Contributions to Animal Biology
Halocynthia Association©, 1994

Studies on italian groundwater crustacean biodiversity

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ABSTRACT - Groundwater crustacean biodiversity in Italy is reviewed, with particular regard to the results of our studies on Italian stygobiotic fauna of Isopods and Copepods.

KEY WORDS: *Ground waters; Crustaceans; Biodiversity*

Surface waters (rivers, lakes, marshes) account for only 3 percent of the fresh water present on land. The remaining 97 percent makes up the groundwater system (phreatic, interstitial, hyporheic): a vast environment hosting a variety of fauna, some of which is highly specialized. The branch of Biology dedicated to the study of groundwater organisms is known as Stygobiology, a neologism that is still surprising to most, including hydrobiologists who have devoted relatively little attention to this environment, despite its remarkable interest, e.g., as drink water reserve.

The present biological knowledge, as compared to the great extent of the problems, is relatively poor, and partially limited to a first, main exploratory approach. Nevertheless, there are important researches regarding structural and functional studies on groundwater ecosystems, but the increasing discoveries of taxa indicate how much is to do in order to realize a comprehensive picture of the general groundwater biodiversity.

As occurs in subterranean terrestrial systems, groundwater fauna is made up of species that have adapted in various ways. Three different ecological levels of specialization can be distinguished: stygoxenes are surface organisms linked to benthic aquatic environments that are only occasionally found under the bottom substratum; stygophiles are benthic forms with low levels of adaptation to the groundwater environments whose stygocoenosis they regularly become a part of (sometimes in relation to developmental stages); finally, stygobies live their entire life cycle in groundwater systems, and therefore present the classic morphological (e.g. no eyes or pigment) and biological (e.g. K strategy) adaptations.

Our attention has been directed mainly at the last level, during years of research carried out in Italy and other geographic areas. The aim of this paper is to provide a summary of the present knowledge of Italian stygobitic fauna in the zoological groups we are specialized in, with particular reference to the results of our studies and the questions these have raised and have introduced into current research projects.

The biological importance of the stygobitic organisms is related to the complexity of their evolution, and therefore to the manner and origin of the conquest of the groundwater environment of each species, as well as to their following evolutionary vicissitudes.

Stygobites have evolved both from marine organisms, which adapted secondarily to groundwater systems, and from surface freshwater organisms. Whatever their origin, it is important to note that once definitively established in ground waters,

stygobies have had their own evolution often spanning millions of years linked exclusively to these environments. Thus the present state of these organisms (level of specialization, role in the various stygobitic biocoenoses, geographic distribution) is the result of two steps which must be analyzed separately: the first is colonization of the various groundwater environments; the second is successive specialization and spread.

As regards the former, that is the origin of the primitive colonization, different models have been proposed: the "refugium model" refers to drastic alterations in surface environments resulting in profound changes in the fauna of a certain region. Only the so called "pre-adapted" forms (species, populations, demes) managed to survive by adapting to groundwater environments as a refuge. The "active colonization model", on the other hand, hypothesizes that the groundwater environment was conquered by surface fresh water species which took advantage of their special adaptive attitude, probably linked to a primitive ecological plasticity. A "two-step model" has been recently proposed by Boutin & Coineau (1990) for explaining the interstitial colonization. This model hypothesizes a first vertical, active transition from marine coastal waters; a subsequent passive transition is suggested into the interstitial environments.

Our studies have focused on certain groups of crustaceans, in particular Isopods and Copepods.

In Italian groundwater systems, isopods are represented by the suborders Asellota and Flabellifera. The former includes a large number of marine families; there are also families partially or completely adapted to both surface and subterranean fresh waters. Stenasellidae is a particularly interesting Asellota family present in Italian ground waters.

The first specimen of stenasellids was brought to light in rather bad conditions after an adventurous exploration of the Gouffre de Padirac at the end of the last century. It led to the description of the first species of stenasellid, *Stenasellus virei,* which long remained a mysterious object for scientists until later discoveries were made. Today, approximately sixty species subdivided into about ten genera have been identified; all are completely stygobitic (that is, no surface eyed form exist) and have thermophilic tendencies. Distribution ranges from Central America to the Far East, with a large number of species present in Europe and Africa. These characteristics attest to the extremely archaic nature of this group. The evident affinities between American forms, some of which were discovered during our studies, and African forms constitute a good example of the complexity of this group's paleogeographic history. In fact, this distribution and the filogenetic relationships can only be explained by going back to the Turonian events that gave rise to the Atlantic Ocean.

Three species are presently known in Italy. *Stenasellus racovitzai* was discovered in 1925 in the muddy bottom of a lake in the "Grotta del Danese", near Grosseto in Tuscany. This isopod is rather large and a brilliant red coloured, an unusual characteristic for a stygobitic species, but a rather common one for a stenasellid. The colour is produced by internal pigments and is thought to serve the respiratory function. This species is of interest because of its strong affinity with *S. buili* from southern France, and because it has been found not only in Tuscany, but also in hyporheic and phreatic environments in Sardinia (in the bed of the Quirra River) and Corsica.

Explaining the presence of stygobitic and freshwater forms of the same species on the continent and on two different islands is no easy matter, but the explanation must be sought in the history of the species after it colonized the groundwater environment. The electrophoretic investigations currently being carried out on known populations of *S. racovitzai* could confirm the hypothesis advanced by Magniez (1983), which suggests that the presence of the species on the two large islands is the result of Miocenic rotation of the Sardinian-Corsican plate and, thus, their separation from a Provençal ancestor common also to *S. buili.* His theory also explains the presence of the species in Tuscany as a temporary expansion of its distribution area owing to a subsequent marine regression.

We have discovered two other species of the same family in southern Sardinia: *S. nuragicus* and *S. assorgiai.* The two species are extremely similar, the only differences being in some female characteristics. The specimens studied were found in different caves at a time when the cave-island concept was deeply ingrained: this gave even more importance to the differences

found. But for many years now, the cavernous system, and of course its groundwater, has been considered a continuum. Investigations carried out in many countries including Italy have revealed the continuity of subterranean aquatic systems and the fact that they are only accidentally interrupted by caves. This led us to state many years ago (Argano and Cottarelli, 1972), at a time of great biospeleological fervour, that while the curiosity of many researchers was making its way into caves, biospeleology was, in some ways, on its way out. Indeed, in many cases, caves should be considered no more than a moment, albeit an extremely important one, for study of the intricate ecological complexity of subterranean realm.

Returning to the two species of *Stenasellus* found in Sardinia, specimens have been found in various man made wells scattered over the Campidano plain and several areas of the southeast coast. These findings not only attest to the lack of geographic barriers, but they also disclose a morphological variability which could not have been imagined from the differentiated characteristics initially detected on the only two available cave samples.

The study currently being carried out on the above variability, associated with electrophoretic analysis, will probably lead to the fusion of these two species into one and make it possible to determine the genetic distance separating it from the Sardinian *S. racovitzai* population.

The other Asellota family present in Italian groundwater systems is Asellidae. The family is widespread in surface water environments throughout the northern hemisphere. The southernmost species, which we studied many years ago, are found in the regions of Mexico bordering on Guatemala. Most of the known species populate subterranean environments and consequently they have a very restricted if not pinpointed distribution. Most of these species seem to have been forced to leave surface waters by temporary environmental changes such as glaciations or droughts. For many stygobitic forms the starting point for subterranean evolution can be found among surface species existing today, but entire groups of species show no affinity at all to present day oculate forms. This is generally considered as an indication that the second group is more archaic.

An example from Italian stygobitic fauna is the recently discovered genus *Chthonasellus* (Argano and Messana, 1991), known for the single species C. *bodoni*, which lives in the hyporheic environment of the Pesio River near Cuneo. The only species with which C. *bodoni* can be compared is *Gallasellus heilyi*, a french stygobitic endemism. However, the two species show no affinity with any of the many other species of the Palaearctic region (in Europe alone, the family of the Asellidae is made up of approximately one hundred species, both subterranean and surface, subdivided into ten genera). Similar structures can be found only in the genus *Caecidotea*, exclusive to the fresh water systems of North America. This leads us to consider the two genera *Chthonasellus* and *Gallasellus* as living evidence of the ancient continuity between the American and European continents, before the formation of the Atlantic Ocean.

Two other genera of Asellidae are present in Italy: *Asellus* and *Proasellus.* Only one surface species of the former, which is an Eurasian genus, has reached Italy: A. *acquaticus.* The genus *Proasellus*, on the other hand, is typically peri-Mediterranean, with a large number of stygobitic species present in Italy. Some belong to exclusively stygobitic groups of species such as *P. cavaticus* and *P. franciscoloi* of the *"cavaticus"* group found in the Central European region, but probably of Alpine origin.

Another group of species in Italy lacking epigean representatives and therefore boasting a long evolutionary history is the *"pavani"* group. It includes three species: *P. pavani, P. acutianus* and *P. deminutus.* The close affinity between these three species and the others of the group not present in Italy suggests a biological distribution that once embraced Corsica as well as the karsic systems of Slovenia, with remarkably complex paleogeographic implications. The ecology of this species is also interesting: *P. acutianus*, for example, which we discovered during studies of a hyporheic environment, has never been found in other environments.

During our studies, we have come upon other interesting species of subterranean *Proasellus*, but the problem to be solved at the moment concerns the most common and apparently banal species: *P. coxalis.* This surface species plays an important role in the fresh water biotic communities of the entire peri-Mediterranean area. An abundant literature is available, describing many aspects of its biology. But it is of interest

here because some stygobitic species of Italian fauna are considered closely related to and probably derived from it.

Morphometric comparisons we performed between various Italian populations and some topotypical specimens gathered in Israel provided the same results as those of a study by Stoch (1989) carried out on museological material: "*P. coxalis* s. str. is not present in Europe". The *Proasellus* species which populate Italian rivers, lakes and marshes probably belong to *P. banyulensis*, and this fact modify our point of view on the evolutionary history of some species of Italy.

Just one example, we have studied a number of stygobic populations in Sardinia (one of which is known as a self-standing species: *P. patrizii* to be totally revised), whose origins have hypothetically been related to the instability of the surface water systems in insular environments. Periods of dryness, even those of geologically imperceptible length, may have driven many forms populating surface waters below ground, resulting in the survival of the so-called "preadapted" (in the stygobitic sense) forms. These phenomena are evidently more probable on islands that do not have large lotic or lentic systems (we have found and studied an analogous situation on Crete).

In Sardinia, the problem now is to establish whether stygobitic populations derive from the species which currently populate surface water systems, or whether they are the result of subsequent colonizing waves. But any attempt to solve this problem calls for prior clarification of the systematic identity of the surface species being referred to. Until a few years ago, this species was *P. coxalis*, but now? Here too, a long job of morphological revision is required, possibly supported by electrophoretic assay of the specimens being gathered.

Microparasellidae is another family of stygobitic Asellota living in interstitial environments. This family is present in Italy with six species, five of which belonging to *Microcharon*, a pantropical genus present also in Europe and in the Mediterranean basin.

According to Coineau (1992), *M. marinus*, a Mediterranean endemism, became isolated after the Messinian closure of Gibraltar Straits. To the same geological event seem to be correlated the speciation of the apulian freshwater species *M. arganoi*. The speciation of the remaining three freshwater species (*M. nuragicus* and *M. silverii* from Sardinian and *M. angelicae* from central Apennines) is probably more recent (Pliocene) or, in particular for *M. nuragicus*, *is* correlated to the eustatic interglacial cycles.

To the same family belongs the genus *Angeliera*. This genus is present in our interstitial waters, both marine and fresh, with the euryhaline species *A. phreaticola*, widespread in the World.

Microcerberidae, recently ascribed to Asellota, is the last family of this suborder living in ground waters (interstitial) of our country with the genus *Microcerberus*.

Two euryhaline species of this genus are widespread along our sandy coastlines (*M. remanei* and *M. arenicola*). *M. ruffoi* is a fresh water species living in the interstitial substrates of the Adige river basin.

Another suborder of isopods whose representatives can be found in Italian ground waters is that of Flabellifera. Two families, Sphaeromatidae and Cirolanidae, whose stygobitic representatives are sea-originated, are present in Italy. Seven different species of the genus *Monolistra*, widely distributed in the karsic systems ranging from the Eastern Alps to Herzegovina, are known in Italy. Electrophoretic comparison by Sbordoni *et al.* (1980) of two of these species with surface Sphaeromatidae, living both in marine and brackish environments, has set the date of their colonization in fresh groundwater systems around the time of the Mediterranean salinity crisis. This brings us back once again to the hypothesis that the stygobitic evolution was realized according to the refugium model.

The Cirolanidae, the other family of the suborder Flabellifera, also include typically marine isopods and a large number of species which secondarily colonized subterranean environments in different parts of the World. Two species of this family are known in Italy.

Only recently has *Sphaeromides virei* become a part of Italian fauna (in the political sense, as it was already known in certain Slovene localities very close to the Italian border), with its discovery in the Grotta di Comarice, near Gorizia (Argano and Gasparo, 1986). The four species of the genus presently known have a biological distribution extending from southern France to Bulgaria.

The other known species, found several years ago in wells dug close to the sea near Siracusa,

Sicily, belongs to the genus *Typhlocirolana*. Morphological studies carried out at the time on specimens did not allow for their clear distinction from Balearic Island populations. They were thus attributed to the species *T. moraguesi* (Argano, 1979). But electrophoretic comparison of the Sicilian populations and some *T. moraguesi* populations from the island of Majorca clearly points to the former's individuality at the specific level (Caccone *et al.*, 1986). It also confirms the hypothesis of relatively recent colonization of fresh and brackish groundwaters by the species of *Typhlocirolana*.

Although the present knowledge of the copepod fauna of Italy, as a whole, is still largely incomplete, subterranean groups are without doubt much better known than surface ones.

Groundwater copepods of Italy are represented by members of the orders Cyclopoida, Harpacticoida and Calanoida, most of which are stygobitic or stygophilic.

They are widely distributed and most successful in nearly all freshwater underground habitats of the country, including springs, wells, interstitial media, alkaline waters and pools in caves; fresh or brackish coastal waters may also harbour representatives of generally marine genera, such as *Halicyclops* and *Neocyclops* among the cyclopids, and *Ectinosoma, Halectinosoma, Arenopontia, Arenosetella* and *Delamarella* among the harpacticoids.

About half of the 130 or so groundwater copepod species appear to be endemic to Italy, the remaining species are most palaearctic or they show a wider or cosmopolitan distribution.

A large amount of specialized taxa can be found amongst the cyclopids and harpacticoids; on the contrary the calanoid order, which as a rule is very limited in the subterranean realm, is poorly represented in the ground waters of Italy where, at present time, a single species, *Troglodiaptomus sketi*, recently discovered in karsic substrates of Venetia (North Italy), is known.

As regard the cyclopids, the genus *Diacyclops* is the most diversified and widespread both in continental and insular part of the country, with more than 30 subterranean species/subspecies which concentrate in the Apennine and Sardinian provinces *(sensu* Pesce, 1985). For the most part *(languidus-languidoides* group) they are stygobitic species, cave dwelling or adapted to psammal and moss media; the others *(bicuspidatus* group) can be regarded as stygophilic or stygoxen species, since they regularly live, beside ground waters, in a wide range of epigean freshwater bodies, such as lakes, rivers, marsh, as well as brackish coastal waters.

Among the nominate genus a conspicuous number of species/subspecies, such as *D. bicuspidatus lucanus, D. crassicaudis var. cosana, D. crassicaudis lagrecai, D. crassicaudis trinacriae, D. cristinae, D. ichnusae, D. languidoides aprutinus, D. lindae, D. maggii, D. nuragicus, D. paolae, D. paralanguidoides, D. ruffoi* and *D. sardous*, are endemic to Italy.

The remarkable stygobitic genera *Speocyclops* and *Graeteriella* include less species, as well as the show a limited distribution in Italy (Alpine or central Apennine provinces and Sardinia).

The genus *Metacyclops* embraces, as well, noteworthy stygobitic species, such as M. *stammeri, M. gasparoi, M. trisetosus* and *M. subdolus;* the first two are endemic respectively to South Italy (Apulia) and Northeastern Italy (Venetia); *M. trisetosus is* known only for Venetia and Dalmatia; *M. subdolus* shows a wider distribution (Balearic islands, Greece, Italy).

The genus *Acanthocyclops*, which is primarily an epigean genus, includes some stygobitic or stygophilic species, endemic to Italy.

Other genera *(Halicyclops, Neocyclops, Eucyclops, Macrocyclops, Paracyclops, Tropocyclops, Cyclops, Thermocyclops, Mesocyclops)* regularly occur in surface waters and only occasionally they can be found in groundwater networks of Italy.

Concerning harpacticoids, this order is represented in Italy by many (about 80) stygobitic species, mostly endemic.

The family Parastenocarididae is an attractive study case since it is represented almost exclusively by stygobitic species, specialists with respect to the morphology, physiology and ecology.

Parastenocaris, a world widely distributed genus inhabiting the ground waters of all the continents, was supposed to be represented in Italy by seven species, sampled in caves (Campania), rivers and lakes (North Italy).

In recent years, considerable attention has focused on this genus, and up to date the number of species already described or in study is remarkably increased (about 30).

Interestingly enough, since the interstitial habitat, although spatially "continuum", is rather

heterogeneous both in biotic and chemiophysical factors, some species seem to be particularly affected by changes in environmental characteristics, and to be patchy distributed along the groundwater systems.

The distribution of 4 species of *Parastenocaris* in the sandbanks of Vico lake (central Italy) is in agreement with the previous hypothesis: three species are more or less homogeneously distributed along the shore, while *P. veneris* can be found only in ground waters, characterized by peculiar pH, 02, temperature and depth values.

The insular endemic *P. ima* (La Maddalena island, Sardinia) was found only in phreatic waters, 70 m deep; this very demanding and specialist taxon was never sampled, although repeated investigations, in more superficial ground waters.

More recently we have highlighted the remarkable morphological affinities among six species of the nominate genus, five from Italy, and one from Portugal, which inhabit only rivermouth interstitial waters. Our study is presently aimed at elucidating the ecological adaptation patterns of these taxa and their population dynamic; further researches will be addressed to clarify if those species are characterized by adaptive convergence phenomena, or if they are phylogenetically closely related by means of different molecular markers, such as allozyme polymorphism, mtDNA RFLP, and sequence data.

Furthermore, faunistic and ecological extensive researches on interstitial fauna in the Mediterranean and extraeuropean regions are dealing with the biogeography of palaearctic stygobites; some examples are represented by the close affinities among some *Parastenocaris* and syncarids *(Exabathynella sp.)* from Sardinia island and Pyrenees mountains, and the recent sampling in Italy of a new species of *Nitocrella*, a genus with a typical Balkanic distribution.

Different colonization models could be pointed out for the Italian groundwater copepods, and they are related to different geological and palaeogeographical events pending the late Miocene and Quaternary.

Particularly, some genera, such as *Halicyclops, Neocyclops, Metacyclops, Nitocrella* and *Parapseudoleptomesochra* are directly sea-originated, through coastal habitats, according to the "Two-step Model Evolution"; other genera reached the continental ground waters from the sea, but through karsic environments. Other ones, viz. *Speocyclops, Graeteriella, Bryocamptus, Elaphoidella, Moraria* and *Diacyclops*, successfully colonized the continental ground waters from surface fresh water bodies, during different geologic ages, as a result of drastic climatic changes.

Concerning the species of Parastenocarididae, they could derive from superficial freshwater bodies and they should be phylogenetically related to the Cylindropsyllidae.

The genus *Ichnusella*, belonging to the last family, which inhabits only fresh and oligohalin waters in rivermouths, could be related to a common meroplanctonic marine ancestor.

As regard the thalassoid forms, we hereby mention only the genus *Arenopontia* (Cylindropsyllidae), which was supposed to inhabit exclusively marine and brackish interstitial waters.

The recent discovery of *Arenopontia* species in fresh waters can be explained by the active migration model (Coineau & Boutin, 1993), which can also be in agreement with the occurrence, in marine genera as *Ectinosoma, Halectinosoma, Arenosetella* and *Delamarella*, of continental groundwater species.

Among the sea-originated taxa, some species of the genera *Nitocrella, Parapseudoleptomesochra, Schizopera* and *Halicyclops*, could have dispersed actively to previously uncolonized areas due to their high degree of salinity tolerance (Active Migration Model).

The origin of the few species of the genera *Thermocyclops* and *Ceuthonectes is* still doubtful, since they show discontinuous or isolated distribution.

Our continuing researches and collections from rithron, phreatic and hyporheic substrata of central Apennine, Sicily and Sardinia are yielding numerous species of both isopods and copepods, so the species richness and diversity of these groups of crustaceans in Italy could be certainly increased in the future, permitting as well a more adequate characterization of the biology of most Italian taxa.

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This paper is dedicated to prof. H. Manelli on occasion of his 70th birthday

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