

Revision of the genus *Halechiniscus* (Halechiniscidae, Arthrotardigrada).

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Abstract : The presence in the Mediterranean Sea of different species belonging to the genus *Halechiniscus*, allows us to further study and more detailed description of this genus. This study has led to a revision of the genus *Halechiniscus* and to a more actual diagnosis with a redescription of its most important morphological and diagnostic characteristics.

Résumé : Une description plus détaillée du genre *Halechiniscus* a été réalisée grâce à la récolte de différentes espèces dans les mers italiennes. Cette étude a aussi permis une révision du genre et un diagnostic plus moderne avec la redescription des plus importants caractères systématiques.

INTRODUCTION

Some of the first described species of marine tardigrades belong to the genus *Halechiniscus*. To date it includes only eight species of which *H. gutteli* is the oldest described species (Richters, 1908).

The other species are : *H. perfectus* and *H. remanei*, both found and described by Schulz in 1955 ; *H. subterraneus* Renaud-Debyser, 1959 ; *H. greveni* Renaud-Mornant and Deroux, 1976 ; *H. tuleari* Renaud-Mornant, 1979 ; and lastly, *H. macrocephalus* and *H. paratuleari* Grimaldi de Zio *et al.*, 1988, which are the most recently discovered and described species. During these eighty years many other species of the phylum Tardigrada have been found and consequently our knowledge of marine tardigrades has become more complete and precise. As new species have been discovered and described, anatomical features and morphological details, previously neglected or emphasized, have gained or lost importance according to the individual case.

Many recent papers by Renaud-Mornant (1982, 1983, 1984 a - b, 1987), Kristensen (1981, 1984), Kristensen & Higgins (1984 a - b), D'Addabbo Gallo *et al.* (1986, 1987), Morone De Lucia *et al.* (1988) and Grimaldi de Zio *et al.* (1987 a - b - c, 1988), on marine tardigrades, in particular Halechiniscidae, provide us with the elements to reconsider old descriptions and, if limited, to complete or improve them.

The discovery of *H. macrocephalus* and *H. paratuleari*, and of a relevant number of specimens of *H. greveni*, in the Mediterranean Sea has given us the opportunity to reconsider this last species and all the *Halechiniscus* genus.

SYSTEMATIC RESULTS

Halechiniscus greveni Renaud-Mornant & Deroux, 1976 (Tab. I ; Figs. 1, 2, 3)

The type locality of *H. greveni* was a sink in "Station Biologique de Roscoff", (Renaud-Mornant & Deroux, 1976). Our records are the first from the natural subtidal biotop. The ultrastructural description of the sense organs of *H. greveni* was also based on materials from the type locality (Kristensen, 1981).

H. greveni found in Sardinia, corresponds to the original description by Renaud-Mornant & Deroux (1976) more than to the more recent one by Kristensen (1981). The morphometrical analysis, however, reveals that the specimens of the mediterranean population are larger than those found elsewhere (Tab. I).

The large number of specimens studied - first and second stage larvae and adults, males and females - provide further details about structures, such as their sense organs, seminal receptacles, placoids and so on (Figs. 1, 2, 3).

Ventral cephalic cirri, which in this species are external as in most *Halechiniscus* species, are different with respect to other cirri because of the scapus which has a closely adherent sleeve-shape rather than a trumpet-shape. Primary clavae are banana-shaped and when they straighten, the ventral edge is corrugated ; this aspect is very common. As specified by Kristensen (1981), they are open at their distal end.

Flat cephalic kidney-shaped buccal papillae partially surround the mouth opening.

Cirrus E has an accordion-shaped proximal portion with folds so thin and close as to be often indistinguishable. Because of this morphology, this detail has been overlooked in previous descriptions. P4 has a large bell-shaped cirrophorus, with a long basal conical portion and a terminal thin tube. This organ, as in the case of primary clava, is open at the terminal end, and therefore, it is probable that these two organs function as chemoreceptors.

Small refractive cotton-reel shaped Van der Land's organs are found inside primary clava and P4, between the cirrophorus and sense-organ.

As is to be expected, there are seminal receptacles in the female ; they are simple vesicles with sinuous ducts which open on either side of the female gonopore. In some specimens the openings are fringed (Fig. 1).

The pharyngeal placoids are different shapes and lengths, as described by Renaud-Mornant and Deroux, but in some first-stage larvae, they are identical.

H. greveni, like *H. gutteli* and *H. perfectus*, has no lateral expansions, whereas all the other species of the genus show more or less evident lateral processes. They also exist in *H. subterraneus* although they are not very evident.

On the basis of these elements the emended diagnosis of the species is the following :

Halechiniscus with tubular arch-shaped primary clava, large cirrophori at the base of all cephalic appendages except for the ventral cirri. Flat buccal papillae on either side of the mouth. Pharyngeal bulb with three placoids ; the ventral one is longer with a knob-shaped terminal apophysis. Large claws, the medial with a dorsal spur. Sexual dimorphism in gonopores and primary clavae which are larger in males than in females. On each side of the female gonopore there are openings of the sinuous ducts of the seminal receptacles. No lateral expansions.

TABLE I

Measurements of larval stages and adults of *Halechiniscus greveni*

Larvae

	1st stage					2nd stage				
	min	max	\bar{x}	σ	n*	min	max	\bar{x}	σ	n*
L	63	75	70	4.4	4	53	97	82.6	17.4	4
W	22	33	28	4	4	24	45	35	8.7	4
mC	20	26	23		3	9	27	20		3
iC	10	17	13		4	9	23	16		4
eC	11	17	14		3	7	16	12		4
lC	22	33	26		4	25	39	32		3
Cl	12	16	14		4	16	22	19		3
P1	5	7	6		4	8	9	8.5		3
P2	7	12	10		3	9	11	10		3
P3	12	12	12		3	13	14	13.5		2
P4	8	10	9		4	8	9	8.5		3
CE	23	65	40		4	34	56	41		3
bT	21					20				

Adults

	Males					Females				
	min	max	\bar{x}	σ	n*	min	max	\bar{x}	σ	n*
L	74	124	89.7	15.9	6	98	185	133.1	21.4	22
W	36	53	42	5.7	6	38	75	61.4	9.4	22
mC	12	34	21		4	21	56	34		12
iC	10	31	16		6	12	45	22		20
eC	9	20	13		6	7	29	16		20
lC	22	40	29		6	19	58	41		21
Cl	28	50	36		6	17	45	30		22
P1	6	9	7.6		3	6	16	10		13
P2	8	12	10		4	8	24	17		14
P3	10	13	11.6		3	10	26	20		18
P4	8	16	11.3		4	8	19	14		18
CE	26	46	34.7		5	21	59	41.6		20
bT	18				1	20	25	23		6

Abbreviations

L	total length	eC	external cirri	P3	third leg papilla
W	width	lC	lateral cirri	P4	fourth leg papilla
mC	medial cirrus	Cl	primary clavae	CE	cirri E
iC	internal cirri	P1	first leg papilla	bT	buccal tube
		P2	second leg papilla	n*	number of specimens

All the measurements are in μm .

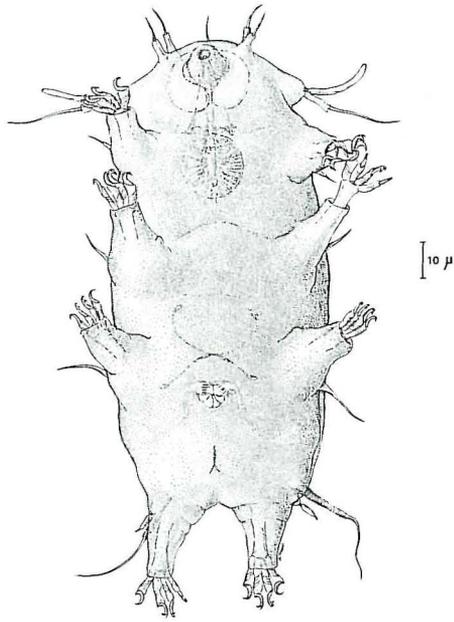


Fig. 1 : *Halechiniscus greveni*. Adult female, ventral view.

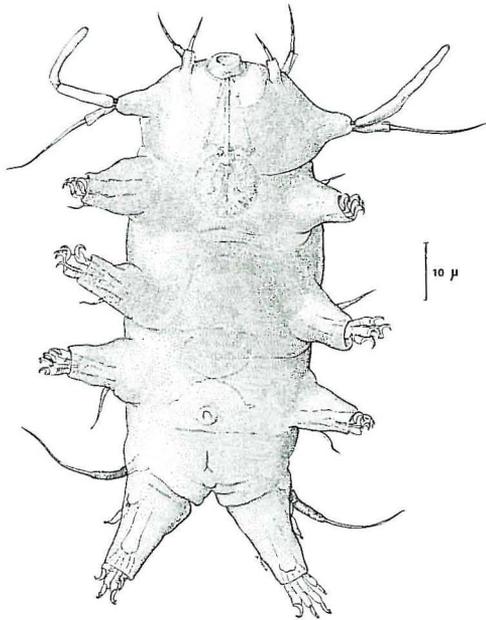


Fig. 2 : *H. greveni*. Adult male, ventral view.

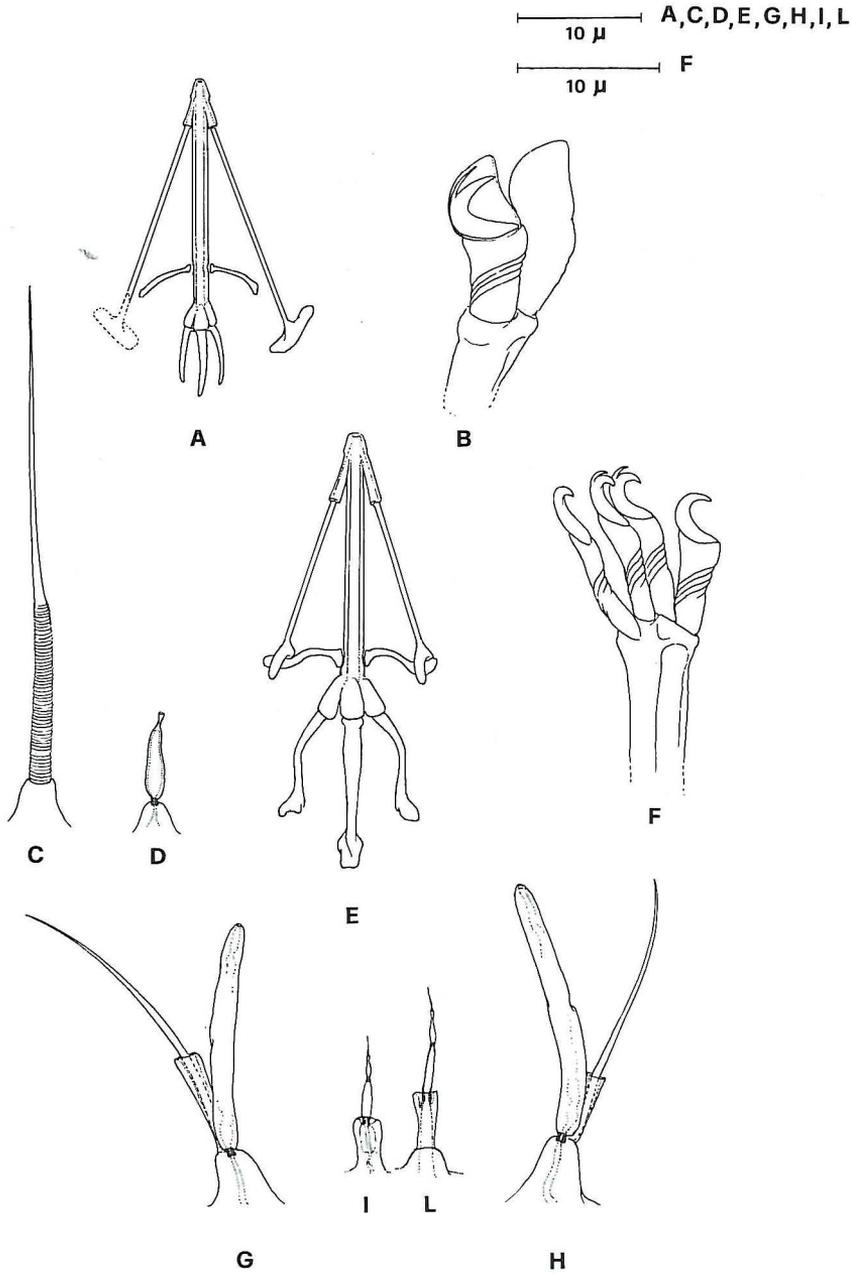


Fig. 3 : *H. greveni*. A : 1st stage larva pharyngeal apparatus ; B : 1st stage larva digits and claws ; C : cirrus E ; D : P4 ; E : pharyngeal apparatus in adults ; F : preadult and adult fingers and claws ; G : female primary clava and cirrus A ; H : male primary clava and cirrus A ; I and L : external and internal cirri.

DISCUSSION

Some of the above mentioned characteristics cannot be used as a basis for the diagnosis of one particular species, but they can refer to all species of the genus, and in many cases these characters are present in all the species.

In most of the recently studied species, a terminal opening has been noted in the primary clavae and P4, whereas inside the bases of these two receptors, a refractory ring known as the Van der Land's organ has been described (Grimaldi de Zio & D'Addabbo Gallo, 1987, Grimaldi de Zio *et al.*, 1988, Morone De Lucia *et al.*, 1988). This diffractive structure was discovered in *Florarctus* by Van der Land (1968) and named first time after him by Kristensen & Higgins (1984) in *Styraconyx*. This structure is certainly related to the function of these two receptors, as well as the terminal opening through which the chemoreceptor receives chemical messages from outside. This is one of the specific anatomical details which cannot be used as a diagnostic basis for the species because it seems to be found frequently in many Arthrotardigrada more so than reported by the literature.

The cirrus E morphology is another feature which merits further study. There is no doubt that the accordion-shaped articulation of this organ is very common in Halechiniscidae and could be considered normal for this family.

This is substantiated by our observations in *H. macrocephalus* and *H. paratuleari* (Grimaldi de Zio *et al.*, 1988). In our description of *H. paratuleari*, this kind of articulation is not mentioned, but nevertheless exists. The feature is undoubtedly a plesiomorphic character because it is known to be present in the most primitive species of marine tardigrades, such as Stygarctidae (Renaud-Debyser, 1965), Neostygarctidae (Grimaldi de Zio *et al.*, 1982), Renaudarctidae (Kristensen & Higgins, 1984 a) and in some species of Echiniscoididae (authors' unpublished material). It is lacking in Batillipedidae where its absence can be considered an apomorphic characteristic due to the environment of the littoral intertidal sand where they live, different from the subtidal sediment in open sea where most Halechiniscidae live.

The cirrus E shape, can be explained by the function it performs : on account of its position in the caudal region, it carries out those functions that are performed in the cephalic region by all cephalic cirri which have a very limited articulation.

In a new species of *Echiniscoides*, perhaps a new sub-species of *E. sigismundi* recently found in the Mediterranean Sea, cirrus A and cirrus E are exactly identical, with the same articulation consisting of a few narrow folds at their bases.

Now, it remains to be demonstrated if in an ancestral species other sensory organs had the same organization ; this hypothesis can be substantiated by the presence of simple omogonphic articulations in P1, P2 and P3 of some Batillipedidae and Halechiniscidae, and, as recently observed, of accordion-shaped articulations in Orzeliscidae (authors' unpublished materials). The presence of accordion-like somatic cirri B, C, D in Coronarctidae (Renaud-Mornant, 1987) should be emphasized. To date, we do not know if these spines were present in the ancestral species or not, and therefore, if the articulation of leg spines is a plesio-

morphic or an apomorphic character. Their absence in Neostygarctidae, Renaudarctidae and many Stygarctidae, could be a documentation of a late adaptation, and their articulation a more recent one ; their presence in some Echiniscoidea, however, could demonstrate the contrary. What we do know is that the origin and function of P4 is different from that of the other three leg spines.

P4 and cirrus E in the caudal region are equivalent to all cephalic sense organ equipment ; furthermore, P4 near the gonopore, may have a role in mating behaviour, as well as the primary clavae which show evident sexual dimorphism ; this situation does not exclude a role in the chemical and physical exploration of the environment.

Another feature to be considered is the presence of seminal receptacles : they were not originally noted and described because of their presence only in adult and mature female where, often, they were not always distinguishable because of their small size and transparency. To date, we are sure that they exist in all Heterotardigrada except for Batillipedidae and some species of Echiniscoididae ; they have been observed in Renaudarctidae, Neostygarctidae, Stygarctidae, Coronarctidae, Orzeliscidae and in recently studied Halechiniscidae. They have another kind of organization in Batillipedidae where one of the two original oviducts was sacrificed to seminal receptacle function (Grimaldi de Zio & D'Addabbo Gallo, 1975).

The buccal papillae need further comment ; they are generally considered secondary clavae. If the position of the cephalic appendages in Stygarctidae (with the exception of *Megastygarctides*) and Neostygarctidae (external-dorsal, internal-ventral cirri) is a plesiomorphic condition, and the presence of three pairs of clavae as in Renaudarctidae (Kristensen & Higgins, 1984) is also a plesiomorphic characteristic, every other position must be considered apomorphic.

In most Heterotardigrada the apomorphic condition is the normal case, in fact the plesiomorphic position exists only in *Neostygarctus*, *Parastygarctus*, *Stygarctus*, *Pseudostygarctus* and in only two species of Halechiniscinae : *H. tuleari* and *H. paratuleari*.

If the apomorphic distribution of the ventral-external and dorsal-internal cirri derives from the migration of the dorsal cirri to the ventral region, secondary clavae connected to them could correspond to the sensory plates found in some Halechiniscinae and Florarctinae. On the contrary, if the distribution derives from a divergence of the ventral cirri, which therefore become external, the ventral sensory plates could correspond to the tertiary clavae perhaps originally connected to them. Anyway, the coexistence of both secondary and tertiary clavae is found only in Renaudarctidae ; in all other Heterotardigrada one pair of clavae is lost.

The medial cirrus is always present in all species known of the *Halechiniscus* genus excepted for *H. gutteli*, but even if it is demonstrated that in Heterotardigrada this cirrus has the tendency to regress, we believe that, as Renaud-Mornant & Deroux observed (1976), in this species it was only overlooked when first described. In any case, a large medial cirrus with a stout cirrophorus is a plesiomorphic characteristic, whereas a reduced medial cirrus, or its absence, is certainly an apomorphic one.

The same reduction tendency can be observed in lateral processes, which at beginning are large as in *H. paratuleari*, but than become smaller as in *H. remanei* and finally, they completely disappear. This morphological detail is very important because it represents a phylogenetic documentation of the close relationship between Stygarctidae and Halechiniscidae (Grimaldi de Zio *et al.*, 1988). Furthermore the lateral expansions also present in Batillipedidae and Orzeliscidae could be interpreted as a residual plesiomorphic characteristic in these tardigrades which colonized in shallow waters, whereas their absence in deep sea species is an apomorphic characteristic.

CONCLUSION

On the basis of the above mentioned elements it is necessary to emend the diagnosis of the family of Halechiniscidae, sub-family of Halechiniscinae and genus *Halechiniscus* as follows :

Family of Halechiniscidae Thulin, 1928

Arthotardigrada 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. Peduncles sometimes present in the digits ; seminal receptacles generally present ; cirrus E often with a basal accordion-shaped articulation.

Sub-family of Halechiniscinae Thulin, 1928

Halechiniscidae usually with complete set of cephalic appendages with cylindrical tibia followed by a small tarsus ; claws either simple or with a dorsal spur ; peduncles absent ; sense organ of the fourth pair of legs (P4) short and non-branched ; vestigial secondary clavae.

Type genus of *Halechiniscus* Richters, 1908

Diagnosis : flat head extending into lateral lobes ; claws either simple or with distal dorsal spur ; calcar always absent ; peduncles absent ; medial cirrus present, primary clava may be longer than the lateral cirrus.

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