

Two new *Proasellus* (Crustacea, Isopoda, Asellidae) species from Sardinia: evidences of an old colonization wave of the island

Roberto ARGANO* & Alessandro CAMPANARO

Dipartimento di Biologia Animale e dell'Uomo, Università di Roma "La Sapienza", Viale dell'Università 32, I-00185 Roma

*Corresponding author e-mail: roberto.argano@uniroma1.it

SUMMARY - Two new *Proasellus* (Crustacea, Isopoda, Asellidae) species from Sardinia: evidences of an old colonization wave of the island - Two new species of *Proasellus* (Isopoda, Asellidae) from Sardinian island are described: *P. ruffoi* n. sp., from surface freshwater, and *P. ezzu* n. sp., stygobian. Up to now, the Sardinian species of *Proasellus* were thought to belong to the *coxalis* group and the island populations were relatively recent. The two new species seem to be morphologically similar to some Iberian species. The preliminary available molecular data support the idea of an ancient presence of the two new species in Sardinia.

RIASSUNTO - Due specie nuove di *Proasellus* (Crustacea, Isopoda, Asellidae) in Sardegna: evidenze di un'antica ondata di colonizzazione dell'isola - Vengono descritte due nuove specie di Isopodi Asellidae del genere *Proasellus* in Sardegna: *P. ruffoi* n. sp., di acque superficiali, e *P. ezzu* n. sp., stigobia. Fino ad ora si riteneva che le specie di *Proasellus* di Sardegna appartenessero al gruppo *coxalis* e che il popolamento dell'isola da parte di questa famiglia fosse di origine relativamente recente. Le due nuove specie costituiscono invece un indizio importante di un'ondata di popolamento molto antico: mostrano affinità con forme iberiche e risultano far parte, in base a dati molecolari preliminari, di un clade arcaico (Miocene).

Key words: *Proasellus*, biogeography, Sardinia

Parole chiave: *Proasellus*, biogeografia, Sardegna

1. INTRODUCTION

Sardinia has traditionally been considered as an ideal place to perform faunistic and biogeographical studies, because of its environmental heterogeneity, its central position in the western Mediterranean area and its peculiar palaeogeographic-palaeoecological history (Cassola 1983).

Crustacean isopods are highly stenoecious and have poor dispersal ability, therefore they are excellent ecological and biogeographical descriptors and useful models for these kind of studies.

This research focuses on Asellidae, a family of surface and subterranean freshwater isopods. Current knowledge on the presence of this family in Sardinia is limited to two species recorded in literature: one species is from surface freshwaters, the other is endemic to a cave in the north-east of the island, and was described on only one female specimen: *Proasellus patrizi* Arcangeli, 1952.

Traditionally, the species considered most common in Sardinia surface freshwater was *Proasellus coxalis*

(Dollfus, 1892). Arcangeli (1942) indicated also a non identifiable subspecies: *P. c. sardous*.

More detailed morphological analyses (Stoch 1989; Moroli 1992) demonstrated that the populations of *Proasellus* occurring in the Italian-Sardinian surface freshwaters are not *P. coxalis*, as usually believed. Stoch (1989) excluded *P. coxalis* from the European fauna and suggested that the species living in Italy is *P. banyulensis* (Racovitza, 1919).

Ketmaier *et al.* (2001) used allozyme markers to compare Italian and Sardinian populations of *P. coxalis* with one population from Jordan, close to the topotypical populations of *P. coxalis* (Dollfus, 1892). The results also confirmed that *P. coxalis* is absent from Italy. The same study and other researches developed using molecular markers (in progress), indicate that Italian and Sardinian *Proasellus* populations do not belong to a single species but to a complex of species, morphologically very similar (see also Stoch *et al.* 1996). Given the lack of reliable diagnostic characters, we prefer to be conservative and refer the sardinian populations to a *P. coxalis*-group rather than to *P. banyulensis*.

Several not yet described subterranean populations of the *coxalis*-group are known for Sardinia (Manicastro *et al.* 1983; Ketmaier *et al.* 2001), besides several surface populations. A preliminary analysis indicated a low morphological differentiation among them. According to the available data, we suggest (Argano *et al.* 1997) for the *coxalis*-group a biogeographical model of colonization of the island based on a relatively recent immigration of taxa from the continent.

We also interpret those subterranean taxa strictly related to the *coxalis*-group, as a relatively recent adaptive answer to the instability of the surface hydrogeological system, and their differentiation as related to genetic events, as e.g. the founder effect.

Moreover, we describe herein two new species: morphological features and molecular evidences (a study on CO-1 sequences is in progress) support their status as distinct species.

Proasellus ruffoi n. sp. is a surface species, which was already reported by Manicastro *et al.* 1983 and by Ketmaier *et al.* 2001 (as *Proasellus* sp.), but the relevant material was subsequently lost. The present description is thus based on newly collected samples. This species lives in the mountain system North of Bosa (north-western Sardinia).

Proasellus ezzu n. sp. is a specialized subterranean species recently discovered in the same area.

2. TAXONOMIC ACCOUNTS

Proasellus ruffoi n. sp.

Material

Villanova Monteleone (SS), a small ephemeral spring in the archaeological area of "domus de janas" of Pottu Codinu. 22.1.1997, Fulvo leg.: 9 females (10.16-14.5 mm), 1 male (6.5 mm); 4.5.1997, Argano leg.: 1 female (6.83 mm), 5 males (5.83-7.5 mm); 25.3.2004, Argano & Campanaro leg.: 22 females (3.5-6.08 mm), 12 males (4.16-5.5 mm).

Villanova Monteleone (SS), spring (40° 30,01 2 N, 008° 28,74 2 E). 30.5.2002, Argano & Campanaro leg.: 2 females (4.16-4.75 mm), 5 males (3.6-7.25 mm).

Description (Fig. 1)

Epigeic species, with developed eyes and brownish in colour.

Flagellum of the antennula of 7 articles with aesthetascs on the last three articles, flagellum of the antenna of about 40 articles. Palmar margin of the propod of the first pereopod in both sexes massive, twice as long as wide, armed with two strong spines and a row of setae, one of which very long. Basis of the first male pleopod subquadrangular and armed with one retinaculum, distal article almost twice as long as

the basis with a series of about 15 short setae of approximately equal length on external margin. Exopod of the second pleopod of the male with one seta on the proximal segment, distal article approximately three times as long as the proximal one, rounded and with about seven long plumose distal setae. Endopod tapered, narrowing in the distal portion with a very short goulot. Exopod of the third pleopod with 3 spines on inner edge. Exopod of the pleopod IV with area long and narrow suboval in shape. Linea transversalis and linea conjungens not visible. Exopod of the pleopod V with two spines in the external margin. Linea duplex transversal, distal margin quite narrowing. Exopod and endopod of uropods subequal, 1.5 times as long as the propodus. Second female pleopod long and narrow, twice as long as large, with subparallel margins. Terminal portion of the appendage with rounded external margin armed with 7 long plumose setae.

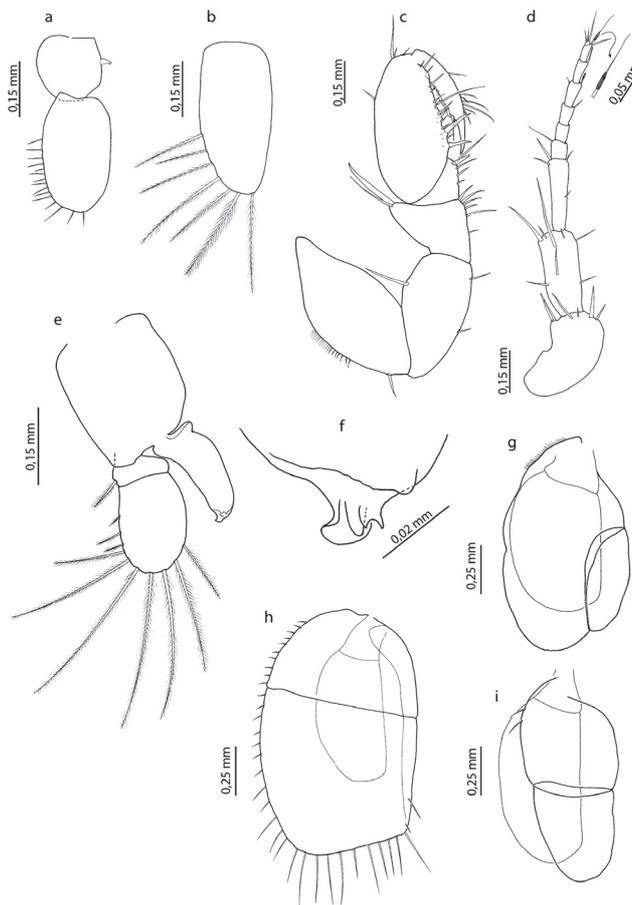


Fig. 1 - *Proasellus ruffoi* n. sp.: a. pleopode I del maschio; b. pleopode II della femmina; c. pereiopode I; d. antennula; e. pleopode II del maschio; f. endopode del pleopode II del maschio, particolare; g. pleopode IV; h. pleopode III; i. pleopode V.

Fig. 1 - *Proasellus ruffoi* n. sp.: a. pleopode I del maschio; b. pleopode II della femmina; c. pereiopode I; d. antennula; e. pleopode II del maschio; f. endopode del pleopode II del maschio, particolare; g. pleopode IV; h. pleopode III; i. pleopode V.

The general structure of the male pleopod II of *Proasellus ruffoi* n. sp. is remarkably similar to the same appendage of some species from the Iberian peninsula, as *P. chappuisi* Henry e Magniez 1968, stygobious, from Cueva del Manantial de Gorbia, Alava province, and *P. ibericus* Braga, 1946 (Pacos de Ferriera, Porto, Portugal), epigean.

Derivatio nominis

The new species is dedicated to Prof. Sandro Ruffo, in the occasion of his 90th birthday, hoping to benefit of his teaching and enjoy his friendship for a long time to come.

Proasellus ezzu n. sp.

Material

Sennariolo (OR), Fontana "Ezza" (an old little building collecting spring water), 40° 12.792 2 N, 008° 33.54 2 E. 2/7/2003, Argano leg.: 4 females (3.34-4.27 mm in lenght), 3 males (2.56-5.3 mm); 25/3/2004, Argano & Campanaro leg.: 1 male.

Description (Fig. 2)

Stygobian, blind and depigmented. Flagellum of the antennula of 4 articles with aesthetascs on the last two articles, antenna almost as long as the entire body with a flagellum of about 40-50 articles. Palmar margin of the propod of the first pereopod of both sexes slender, almost glabrous, with only one strong spine and four minor setae. Basis of the first pleopod male armed with one retinaculum, distal article twice as long as the basis. Proximal segment of exopod the pleopod II male with one outer seta, distal article approximately four times as long as the proximal one and with six plumose distal setae. Endopod of the same appendage massive, twice as long as large, with a little goulot and the distal apophysis right angled. Second pleopod female subtriangular with 4-5 long plumose setae on the external margin. Exopod of the third pleopod with 1-2 spines on inner edge. Area of the fourth pleopod very small, suboval in shape, placed in the middle of the inner margin.

Exopod of pleopode V absent. Uropods approximately as long as the pleotelson, with subequal rami.

The massive structure of the endopod of the second male pleopod of the new species seems to be very similar to the same appendage of Iberian hyporheic species *P. ebrensis* Henry & Magniez 1992, from Ebro Valley (Burgos province), partially adapted to the subterranean aquatic environment. The shape of the area of the fourth pleopod and the absence of the exopod of the pleopod V in *Proasellus ezzu* n. sp. are distinctive characters of the new species.

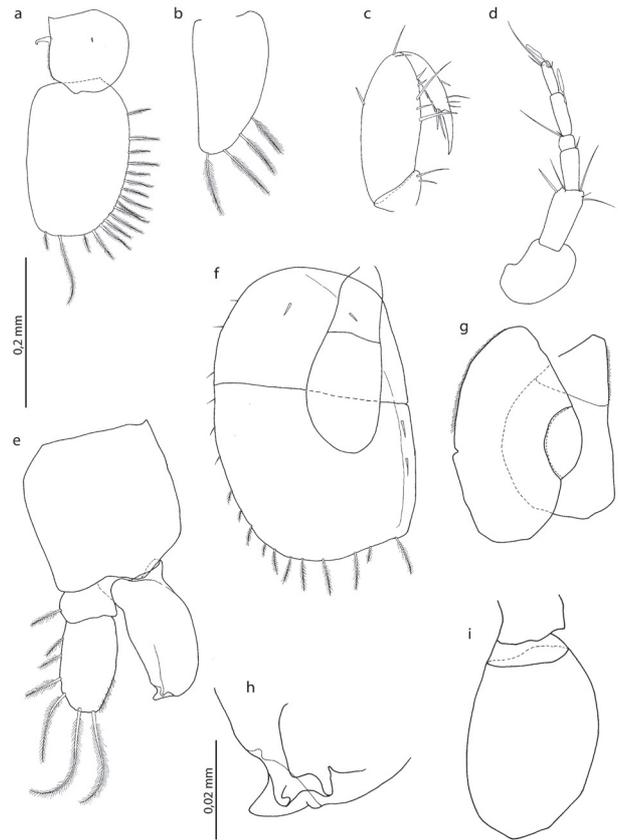


Fig. 2 - *Proasellus ezzu* n. sp.: a. male pleopod I; b. female pleopod II; c. propod of the pereopod I; d. antennula; e. pleopod male II; f. pleopod III; g. pleopod IV; h. endopod of the pleopod II male, detail; i. pleopod V.

Fig. 2 - *Proasellus ezzu* n. sp.: a. pleopode I del maschio; b. pleopode II della femmina; c. propode del pereopode I; d. antennula; e. pleopode II del maschio; f. pleopode III; g. pleopode IV; h. endopode del pleopode II del maschio, particolare; i. pleopode V.

Derivatio nominis

The specific epithet "ezzu" is the name of the toponymic locality and, in Sardinian language, means "old, ancient", a name that well fits the new species, witness of ancient geological events.

3. CONCLUSIVE REMARKS

The phylogenetic systematic of the family Asellidae, and the consequent biogeographical considerations, are based mainly on the morphology of the copulatory pleopods. These appendages are considered good markers of reproductive isolation among species, as well as significant phylogenetic characters. The "strength" of the following comprehensive scenario is at the moment being tested using molecular analysis.

From a morphological point of view, the similarity between the two new Sardinian species and some

species living in north-western Spain suggests a relationship dating before the separation of the Sardinia-Corsica microplate from the Pyrenean region (Boccaletti *et al.* 1990; Alvarez 1972). Therefore, the two new species should belong to a previous colonization wave than the more recent colonization by the other species of the *coxalis*-group.

At present we have a study in progress on the molecular phylogeny (based on sequences from the mitochondrial gene for CO-I) of surface and subterranean populations of *Proasellus* from continental Italy and Sardinia. Preliminary results (Campanaro *et al.* 2004) indicate that some surface (e.g., Villanova Monteleone) and subterranean populations (e.g., Sennariolo), belong to a clade with an ancient divergence from the *P. coxalis*-complex. A preliminary dating by a molecular clock derived from *Stenasellus* (Ketmaier *et al.* 2003: the Stenasellidae are closely related to the Asellidae) indicates a separation between the clades in the early Miocene (about 28 Myr). In that time period great geological movements affected the Mediterranean land masses (e.g. the separation of Sardinia-Corsica microplate from Pyrenean region) starting those modifications that produced the present geography. Although direct comparison with the Iberian populations (that seem the most closely related) are still needed, these preliminary results support the idea of an ancient presence of the two new species in Sardinia.

REFERENCES

- Alvarez W., 1972 - Rotation of the Corsica-Sardinia microplate. *Nature*, 235: 103-105.
- Arcangeli A., 1942 - Il genere *Asellus* in Italia con speciale riguardo alla diffusione del genere *Proasellus*. *Boll. Mus. Zool. Anat. Comp. R. Univ. Torino*: 44 pp.
- Argano R., Messana G., De Matthaëis E., Cobolli M. & Ketmaier V., 1997 - Uso di marcatori biochimici per lo studio del popolamento stigobio di Sardegna: gli Asellota (Crustacea Isopoda). *Biogeographia*, 19: 157-171.
- Boccaletti M., Ciaranfi N., Casentino D. *et al.* 1990 - Palinspastic restoration and palaeogeographic reconstruction of the peri-Tyrrhenian area during the Neogene. *Palaeogeography, Palaeoclimatology, Palaeoecology*, 77: 41-50.
- Campanaro A., Ketmaier V. & Argano R., 2004 - COI data indicate a complex evolutionary history for Italian Subterranean and surface populations of *Proasellus* (Crustacea, Isopoda). SWSB Symposium on World Subterranean Biodiversity, Villeurbanne. *Book of Abstracts*: 24.
- Cassola F., 1983 - L'esplorazione naturalistica della Sardegna. *Lav. Soc. It. Biogeogr.*, n.s., 8: 5-34.
- Ketmaier V., Argano R., Cobolli M., De Matthaëis E. & Messana G., 2001 - A systematic and biogeographical study of hypogean and epigean populations of the *Proasellus coxalis* group from Sardinia, Central Italy and Jordan: allozyme insights. *J. Zool. Syst. Evol. Research*, 38: 1-9.
- Ketmaier V., Argano R. & Caccone G., 2003 - Phylogeography and molecular rates of subterranean aquatic Stenasellid Isopod within a peri-Tyrrhenian distribution. *Mol. Ecol.*, 12: 547-555.
- Manicasteri C., Baldari F. & Argano R., 1983 - Aspetti biogeografici del popolamento isopodologico della Sardegna. *Lav. Soc. It. Biogeogr.*, n.s., VIII (1980): 251 - 269.
- Moroli M., 1992 - Uno studio morfometrico multivariato della variazione in *Proasellus coxalis* (Dollfus, 1892) (Crustacea Isopoda Asellota). Tesi di Dottorato di Ricerca in Biologia Evoluzionistica, IV Ciclo. Università di Roma "La Sapienza": 89 pp.
- Stoch F., 1989 - Critical remarks on the southern Italian asellids described by E. Dudich (1925) with redescription some taxa (Crustacea, Isopoda, Asellota). *Miscellanea Zool. Hung.*, 5: 61-69.
- Stoch F., Valentini F. & Volpi E., 1996 - Taxonomic and biogeographic analysis of the *Proasellus coxalis*-group (Crustacea, Isopoda, Asellidae) in Sicily, with description of *Proasellus montalentii* n. sp. *Hydrobiologia*, 317: 247-258.