m}$  long and 124  $\mu\text{m}$  wide.

The body is distinctly divided into five parts : head and four trunk segments, the last of which is the caudal region. Each part is covered with a dorsal plate extending into long lateral processes and largely surrounded by numerous flexible spikes (Fig. 1 ; Tabl. I).

TABLE I

*Neostygarctus acanthophorus* : measurements (in  $\mu\text{m}$ ) of larvae and adults

	II stage larvae			male	female
L	89	116	147	182	188
W	108	93	120	124	126
mC	45	40	43	57	61
iC	31	13	20	23	27
eC	31	26	37	43	47
IC	42	38	35	55	62
ICl	8	7	10	9	10
IIClW	6	9	6	6	9
LC	14	10	16	16	15
SI	34	44	55	45	55
SII	36	34	44	36	38
SIII		37	37	39	38
SIV	29	32	35	32	31
SV	17	22	26	10	12
CE	94	81	108	102	119
P4	4	3	6	6	7
a	12	8	15	16	14

### Abbreviations

L = total length	IIClW = secondary clavae	CE = cirri E
W = total width	LC = lateral cephalic cirrophorus	P4 = sense organ of the fourth pair of leg
mC = medial cirrus	SI = I dorsal spike	a = anus area
iC = internal cirri	SII = II dorsal spike	g = gonopore
eC = external cirri	SIII = III dorsal spike	sr = seminal receptacle
IC = lateral cirri	SIV = IV dorsal spike	
ICl = primary clavae	SV = V dorsal spike	

The cephalic plate extends laterally into two long arms ending in the cirrophorus of both the lateral cirrus (55  $\mu\text{m}$ ) and the primary clava (9 x 3  $\mu\text{m}$ ) (Fig. 2 D). The primary clava looks like a bud of the terminal part of the cirrophorus, it has a very short spike-like terminal opening and a not very evident oblique base. No Van der Land's organelle is distinguishable inside it.

The frontal edge of the cephalic plate is divided in lobes bearing the other cephalic sense organs which are : internal ventral cirri (23  $\mu\text{m}$ ) with a small base, external dorsal cirri

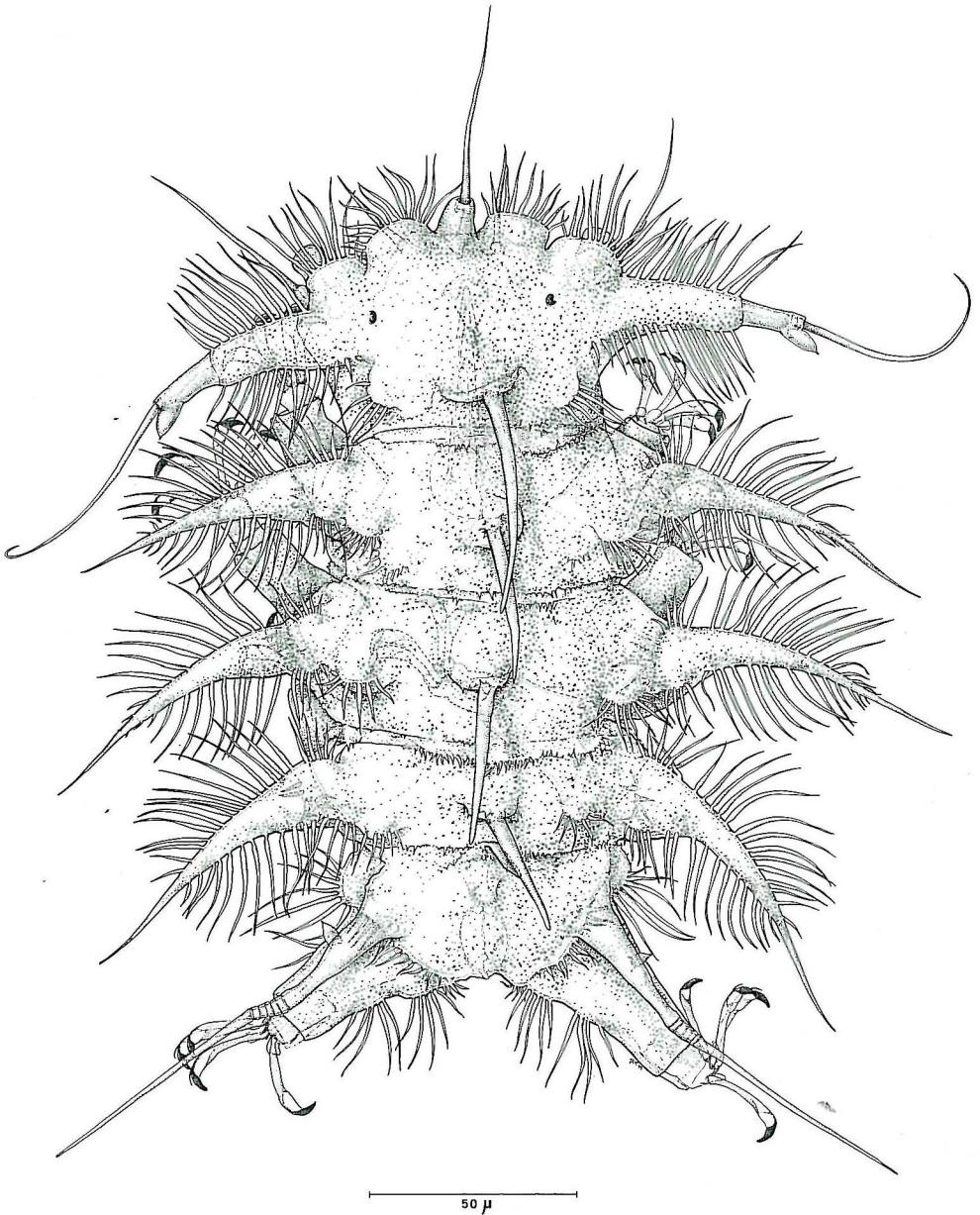


Fig. 1 : *Neostygartus acanthophorus* : Adult male.

(43  $\mu\text{m}$ ) with an evident cirrophorus and, close to the external cirri, the secondary clavae which are bulb shaped (5 x 6  $\mu\text{m}$ ) and have a thick wall. Dorsally there is a very strong and evident medial cirrus (46  $\mu\text{m}$ ) with a high cirrophorus (11  $\mu\text{m}$ ) (Fig. 2 C). Two kidney-sha-

ped brown eyes, connected to the protocerebrum, are also present. All the cephalic cirri consist of a very short scapus and a long flagellum. The caudal edge of this plate has two roundish lateral lobes surrounded by spines and a medial one with a very long dorsal spike (45  $\mu\text{m}$ ). The plate surface is covered with numerous very short spikes while the lateral cirrus cirrophorus is completely smooth. Behind the cephalic region, there are three body segments, of about the same shape and size, each bearing a pair of legs. Each segment has long, conical lateral processes surrounded by spikes of different lengths. At the base of each process there is, on each dorsal plate, a swelling edged by spikes. Furthermore each plate bears a medial lobe with a stout spike. The first one being 36  $\mu\text{m}$  long, the second 39  $\mu\text{m}$ , the third 32  $\mu\text{m}$ .

The caudal region is covered with a subtriangular plate which has two lateral swollen lobes with a few long spines round them, a strong medial spine (20  $\mu\text{m}$ ), and two long dorso-lateral processes which are the cirrophori of the cirri E. The latter are 102  $\mu\text{m}$  long and are strong. They have a large articulated accordion-plated portion (8  $\mu\text{m}$ ) of only five folds. The cirri E cirrophori are smooth like those of the lateral cirri in the cephalic region.

This region bears the fourth pair of legs. They are the ones with an evident sense organ, the P4, which is a stocky papilla with a very short terminal spike. The P4 is very similar to the primary clava and, also in this case, no Van der Land's organelle is distinguishable inside it (Fig. 2 E).

No spines are present on the first three pairs of legs, whereas long spines are evident on the medial side of the fourth pair.

Each leg bears four claws, which are very peculiar. Each claw is rigid and looks like a hollow tube. It has an articulated basal fold and a terminal flat portion which bears a hook. The hook, which in both the two medial claws has a dorsal accessory seta, is the most sclerified and solid portion of the claw (Fig. 2 E).

Segmental folds, rather than plates, can be distinguished in the ventral region where, furthermore, no spikes are present.

The pharyngeal apparatus consists of a sclerified tube (35  $\mu\text{m}$ ), the pharyngeal bulb (31 x 25  $\mu\text{m}$ ) and two slender stilets (46  $\mu\text{m}$ ) with very short cuticular sheaths and a small furca, which are external to the bulb. The three placoids are long, lightly undulating and without an apophysis (Fig. 2 A).

The terminal part of the genital duct can be protruded and the male gonopore, which is close to the anus, is covered with a crescent fold (Fig. 3 A).

The anus is covered with two lateral folds.

#### Description of the female

The female is like the male with the exception of the gonopore which is a rosette-like opening, 6  $\mu\text{m}$  from the anus ; furthermore, there are two flat roundish seminal receptacles, with very curled ducts which open close to the gonopore (Figs. 2 B, 3 B).

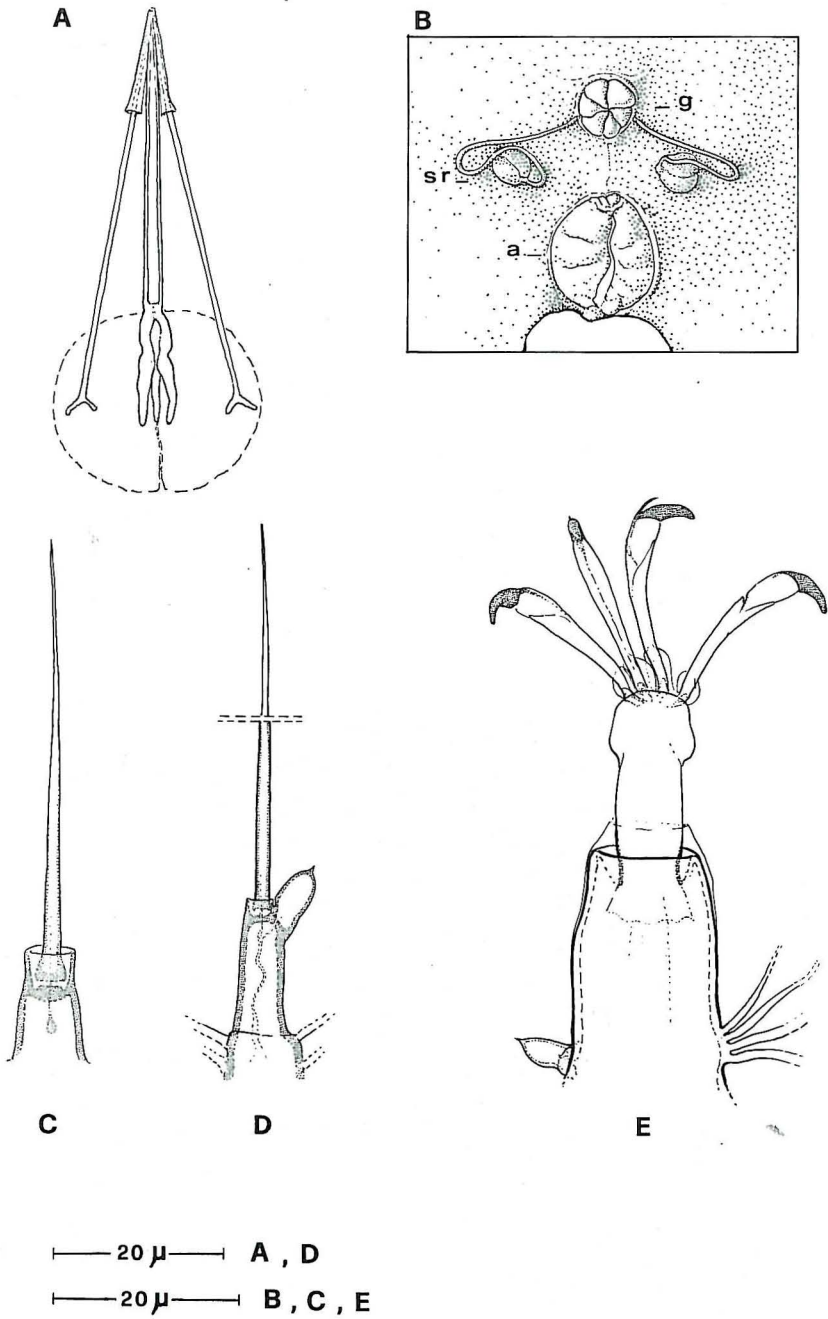


Fig. 2 : *Neostygarctus acanthophorus* : A - Bucco-pharyngeal apparatus ; B - Female gonopore (g) with seminal receptacle (sr) and anal region (a) ; C - Medial cirrus ; D - Lateral cirrus and primary clava ; E - Leg of the fourth pair with P4.

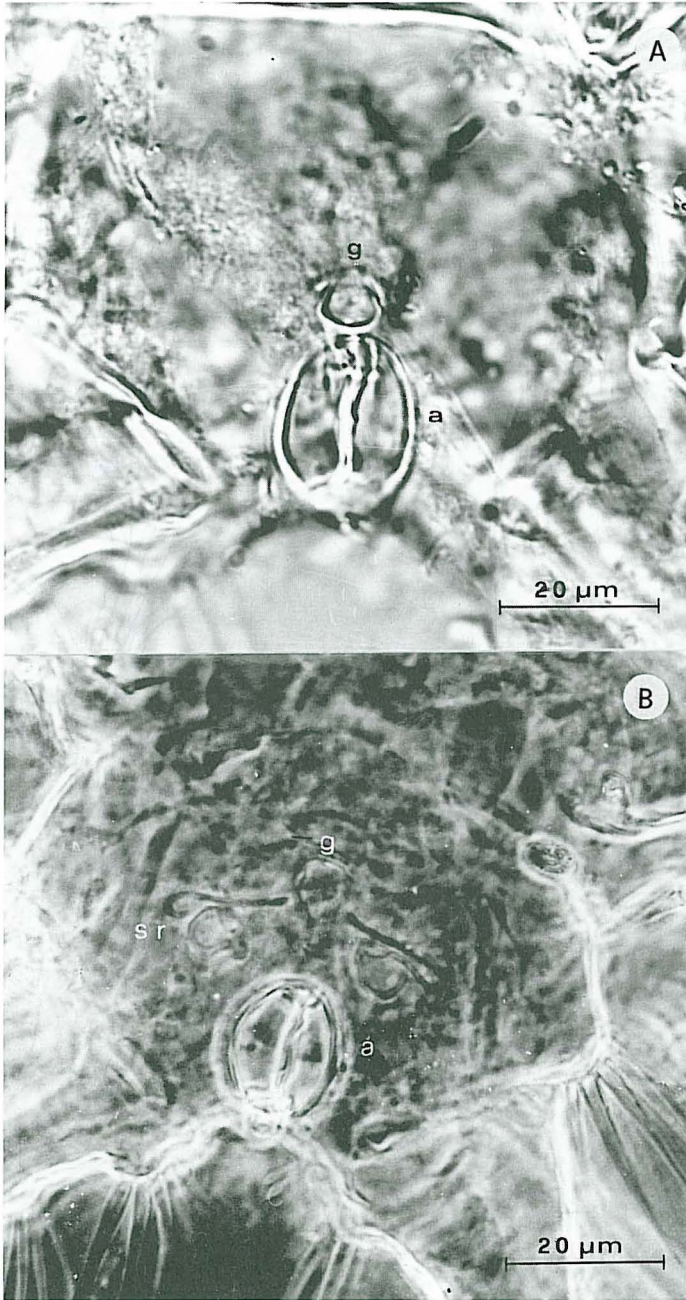


Fig. 3 : *Neostygartus acanthophorus* : A : Adult male ; g : gonopore ; a : anus ; B : adult female ; g : gonopore ; sr : seminal receptacle ; a : anus.

## Discussion and conclusion

From a phylogenetic point of view, Neostygarctidae is a very interesting family because it shares many plesiomorphic characteristics with the Renaudarctidae and Stygarctidae families, the latter undoubtedly the stem group of all Heterotardigrada (D'Addabbo Gallo *et al.*, 1987 ; Grimaldi de Zio *et al.*, 1988 ; Kristensen, 1987 ; Kristensen & Higgins, 1984 ; Renaud-Mornant, 1984).

Furthermore, it has preserved many plesiomorphic characteristics, such as the strong middorsal spikes, which in all the other Arthrotardigrada have disappeared.

The morphology of the skeletal apparatus of *Neostygarctus* is very similar to that of *Parastygarctus*. The shape of the segmental sclerotized plates is, in fact, identical to those of *Parastygarctus* although they are surrounded by and covered with numerous spines and have a large middorsal segmental spike. Furthermore, as in Stygarctidae the ventral region has lost the sclerotized plates, preserving evident segmental folds. This characteristic separates Neostygarctidae from Renaudarctidae, where, on the contrary, both dorsal and ventral plates do exist (Kristensen & Renaud-Mornant, 1984).

The distribution of the cephalic appendages, is as in *Parastygarctus*, but the cephalic plate, less deeply divided in lobes than in *Parastygarctus*, is an apomorphic characteristic. The secondary clava is as in *Mesostygarctus* Renaud-Mornant, 1979, whereas the primary clava and the lateral cirrus have a smooth cirrophorus located at the end of the head lateral process. This cirrophorus, and the very strong primitive cirrophorus of the medial cirrus, could be regarded as plesiomorphic characteristics which in Stygarctidae and Renaudarctidae are reduced or have disappeared. Furthermore, the primary clava of *Neostygarctus* has a very primitive shape, quite different from the primary clava of all Stygarctidae and Renaudarctidae. The middorsal segmental spikes must also be considered as an evident plesiomorphic element (Kristensen, 1987), whereas an apomorphy is the absence of sense organs on the first three pairs of legs.

*Neostygarctus* also preserves a very primitive organization of cirrus E : large base and an articulation with few, large folds ; all the kinds of articulation derive from this pattern in Arthrotardigrada and Echiniscoidea. In *Renaudarctus* cirrus E organization is as in some Styraconyxinae, quite different from *Neostygarctus* (Kristensen & Higgins, 1984).

Another characteristic, which according to Kristensen (1987) is apomorphic, is the presence in *Neostygarctus* of black eyes connected with the protocerebrum.

The morphology of the pharyngeal apparatus in Neostygarctidae has many apomorphic characteristics compared with that of Stygarctidae : the stilets are slender, external to the pharyngeal bulb, with very short cuticular sheaths and small furcae. No stylet supports are evident. The buccal tube, CaCO<sub>3</sub>-incrusted, is relatively long and narrow. The large pharyngeal bulb, wider than high, has three bar like, CaCO<sub>3</sub>-incrusted placoids, completely different from those of *Parastygarctus* or *Stygarctus* or *Renaudarctus*.

No sexual dimorphism is evident, with the exception of the gonopores. This is an apomorphic condition, but there are large seminal receptacles with long curled ducts, which is a primitive characteristic that Neostygarctidae and Renaudarctidae share with Stygarctidae.

A peculiar feature of Neostygarctidae is the claws which look like fingers with terminal hooks, but are not fingers like those of Renaudarctidae, Halechiniscidae or Batillipedidae : they are hollow, directly connected to the foot, with a basal fold, and have a terminal solid portion. This could be either the direct evolution of the ancestral annelidean articulated cheta, or the evolution of a simple claw like that of *Parasygarctus*. In this case, when Neostygarctidae and Renaudarctidae separated from Stygarctidae, they evolved individual claw types.

On the basis of all these elements, we can conclude that, even though Renaudarctus and Neostygarctus are undoubtedly closely related to each other (Kristensen & Higgins, 1984), they represent the two different families Renaudarctidae and Neostygarctidae, because of the strong differences between them as well as those between them and Stygarctidae.

#### REFERENCES

- D'ADDABBO GALLO, M., M. R. MORONE DE LUCIA & S. GRIMALDI DE ZIO, 1987. Heterotardigrada of the Amendolara Shoal, High Ionian Sea. In : R. Bertolani (ed.), *Biology of Tardigrades. Selected Symposia and Monographs U.Z.I., 1, Mucchi, Modena* pp. 103-110.
- GRIMALDI DE ZIO, S., M. D'ADDABBO GALLO & M.R. MORONE DE LUCIA, 1982. *Neostygarctus acanthophorus* n.gen., n.sp., nuovo Tardigrado marino del Mediterraneo. *Cah. Biol. Mar.*, 23 : 319-323.
- GRIMALDI DE ZIO, S., M. D'ADDABBO GALLO & M.R. MORONE DE LUCIA, 1987. Adaptive radiation and phylogenesis in marine Tardigrada and the establishment of Neostygarctidae, a new family of Heterotardigrada. *Boll. Zool.*, 54 : 27-33.
- GRIMALDI DE ZIO, S., M. D'ADDABBO GALLO & M.R. MORONE DE LUCIA, 1988. Two new Mediterranean species of the *Halechiniscus* genus (Tardigrada, Heterotardigrada). *Boll. Zool.* 3 : 205-211.
- KRISTENSEN, R. M., 1987. Generic revision of the Echiniscidae (Heterotardigrada), with a discussion of the origin of the family. In : R. Bertolani (ed.), *Biology of Tardigrades. Selected Symposia and Monographs U.Z.I., 1 Mucchi, Modena* pp. 261-335.
- KRISTENSEN, R.M. & R.P. HIGGINS, 1984. 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. & J. RENAUD-MORNANT, 1983. Existence d'Arthrotardigrades semibenthiques de genres nouveaux de la sous-famille des Styraconyxinae subfam. nov. *Cah. Biol. Mar.*, 24 : 337-353.
- RENAUD-MORNANT, J., 1979. Tardigrades marins de Madagascar II. Stygarctidae et Oreellidae. III. Considérations écologiques générales. *Bull. Mus. natn. Hist. nat., Paris.* 1 : 339-351.
- RENAUD-MORNANT, J., 1984. Nouveaux Arthrotardigrades des Antilles. *Bull. Mus. natn. Hist. nat., Paris.* 6 : 975-988.