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Contribution towards the study of the phylogeny of the genus Proasellus. Observations at the scanning electron microscope of the copulatory pleopods of P. istrianus and P. ibeficus (Crustacea Isopoda)

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Genetica. — *Contribution towards the study of the phylogeny of the genus* Proasellus. *Observations at the scanning electron microscope of the copulatory pleopods of* P. istrianus *and* P. ibericus *(Crustacea Isopoda).* Nota di Emanuela Valeria Volpi, Rachele Antolini e Flora Valentino, presentata (*) dal Socio G. Montalenti.

Abstract. — In 1972 Henry e Magniez formulated an hypothesis about the probable evolution of the extremity of the endopodite of male second pleopod (copulatory pleopod) in the species of *Proasellus* belonging to the *meridianus* line.

In the scheme proposed by them, a rather easy structure, typical of some endemic species relicts of an ancient fauna of *Proasellus*, is the starting point. After an intermediate stage, they describe the structure present in *P. meridianus*, modern species, as the most functional.

Referring to this evolutive scheme we realized a study by scanning electron microscope of this appendix in *P. ibericus* and *P. istrianus*. Our observations confirm the attribution of these two species, proposed by Henry e Magniez, respectively to the most primitive evolutive stage and to the intermediate stage.

Key words: Proasellus istrianus; Proasellus ibericus; Phylogeny.

Riassunto. — *Contributo allo studio della filogenesi del genere* Proasellus. *Osservazioni al microscopio elettronico a scansione dei pleopodi copulatori di* P. istrianus *e* P. ibericus *(Crustacea Isopoda).* Nel 1972 Henry e Magniez hanno formulato un'ipotesi riguardante la probabile evoluzione dell'estremità dell'endopodite del pleopode secondo maschile (pleopode copulatore) nelle specie di *Proasellus* appartenenti alla linea *meridianus*.

Essi propongono uno schema in cui, partendo da una struttura piuttosto semplice, tipica di alcune specie endemiche relitti di un'antica fauna di *Proasellus*, si arrivi, attraverso uno stadio intermedio, alla struttura maggiormente funzionale presente in *P. meridianus*, specie moderna a vasta diffusione.

Riferendoci a questo schema evolutivo abbiamo realizzato uno studio al microscopio elettronico a scansione di questa appendice in *P. ibericus* e *P. istrianus*.

Le nostre osservazioni confermano l'attribuzione di queste due specie, proposta da Henry e Magniez, rispettivamente allo stadio evolutivo più primitivo e a quello intermedio.

Introduction.

The genus *Proasellus* (Dudich, 1925) has been considered for many years a subgenus of the genus *Asellus*.

In 1970 Henry and Magniez elevated it to the rank of genus, maintaining that it's a line whose origin and age are very different from those of the genus *Asellus*.

The genus *Proasellus* is a diversified mixture of numerous perimediterranean and Atlantic species. Its distribution area includes Europe to the south of an imaginary line joining the Black Sea to Great Britain, the eastern Mediterranean area and North Africa.

Racovitza (1919) was the first to establish the great taxonomic value of the male second pleopods (sexual or copulatory pleopods) and in fact the morphology of these appendixes is a fundamental character for the definition of the genera and species of Asellidae (Henry and Magniez, 1969).

(*) Nella seduta del 14 gennaio 1989.

In 1949 Chappuis, notwithstanding the apparent morphological homogeneity among the subgenus and notwithstanding the modest number of species described up to that period, formulated, on the basis of observations conducted on the second pair of male pleopods, the hypothesis of a probable polyphylesis of the subgenus *Proasellus*, recognizing no less than four distinct evolutive groups in its ambit.

Confirmation of Chappuis' idea followed in the findings of Henry and Magniez (1972), which are even more significative if one takes into account the large number of species observed by these Authors before intervening in favour of the polyphyletic hypothesis of Chappuis. However they divide the species of the genus *Proasellus* into only three evolutive lines: *coxalis, meridianus* and *antica*.

Proasellus meridianus (Racovitza, 1919), which can be found in the most parts of Western Europe, is the «type» species of the entire genus as well as being the form which heads the species belonging to the *meridianus* line. They are endemic species, relicts of an ancient fauna of *Proasellus* spread over the whole Mediterranean area prior to the expansion of *P. coxalis*, which enter naturally in this line whose evolutive point of arrival is, according to Henry e Magniez (1972), *P. meridianus*.

These Authors, being unable to describe a type of male second pleopod structure valid for all the forms that constitute this line, have proposed a probable evolutive scheme of the extremity of the endopodite of this appendix, departing from the endemic forms to arrive at *P. meridianus*, a modern species, widely spread.

Referring to this evolutive scheme, we have realized a study at the scansion electronmicroscope (SEM) of the extremity of the endopodite of the second pleopod in some male examples of *Proasellus* of the *meridianus* line.

The distal portion of the endopodite presents a terminal apophysis (also called tergal apophysis) and a species of tube, the «goulot» by French authors, often open in a

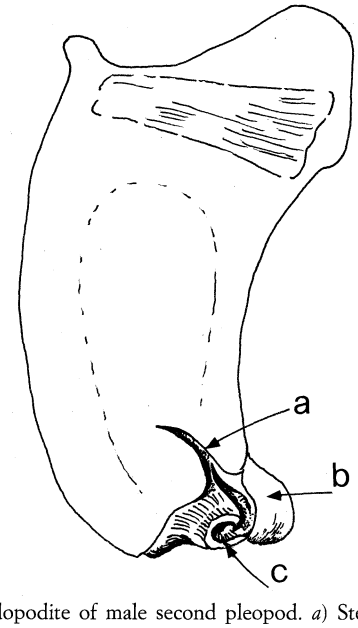


Fig. 1. – Genus *Proasellus*: endopodite of male second pleopod. *a*) Sternal cleft; *b*) Tergal apophysis; *c*) «goulot» (from Henry, 1976).

longitudinal slit (sternal cleft) that constitutes the outlet of the internal vesicle of the endopodite. The form of the terminal apophysis and above all, the «goulot», and their reciprocal relations are the most important characters in the diagnosis of the species, regarding the reconstruction of the phylogenetic relationships (Argano, 1979) (fig. 1).

Materials and methods.

We examined specimens of *P. ibericus* collected at Cabeceiras de Basto (Portugal) in November 1987, specimens of *P. istrianus* taken at the Contovello spring near Trieste (Italy) in June 1987 and specimens of *P. meridianus* collected at York (England) in September 1986.

The validity of our method of observation (SEM) has already been underlined by Henry and Magniez in a 1969 study and our procedure for the setting up of the preparations follows that indicated by those Authors in that work.

We preferred to conduct our observations on the whole pleotelson, bearing upward the distal extremity of the pleopods.

A long fixing in alchool at 70° was followed by dehydration in pure alchool and exsiccation by air.

The specimens were then glued to the SEM supports and metallized.

Observation of the samples was effected with a scanning electron microscope type Cambridge 2000.

Observations and discussion.

According to the evolutive scheme proposed by Henry and Magniez (1972), there are some species at the base of the *meridianus* line, whose male second pleopod differs considerably from that of *P. meridianus*, displaying a much more primitive structure.

Henry (1976) referring to a general morphological characteristic common to all species belonging to this evolutive stage, writes of a «goulot» of mediocre diameter which increases distally and whose border does not refold to form the «corolla» typical of *P. meridianus* (fig. 2 and 3). The angle between the tergal apophysis and the «goulot» is not very obvious.

As representative species of this first evolutive stage, we photographed at the SEM *P. ibericus* (fig. 4 and 5), an endemic species of Portugal, first described by Braga in 1946. In his work Braga writes—referring to the distal part of the endopodite—of a tight sternal opening that continues in a short «goulot» and of a tergal apophysis long, large and inclined towards the outside.

The particulars indicated by Brage and Henry are easily discernible in our photographs and confirme the evolutive considerations about *P. ibericus*.

Always according to Henry, the following evolutive stage includes a series of forms in which the endopodite of the copulatory pleopod has a general appearance reminiscent of *P. meridianus*; the «goulot» increases in length and diameter. The distal aperture accentuates in a marked manner and outlines the corolla aspect. The angle between the «goulot» and the apophysis widens.

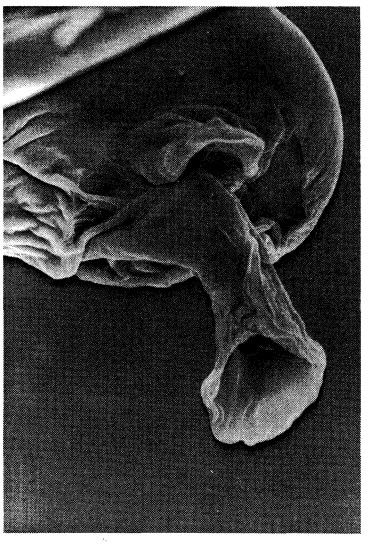
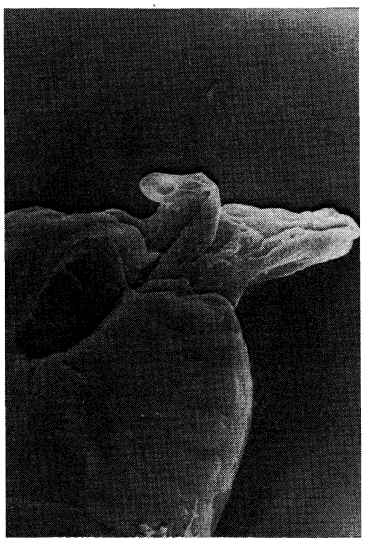


Fig. 2 and 3. – *P. meridianus*: endopodite of male second pleopod.



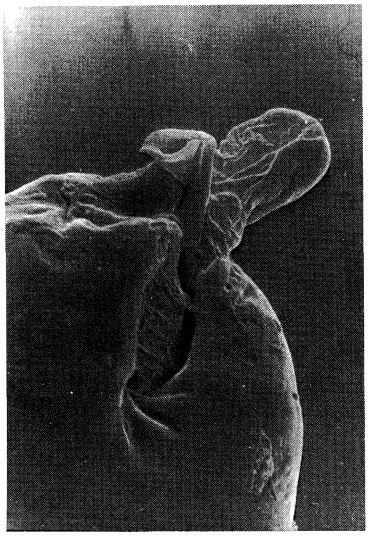


Fig. 4 and 5. – *P. ibericus*: endopodite of male second pleopod.

As representative species of this second stage we have considered *Proasellus istrianus* (fig. 6 and 7), endemic species of Triestine and Istrian Carso, whose systematic position is at present in dispute among zoologists. Stammer (1932) first described *P. istrianus*, emphasizing the morphological similarities regarding *P. meridianus*.

Following an attentive re-examination of *P. istrianus* specimens, Stoch (1985) maintains that it is difficult to establish the position of this material in the ambit of the genus *Proasellus*. He asserts that the similarity to *P. meridianus*, evidenced by Stammer, does not include a number of important particulars, among which, the shape of the «goulot», «... As regards the last named, even if a resemblance is revealed in the ample

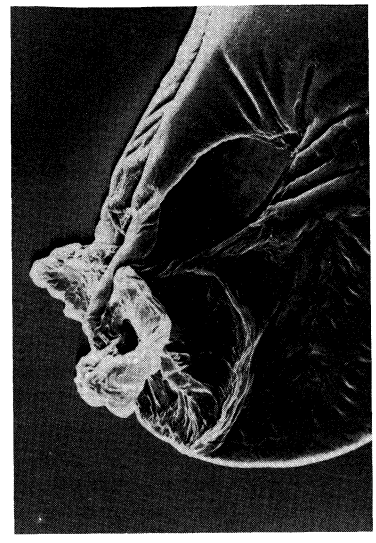


Fig. 6 and 7. – *P. istrianus*: endopodite of male second pleopod.

terminal opening, the absence of the tubular structure and the shape of the sternal cleft, very large and rounded, suggest that this species is not of the *meridianus* group...; however, it seems probable that *P. istrianus* belongs to a distinct phyletic line, rather isolated in the ambit of the genus, and perhaps including some other endemisms of the Illyrian-Balkan regions, presenting on the one hand an uncertain affinity with the *meridianus* group, and on the other hand an affinity with the *coxalis* group» (Stoch, 1985).

In an attempt to contribute to the clarification of this problem, we have studied

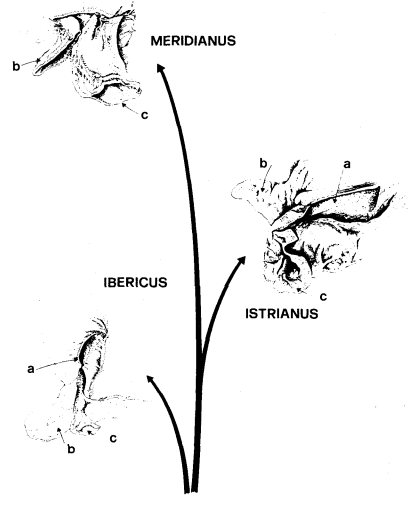


Fig. 8. – Evolutive scheme in which, on the base of the shape of endopodite of the male second pleopod, the relationship existing among the different species of the *meridianus* line is indicated.

(Valentino *et al.*, 1987) the chromosomal complement of *P. istrianus* in such a way as to be able to effect a comparison with the chromosomal complement of *P. meridianus* which is $2n = 10$ (Muldal, 1951; Rocchi-Brasiello, 1967) and with the chromosomal complement of *P. coxalis*, which is $2n = 12$ (Montalenti and Rocchi, 1964). The chromosomal complement consists of five pairs of chromosomes, all metacentric or sub-metacentric. The morphological confrontation between the chromosomal complement of *P. meridianus* and that of *P. istrianus*, showed them to be equal.

In our opinion, the insertion of *P. istrianus* in the *meridianus* line, actuated by Henry and Magniez, is valid, and in particular its placing, relative to the conformation of the endopodite, in an intermediate evolutive stage.

P. meridianus signals the end of this evolution (fig. 2 and 3). In fact, we note in it the growth of the diameter and length of the «goulot» which broadens in a marked manner at the extremity and forms a species of tube whose edges are tightly covered but not sealed. The tergal apophysis with the «goulot» forms an angle that can reach 180°.

P. meridianus presents therefore the most functional sexual structure which signals the point of arrival of this morphological evolution, shown in the diagram of fig. 8.

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