Hydrobiologia **317:** 247–258, 1996.

Taxonomic and biogeographic analysis of the *Proasellus coxalis*-group (crustacea, isopoda, asellidae) in Sicily, with description of *Proasellus montalentii* n. sp.

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Received 23 June 1994; in revised form 25 April 1995; accepted 10 May 1995

Key words: Isopods, *Proasellus*, Sicily

Abstract

The genus *Proasellus* is widespread in ponds, ditches and rivers of Sicily. A detailed morphological analysis of several samples of asellids collected in Sicilian freshwaters resulted in the determination of three species of the *Proasellus coxalis*-group: *P. banyulensis italicus* (Dudich, 1925), *P. montalentii* n. sp. and *P. wolfi* (Dudich, 1925), which is elevated to specific rank. The three species can be distinguished on the basis of the sutures of pleopod V exopod. *Proasellus montalentii* inhabits the western part of Sicily, while the range of *P. wolfi* is confined to the Iblean region. Both species are more closely related to the North African taxa of *Proasellus coxalis*-group, while *Proasellus banyulensis italicus* is very similar to the populations found in peninsular Italy. These patterns are explained supposing multiple colonizations of Sicily during Pliocene connections.

Introduction

Proasellus coxalis (Dollfus, 1892) has long been considered a polytypic species, widely distributed in freshwater environments (sources, streams, ponds, wells and cave waters) around the Mediterranean basin (including large and small islands) and in several rivers and channels in central Europe. Since its description, almost all researchers have stressed its morphological variability, and 28 subspecies were described; for more detailed information see the works of Racovitza (1919), Arcangeli (1942), Braga (1948), Herhaus (1977), Stoch (1985, 1989) and the references they cited.

Considering the interesting taxonomic problems *Proasellus coxalis* gives rise to, a detailed morphological study and a taxonomic revision of this polytypic species has been carried out (Stoch, unpubl.). At the same time interbreeding experiments were performed between some epigean populations from Sicily and peninsular Italy (Pomponi, 1984; Volpi *et al.*, 1989). The first results of the research indicated that several different species are concealed under the name *Proasellus coxalis* (Stoch, 1989). On the other hand it is possible that some taxa described as subspecies of *Proasellus coxalis* have no taxonomic value and must be considered as simple ecotypes. All the members of *Proasellus coxalis*-group share the same shape of the male second pleopod endopod, which was used as the unique diagnostic character within the genus *Proasellus* since the revisory work of Henry & Magniez (1976).

The present paper reports the results of the taxonomic study carried out on the interesting material collected in Sicily by one of us (F.V.) and R. Gerecke. Members of the *Proasellus coxalis*-group are widespread in almost all kinds of freshwater environments of this islands, excluding fast running streams (cave waters: Caruso, 1982; epigean waters, with scarce hydrodynamism: Riggio, 1978). Following the opinion of Arcangeli (1942), the material from Sicily should have been attributed to *Proasellus coxalis wolfi* (Dudich, 1925), described from the source of the river Ciane (province of Siracus(a). The specimens examined can be attributed to three different species, one of which is new to science, while typical *Proasellus cox-*

alis is not represented in our samples. In the following part of the present paper, the taxa found in Sicily are described and illustrated, and a biogeographic analysis is discussed.

Descriptive part

Proasellus banyulensis italicus (Dudich, 1925)

Material S. Cipirello, T. Iato, near L. Poma (province of Palermo), station 149, leg. R. Gerecke: 2.IX.1985, several specimens; Partinico, F. Iato, near SS 13 (province of Palermo), station 205, leg. R. Gerecke: 15.X.1985, several specimens; F. Eleuterio, road Misilmeri- Bolognetta (province of Palermo), station 49, leg. R. Gerecke: 01.VI.1985, 1 ♂.

Remarks The type of material of this taxon, originally described by Dudich (1925) – sub *Asellus italicus* – from Napoli, has been recently redescribed (Stoch, 1989) and does not belong to *Proasellus coxalis* as supposed by Arcangeli (1942). The morphological characters of the material examined in this work are almost identical to those of the specimens described from peninsular Italy.

Proasellus montalentii n. sp.

Material San Domenico (province of Palermo), in a little brook, leg. F. Valentino: 9.IV.1987, several specimens; 7.XII.1987, several specimens; Castronuovo, P. del Riso, Sicani (province of Palermo), station 235, leg. R. Gerecke: 8.XI.1985, 1 ♂; River Torto, near Felsen, Roccapalumba (province of Palermo), station 75, leg. R. Gerecke: 25.VI.1985, 1 ♂, 1 ♀; Source near M. Canale (province of Enna), station 157, leg. R. Gerecke: 7.IX.1985, 1 ♂, 1 ♀; pit near Massa Intronata, M. Zimmara (province of Enna), leg. R. Gerecke, station 154: 7.IX.1985, 1 ♂; drinking through near Enna (province of Enna), leg. F. Valentino: 10.XI.1985, several specimens.

Loc. typ. San Domenico, Palermo, Sicily (Italy).

Type material Holotype, one male (collected 9.IV.1987), preserved in alcohol 75%, appendages dissected and mounted on a slide in Faure medium; paratypes, 20 specimens, preserved in alcohol 75%; type material deposited in the collections of the Natural History Museum in Verona, Italy. The remaining material is deposited in the collection of the first author (F.S.).

Etymology The new species is dedicated to the late Prof. G. Montalenti, to commemorate his contributions to the knowledge of several aspects of the genetics and evolution of the asellids.

Description of type series Eyes normally developed, pigment brownish. Body length (excluding uropods) of the mature male 5–8.9 mm; length of the holotype 8.9 mm; maximal width at the level of the third pereionite (ratio length/width 3.63). Mean length of the ovigerous females 6.5 mm; mean maximal width 2 mm; mean ratio length/width of the female 3.25.

Margins of head, pereonites and telson moderately setose; the range of 'sensorial setae' indicated by Racovitza (1919) on seventh tergite in *Proasellus coxalis peyerimhoffi* are lacking (only two setae are present). Coxal plates visible dorsally except for pereionite VII, as in Figs 1f–g.

Pleotelson broader than long, mean length/width ratio 0.93 in the male, 0.88 in the female; caudal lobe pronounced.

Antennula with flagellum of 9 articles; aesthetasks present on the last three articles (Fig. 1a). Antenna with flagellum of 65 articles in the holotype, but the number is highly variable. An ovigerous female carries 41 articles in the left flagellum, 50 in the right one.

Mandibles, maxillae and maxillipeds as in Figs 1b–e, typical of *Proasellus coxalis*-group. Maxilliped (Fig. 1e) with 4–5 coupling spines (retinacul(a). Second article of palp wide; all the segments are densely setose. Maxilliped of ovigerous females with oostegite bearing 12 apical setae.

Pereopod I propodus of the male (Fig. 2a) about 1.8 times as long as wide; palmar margin provided with three strong spines ('phanères ensiformes' of Racovitza, 1919), and two longitudinal rows of setae, arranged as shown in Fig. 2a. Dactylus reaching the distal end of carpus, bearing 7 posterior spines, accompanied by 2 short setae and a long subungueal seta. Anterior margin of dactylus with a distal row of 6–8 long setae.

Pereopod I propodus in the female (Fig. 2b) more slender, approximately 2 times as long as broad, with 3 stout spines and a row of setae. Dactylus bearing 4–5 strong spines accompanied by 3 short setae and a longer subungueal seta.

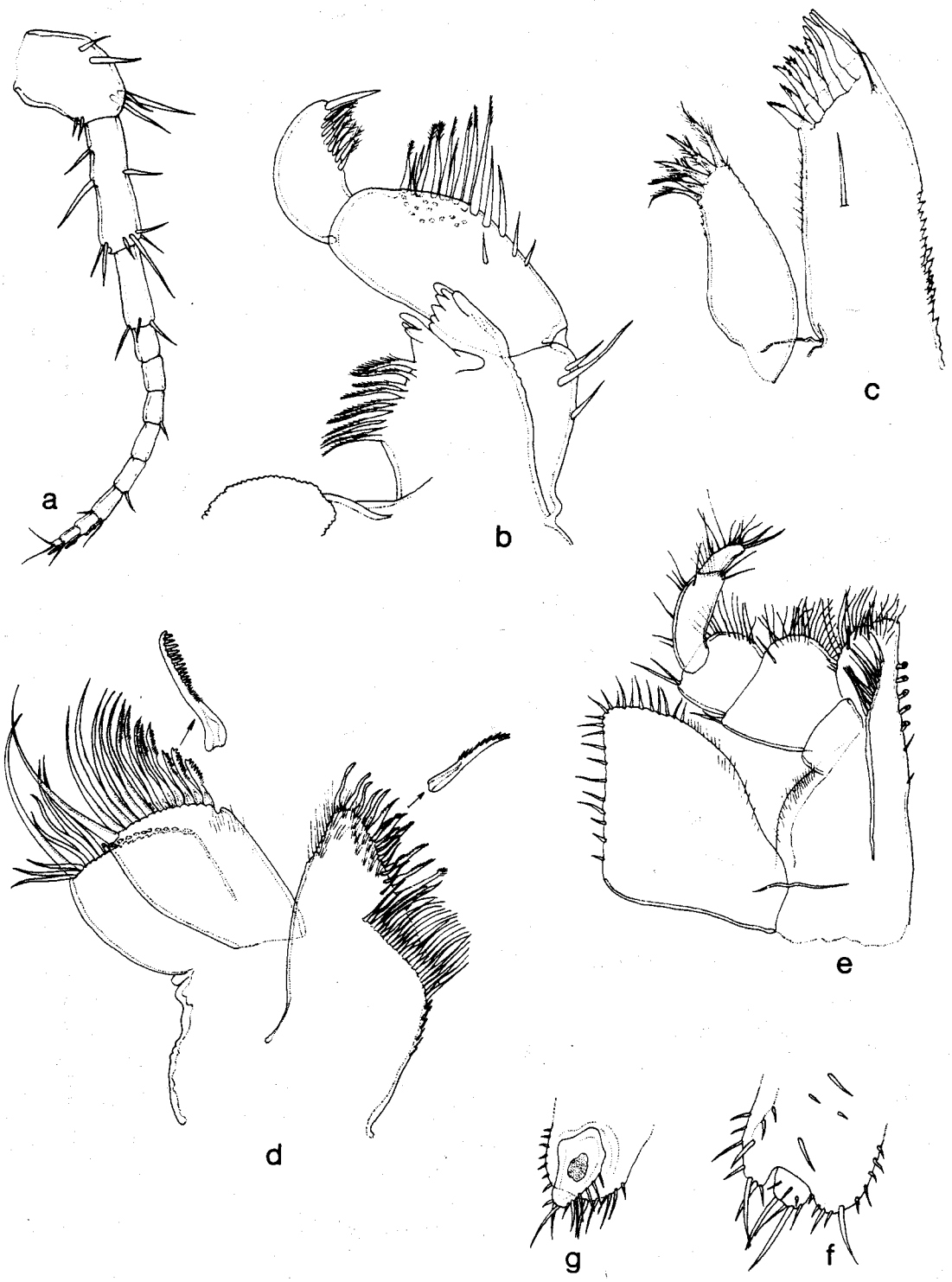

Fig. 1. *Proasellus montalentii* n. sp., S. Domenico (Palermo); holotype (male). (a) antennula; (b) mandible; (c) maxilla I; (d) maxilla II; (e) maxilliped; (f) left coxa and pereionite IV (dorsal view); (g) pereionite VII and coxa, ventral view.

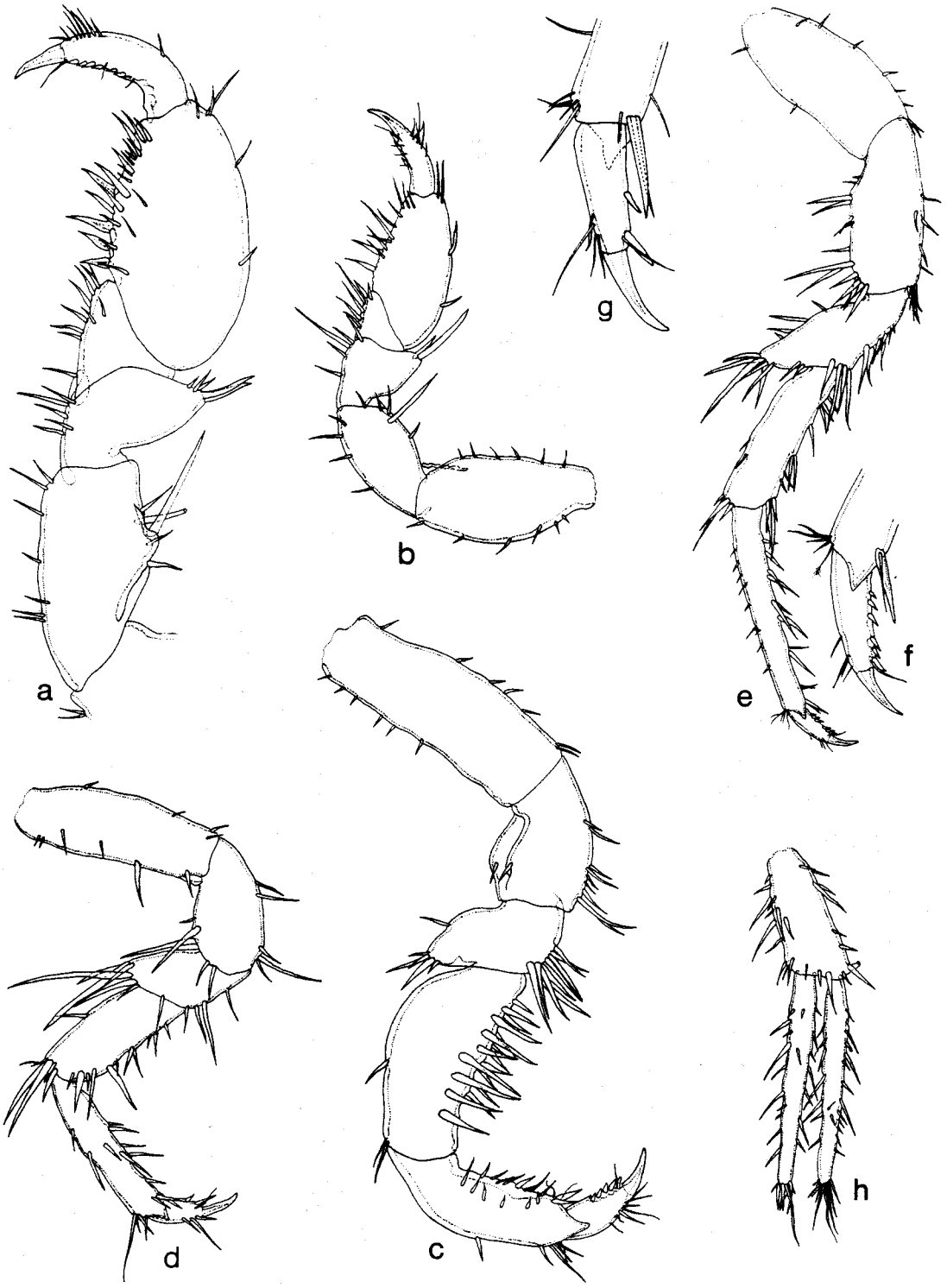

Fig. 2. *Proasellus montalentii* n. sp. Holotype (male) and paratype (ovigerous female mm 6.7): (a) pereopod I (male); (b) pereopod I (female); (c) pereopod IV (male); (d) pereopod IV (female); (e) pereopod VII (male); (f) dactylus of pereopod VII (male); (g) dactylus of pereopod VII (female); (h) uropod (male).

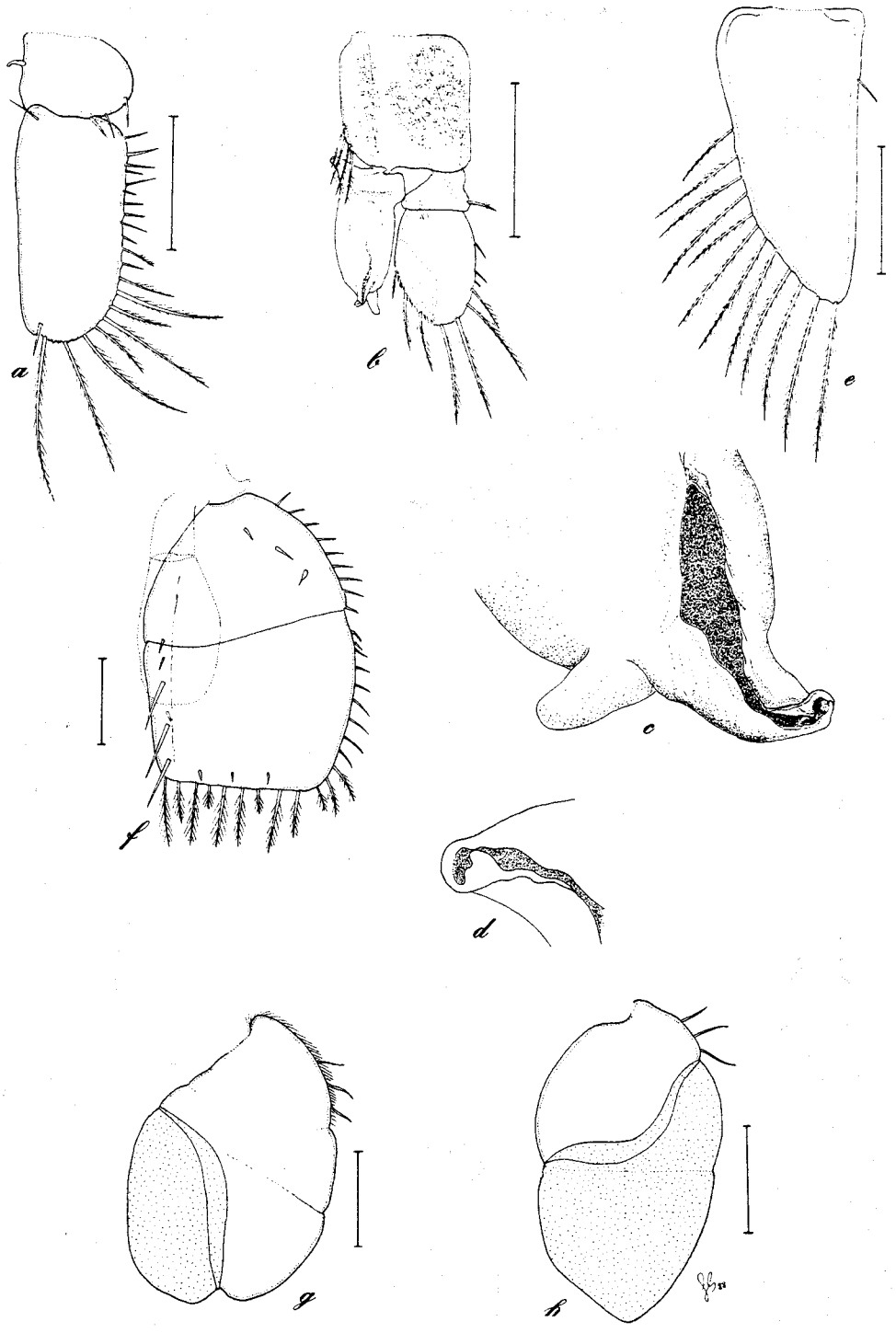

Fig. 3. ***Proasellus montalentii*** n. sp. Holotype (male): (a) pleopod I; (b) pleopod II; (c) distal part of pleopod II endopod (from S.E.M. analysis); (d) 'goulot' (from S.E.M. analysis); (f) pleopod III exopod; (g) pleopod IV exopod; (h) pleopod V exopod; paratype (ovigerous female, mm 6.7): (e) pleopod II.

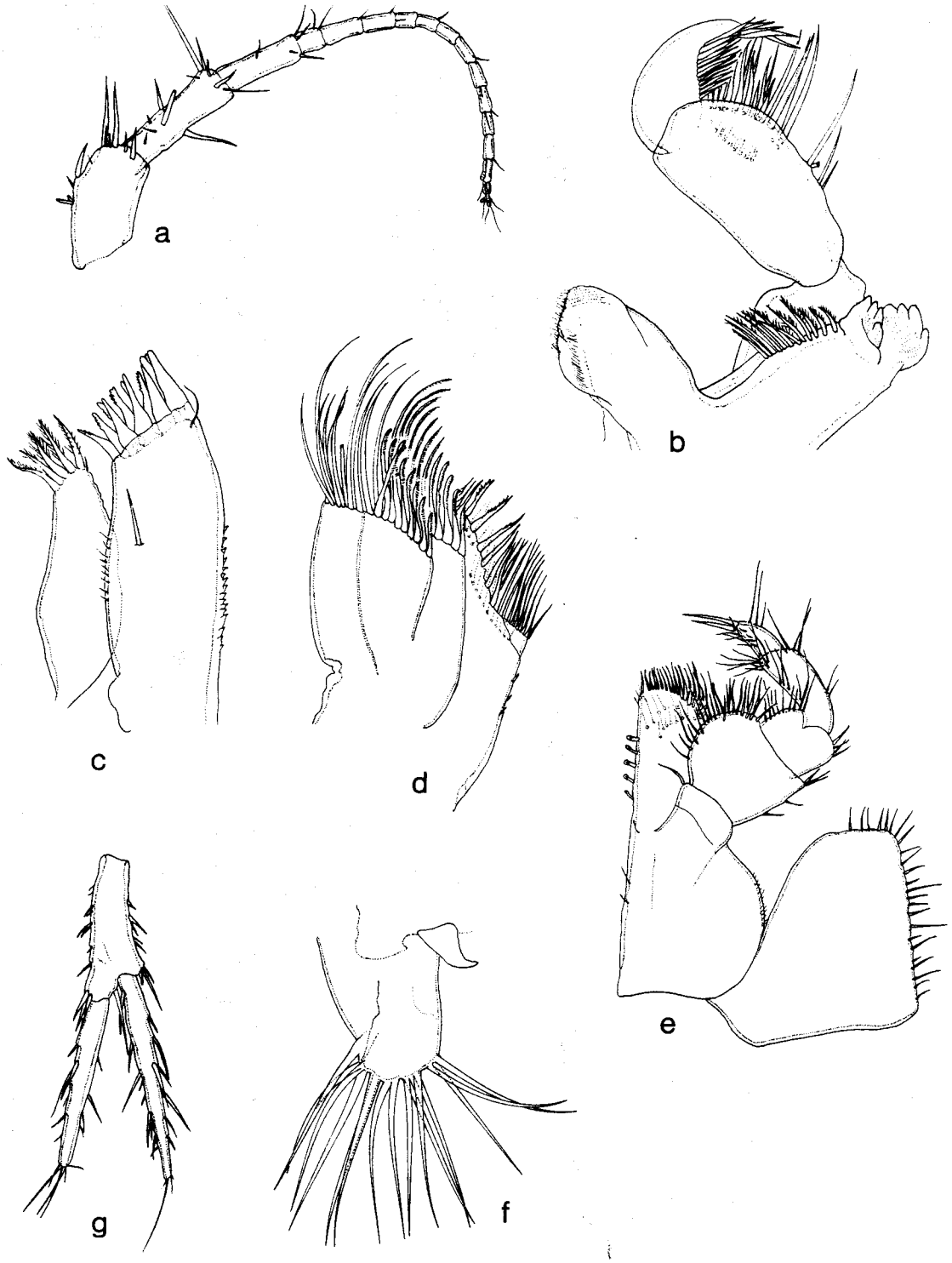

Fig. 4. *Proasellus wolfi* (Dudich), Anapo River. Male, mm 8.4: (a) antennula; (b) mandble; (c) maxilla I; (d) maxilla II; (e) maxilliped; (g) uropod. Ovigerous female, mm 6.9: (f) oostegite of maxilliped.

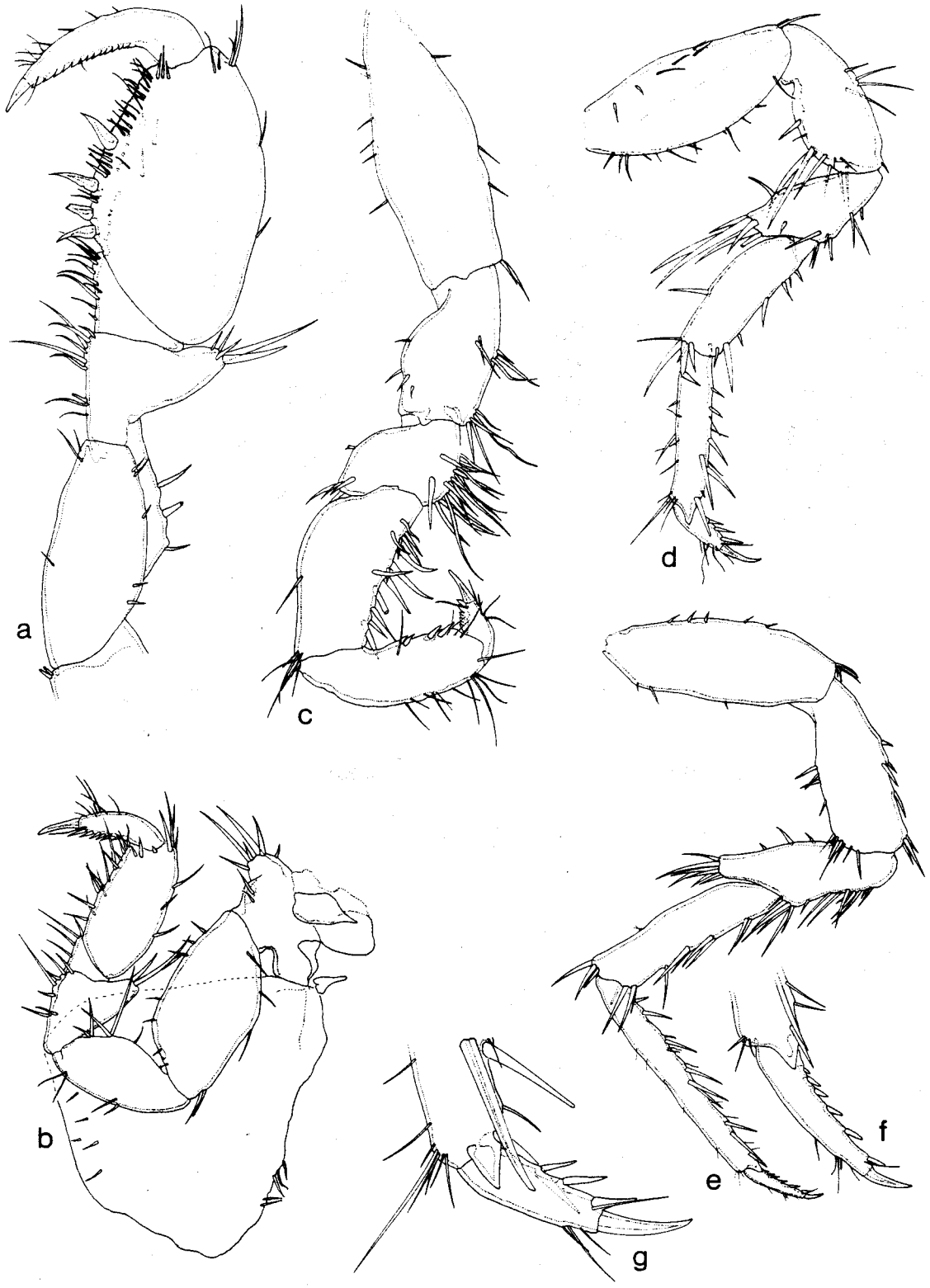

Fig. 5. *Proasellus wolfi* (Dudich), Anapo River. Male, mm 8.4 and ovigerous female, mm 6.9: (a) pereopod I (male); (b) pereopod I (female); (c) pereopod IV (male); (d) pereopod IV (female); (e) pereopod VII (male); (f) dactylus of pereopod VII (male); (g) dactylus of pereopod VII (female).

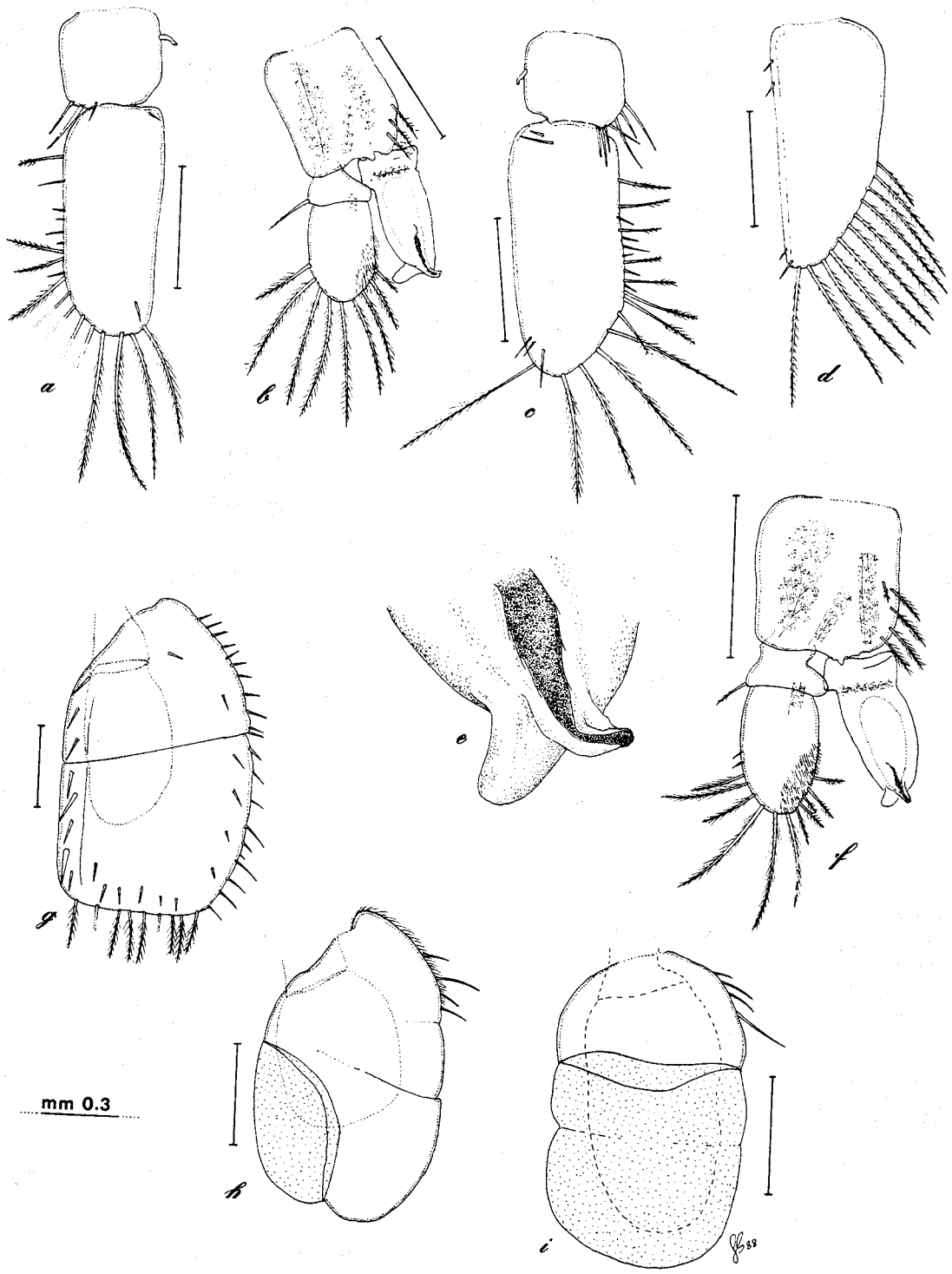

Fig. 6. *Proasellus wolfi* (Dudich). Lectotype, Ciane River, male, mm 6.6: (a) pleopod I; (b) pleopod II; (g) pleopod III; (h) pleopod IV; (i) pleopod V. Anapo river, male, mm 8.4: (c) pleopod I; (f) pleopod II. Male, Ciane river: (e) endopod of pleopod II, distal part (from S.E.M. analysis). Ovigerous female, Anapo River, mm 6.9: (d) pleopod II.

Pereopod IV sexually dimorphic (Figs 2c–d). Armature of the carpus in the male as in Fig. 2d; dactylus with 5 short spines and a subungueal seta.

Dactylus of pereopods V–VII shorter than in *Proasellus banyulensis* and *P. wolfi*. Dactylus of pereopod VII (Figs 2e–g) armed with 6 posterior spines in the male, and with 2 stout spines in the female; a subungueal seta is always present.

Pleopod I of the male as in Fig. 3a. Basis armed with one retinaculum, distal margin with 3–5 setae. Exopod approximately 2 times as long as wide, with no rows of ventral spines. One spine is present on inner distal margin, and another one on inner proximal angle. Outer setae of the holotype as in Fig. 3a.

Pleopod II of the male (Fig. 3b) with subquadrangular basis. Proximal segment of exopod with one outer seta, typical of *Proasellus coxalis*-group; distal article rounded, with 6 long plumose distal setae in the holotype, but their number is variable. Endopod approximately as long as the exopod (exp/enp ratio = 1.07); distal apophysis short. Distal part of the endopod ('goulot' sensu Racovitza, 1919) (Figs 3c, d drawn from S.E.M. photographs), wider than in *Proasellus wolfi* and *Proasellus banyulensis* (see figures in Stoch, 1989), closely resembles that of the North African species (*Proasellus coxalis peyerimhoffi* and related tax(a).

Pleopod II of the female as in Fig. 3e; the mean number of long plumose setae is 12; the inner margin is armed with a short proximal spine.

Pleopod III exopod (Fig. 3f) with a row of 3–4 very stout spines on inner edge, typical of this species.

Pleopod IV exopod (Fig. 3g) approximately 1.4 times as long as wide, with 2–3 setae on the outer proximal edge. Area very large; linea areae parallel to the inner margin, or slightly oblique; the other two lines (linea transversalis and linea conjungens, 'false sutures' of the American authors) are hard to detect, and are marked only close to the outer margin. The respiratory surface area is slightly smaller in the populations of the province of Enna.

Pleopod V exopod (Fig. 3h) approximately 1.8 times as long as wide, with three outer proximal setae; distal margin somewhat sharp, differing from that of *Proasellus coxalis*. Linea articularis poorly marked. The shape of linea duplex is very characteristic, and can be considered diagnostic for this species; it runs obliquely from an incision of the inner margin – very close to that of the linea articularis – to an incision of the outer margin, slightly proximal to one fifth length of the margin. The ratio y1/y2 (y1 = outer distance between linea duplex and linea articularis; y2 = inner one) is very high (approximately 10 or more).

Uropods (Fig. 2h) about 93% of the pleotelson length in the male, 83% in the female. Exopod and endopod subequal in length.

Remarks The morphological characters of this species, and first of all the structure of the male second pleopod, indicate that *Proasellus montalentii* lies with the *Proasellus coxalis*-group. As already pointed out in a previous paper (Stoch, 1989), the discriminant characters of the closely related species included in this group are scarce, and are based primarily on the shape of linea duplex on pleopod V exopod. *Proasellus montalentii* can be readily separated from the other species of the group by the linea duplex, which is very oblique, and the shape of the area of pleopod V exopod. Other useful taxonomic characters are: (1) exopod and endopod of male pleopod II subequal in length (character shared by *Proasellus wolfi*); (2) 'goulot' wide, distal apophysis short; (3) area of pleopod IV small; (4) low number of spines (seven) on the dactylus of male pereopod I; (5) shorter dactyls of pereopods 5–7. These characters are rather constant in the specimens examined, except for the area of pleopod IV which is slightly variable between the populations inhabiting different hydrographic basins, although constant within the same population.

The complete reproductive isolation of this species from *Proasellus banyulensis italicus*, as well as from *Proasellus wolfi* and *Proasellus banyulensis polychaetus* from southern Italy has been demonstrated by laboratory interbreeding experiments (see detailed analysis in Volpi *et al.*, 1989). Other data were obtained using electrophoretic techniques (Pomponi, 1984). The mean genetic distance (Nei's formul(a) calculated between *P. montalentii* and the other two Sicilian species is very high (lying within the range 0.93–1.06), while the distance from the North African populations is slightly smaller (Pomponi, 1984).

Proasellus montalentii is widespread in Sicily, and has been discovered in streams, sources and ponds in the provinces of Palermo and Enna; it seems to be absent from the Iblean region where *P. wolfi* occurs.

Proasellus wolfi (Dudich, 1925)

Asellus banyulensis wolfi, Dudich, 1925a: 239; *Asellus coxalis cyanophilus*, Dudich, 1925a: 296; *Asellus (Proasellus) coxalis wolfi*, Arcangeli, 1942: 186; *Proasellus wolfi*, Stoch, 1989: 66.

Material Source of River Ciane (labelled Kyane folyò) near Siracusa (Province of Siracus(a), leg. E. Wolf: 23.IV.1925, 25 specimens (type material, deposited in the Zoological Department of the Hungarian Museum of Natural History, Budapest, No 1632); ibidem, leg. F. Valentino: 6.IV.1987, 1 ♂, 1 ♀; 7.I.1988, several specimens (coll. F.S.); Anapo River (Province of Siracus(a), collector unknown: : V.1889 (deposited in the Collection Dollfus, Laboratory of Zoology, Arthropodes, National Museum of Natural History, Paris, No Is. 2757); ibidem, leg. F. Valentino: 6.IV.1987, several specimens (coll. F.S.); Source in Cava Candelaro, Rosolini, east from the road toward Noto (Province of Siracus(a), leg. R. Gerecke: 30.IX.1985, 1 ♂.

Loc. typ. Source of Ciane River, province of Siracusa, Sicily, Italy.

Type material Lectotype, one male from the collection Dudich, preserved in alcohol 75%, pleopods partially dissected and mounted in Faure medium on a microscopic slide; paralectotypes, 24 specimens. Material deposited in the Hungarian Museum of Natural History in Budapest.

Description The following description supplements that of Dudich (1925(a).

Eyes normally developed, pigment brownish. Body length of the lectotype 6.6 mm; maximal length of the males 8.4 mm, of the females 6.9 mm. Length/wide ratio of the lectotype 3.56 (maximal width at the level of the third pereionite); the non ovigerous females have a mean length/width ratio of 4.06, the ovigerous females of 2.7.

Sensorial setae *sensu* Racovitza (1919) absent. Coxal plates visible dorsally on pereonites I–VI.

Pleotelson approximately as long as wide; the ratio length/width varies with the total length of the body: the lectotype has a ratio of 1.07, one male of 8.4 mm has a ratio of 0.95. In the females the ratio is lower (approximately 0.88). The caudal lobe is pronounced.

Antennula (Fig. 4(a) with flagellum composed of 12 articles; the last three segments bear aesthetascs. Number of articles of the antennal flagellum variable; the lectotype carries 32 articles in left flagellum, 47 in the right one; a female of 7.0 mm has 34 articles in the right flagellum.

Mandibles, maxillae and maxillipeds typical of *Proasellus coxalis*-group (Fig. 4b–e). Maxillipeds (Fig. 4e) with 4–5 retinacula; the oostegite of the maxilliped in ovigerous female bears 13–15 setae (Fig. 4f).

Pereopod I of the male (Fig. 5(a) with propodus about 1.7 times as long as wide, carrying four stout spines; dactylus slender, reaching the margin of the carpus, with 13–14 spines, accompanied by 1–4 short setae and a longer subungueal seta.

Pereopod I propodus in the female (Fig. 5b) more slender (approximately 2 times as long as wide); dactylus with 6–8 spines.

Pereopod IV sexually dimorphic (Figs 5c–d). Carpus armed with several long spines; armature simpler than in *Proasellus montalentii*. Dactylus with 5 spines and a long subungueal seta.

Pereopod VII as in Fig. 5e. Dactylus slender than in *Proasellus montalentii*, armed with 6 spines in the male (Fig. 5f), 3 stout spines in the female (Fig. 5g).

Basis of pleopod I in the male (Fig. 6a, (c) armed with 1 retinaculum; distal margin carrying 3–7 setae. Exopod approximately 2 times longer than wide, with a variable number of plumose distal setae (12 or more); ventral spines lacking, except those cited for *Proasellus montalentii*.

Pleopod II of the male (Figs 6b, f) with subquadrangular basis, bearing 3–4 setae; exopod and endopod of approximately equal length, as in *Proasellus montalentii* (ratio exp/enp 1.04–1.09). Distal article of exopod rounded, with 9–10 plumose setae; tergal apophysis of endopod short. The lips of the distal slit of 'goulot' are not so close together as in *Proasellus coxalis*, and do not form the rounded opening of *Proasellus montalentii*, but the distal part of the pipe ('fente sternale' *sensu* Racovitza, 1919) is wide and open (Fig. 6e). This shape is close to that of *Proasellus coxalis peyerimhoffi*.

Pleopod II of the female (Fig. 6d) with 10–12 plumose setae and two short proximal spines in the inner margin.

Pleopod III exopod (Fig. 6g) with inner range of spines less stout than in *Proasellus montalentii*.

Pleopod IV exopod (Fig. 6h) 1.55–1.63 times as long as wide, with elongated area, very similar to that of *Proasellus coxalis peyerimhoffi*; linea transversalis very well defined, linea conjungens clearly visible near the outer margin. Proximal part of outer margin with 4–5 setae.

Pleopod V exopod (Fig. 6i) 1.7–1.9 times as long as wide, bearing 4 outer setae. Distal margin rounded. The shape of linea duplex resembles that of *Proasellus coxalis peyerimhoffi*, with the two parameters y1 and y2 approximately equal in length (their ratio is 1.15 in the lectotype, approximately 0.8 in the material from

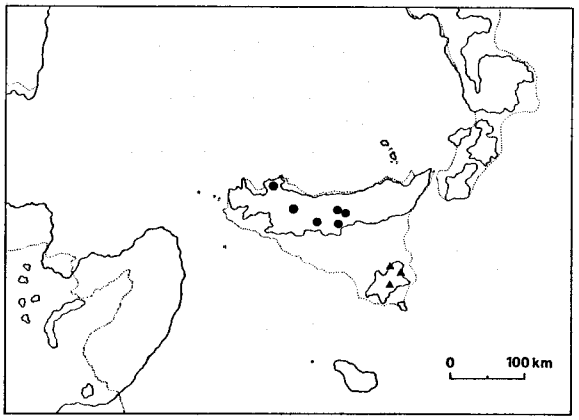

Fig. 7. Sampling stations of *Proasellus montalentii* (circles) and *Proasellus wolfi* (triangles) in Sicily superimposed on the distribution of landmasses and seas during the lower Pliocene (from AA.VV., 1957). Dashed line indicates the actual coast line.

Anapo). The distance between the incisions formed by linea duplex and linea articularis are higher than in *Proasellus coxalis peyerimhoffi.*

Uropods as long as 90% of the pleotelson (Fig. 4g), with endopod and exopod subequal in length; spines on the basis long and stout.

Remarks *Proasellus wolfi* was described by Dudich (1925(a) as a subspecies of *Proasellus banyulensis.* The detailed morphological analysis carried out in the present paper demonstrates that this taxon has to be considered a good species, clearly distinguished from *Proasellus montalentii* and *P. banyulensis* by the conformation of 'goulot' and the shape of linea duplex of pleopod V. The degree of variability of these characters in the specimens examined is negligible. The morphological differentiation of the three species has been confirmed using laboratory crossings (Volpi *et al.*, 1989) and electrophoretic techniques (Pomponi, 1984).

Proasellus wolfi is closely related to *Proasellus coxalis peyerimhoffi* by the shape of pleopods IV and V; the two taxa differ by the shape of linea duplex of pleopod V and the minute characters pointed out by Racovitza (1919) and Dudich (1925(a). The high genetic distance from the North African *Proasellus coxalis peyerimhoffi* (Pomponi, 1984) suggests that *Proasellus wolfi* has to be considered an independent species, and not a subspecies of *Proasellus coxalis*, even if the discriminating characters for these two taxa are scarce.

Asellus coxalis cyanophylus was described by Dudich (1925(a) from the Ciane River source; unfortunately the slide containing the first pair of pleopods of a male (all the existing material) was not found in the Museum of Budapest (Stoch, 1989), and has to be considered lost (Forrò, pers. comm.). The only known specimen was collected by E. Wolf intermixed with *Proasellus wolfi* in the source of Ciane River; the distinctive characters pointed out by Dudich (1925(a) are the shape and number of setae of the first pair of pleopods, a highly variable character, insufficient for a diagnosis. These features fall within the range of variability of those of *Proasellus wolfi*; so we agree with Arcangeli (1942) in considering this taxon as synonymous with *Proasellus wolfi.*

The range of *Proasellus wolfi* in Sicily seems to be restricted to the Iblean area (province of Siracus(a).

Bibliographic notes

The relationships of *Proasellus montalentii* and *Proasellus wolfi* with the North African taxa pointed out in the descriptions above, and the high degree of morphological differentiation from the European *Proasellus banyulensis* in the shape of linea duplex of pleopod V, pose some problems regarding the origin of the Sicilian species from their North African ancestors. The most ancient colonizer seems to be *Proasellus montalentii*, well differentiated from the North African species, while *Proasellus wolfi*, closely related to *Proasellus coxalis peyerimhoffi*, probably invaded the Iblean region more recently. The presence of some populations of the widespread *Proasellus banyulensis* in Sicily, which are morphologically almost identical to the peninsular ones, must be considered of a more recent origin.

This hypothesis of multiple colonizations of the Sicilian freshwaters has a paleogeographical basis. Indeed Ruggieri (1973), in his synthesis, pointed out that the history of Sicily begins with the Middle Pliocene, when a violent orogenetic crisis raised this island above sea level, and therefore the origin of its freshwater fauna cannot be explained by the presence of miocenic taxa. In this author's opinion, the colonization of Sicily was carried out first of all by North African taxa, mainly during Pleistocene connections. If we check the distribution of the localities where *Proasellus montalentii* and *Proasellus wolfi* were collected, superimposed on the distribution of landmass and sea during the lower Pliocene (Fig. 7),

we find that the Iblean region (where *Proasellus wolfi* occurs) was separated from the areal of *Proasellus montalentii* as a distinct island. Since the morphological data indicate that these species originated from North African ancestors independently of each other, we exclude their derivation from a common ancestor widespread in Sicily. We suggest to explain this situation with the hypothesis of successive colonizations of the two islands during late Pliocene, postulating the existence of connections between these islands and northern Africa.

Nevertheless, as already pointed out, the presence of *Proasellus banyulensis* in Sicily (as well as in Northern Africa, Sardinia, Madeira and other islands: unpublished dat(a) must have a more recent origin; even though for Sicily a Pleistocenic colonization could be contemplated (Calabria-Sicily connection during Calabrian-Sicilian: Ruggieri, 1973), we cannot exclude the role of passive transport (Herhaus, 1977), which is surely the best interpretation of the presence of this species in Sardinia and Madeira, and could also explain the small range it occupies in Sicily.

Acknowledgements

The authors are indebted to the following colleagues and institutions for providing material or helpful information: Prof. G. Nascetti, Department of Biology, University of Viterbo, Italy; Prof. R. Argano, Dipartimento di Biologia Animale e dell'Uomo, Università degli Studi 'La Sapienza', Roma, Italy; Dr L. Forrò, Hungarian Natural History Museum, Zoological Department, Budapest, Hungary; Dr O. Afonso, Instituto de Zoologia 'Dr Augusto Nobre', Faculdade de Ciências, Universidade do Porto, Portugal. Many thanks are due to Prof. D. Caruso (University of Catani(a) and Prof. G. Ortolani (University of Palermo) for the facilities provided in the field work in Sicily, and to Dr R. Gerecke (Tubingen, Germany) who kindly put at our disposal the material he collected in Sicily in 1985. We are indebted to Prof. B. Sket (Ljubljana, Slovenij(a) and an anonymous reveiwer for the useful suggestions which allowed substantial improvements to the manuscript.

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