Explorations of the systematics and deep history of stygobiont amphipods
Vonk, R.

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Mediterranean freshwater ingolfiellids from wells and caves
THE PHREATIC AQUIFER OF THE 'PLANAA DE CASTELLÓN' (SPAIN): FIRST IBERIAN RECORD OF INGOLFIELLID AMPHIPODA

ABSTRACT. The stygobiont Ingolfiella (Tyrrhenidiella) cf. catalanensis Coineau 1963 is described from a shallow well in the Mediterranean coastal plain of Castellón (Spain). The record confirms the presence of a western Mediterranean species group of Ingolfiella, and the geographical homogeneity of the subgenus Tyrrhenidiella Ruffo & Vigna-Taglianti, 1989.

Key words: stygobiont, phreatic groundwater, taxonomy, Ingolfiella.

INTRODUCTION

During ecological investigations of phreatic groundwaters of the northeastern area of the 'Plana de Castellón' (Province of Castellón, Spain) the presence of the amphipod family Ingolfiellidae on the Iberian Peninsula was ascertained for the first time. Backgrounds of the groundwater ecosystem in which the animal has been found are given by Notenboom et al. (in press). Unfortunately only one single specimen was found, making a proper and complete diagnosis of the species impossible. The record is considered worth being reported mainly because of its zoogeographic interest. A description of the material, undoubtedly a specimen of Ingolfiella, is given in order to contribute to the taxonomy of the genus. As far as the poor material enables, affinities within the genus are established.

In the western Mediterranean realm the Ingolfiellidae are re-
presented by three species: *Ingolfiella catalanensis* Coineau, 1963, from interstitial waters of the Tech valley (Southern France: Pyrénées-Orientales); *I. thibaudi* Coineau, 1968, from interstitial fresh waters of alluvia in the Gard and Ardèche valleys (Southern France); and *I. cottarelli* Ruffo & Vigna-Taglianti, 1989, from a freshwater pool in a cave on the island of Tavolara (Italy: northeastern Sardinia). The Spanish record originates from fresh shallow ground water of an alluvial coastal plain at short distance from the sea.

**MATERIAL AND METHODS**

*Material* - The specimen was found in a sample taken in summer (July 20, 1992) from a domestic well near ‘Camino Donacion, 142’, Castellón (station 92-7/4). ZMA coll. nr. Amph. 201.539, specimen dissected and embedded on two slides with cover slips in Faure.

*Methods* - Samples were taken semi-quantitatively by extracting water from the deeper parts of the well with a small centrifugal pump operated by a gasoline motor. The water was filtered through a plankton net of 41 μm mesh. Samples were fixed in the field in 4% formaldehyde to which bengal rose was added for staining animals. In the laboratory macro- and meiofauna organisms were counted and sorted under stereo microscope (magnification 40-100×) and specimens were transferred to 70% ethanol for long-term storage.

**TAXONOMY**

*Ingolfiella (Tyrhenidiella) cf catalanensis* Coineau, 1963

*Description* - Body length of the only, male, specimen 2.1 mm. Ocular lobes absent. Body elongate, segments laterally compressed (Fig. 1).

First antenna (Fig. 2a) with 3 strong peduncle segments, a 3-segmented accessory flagellum and 5-segmented flagellum: aesthetascs on the second, third and fourth segment of the flagellum.

Second antenna (Fig. 2b) with 5-segmented peduncle and 5-segmented flagellum.

Right mandible with three spinules at the base of the lacinia mobilis; left mandible (Fig. 3a) with two spinules at the base of the lacinia mobilis. First maxilla (Fig. 3b) with rounded inner lobe bearing 2 setules; outer lobe with 3 distal denticulate spines, 3 distal setae and 1
submarginal seta; 2-segmented palp with 2 apical setae. Second maxilla
(Fig. 3c) with 3 apical seta on both outer and inner lobe. Maxilliped
(Fig. 3e) with 5-segmented palp bearing few irregularly placed setae;
endite with 1 small apical setule.

Coxal gills (Figs. 2f,g,h) present on pereopods 3-5, their surface
pustulate with an outwardly bulging relief of conspicuous half-circles:
these are probably ectoparasites. First gnathopod (Fig. 2c) with 1 seta
anterodistally on basis; 1 seta anterodistally on ischium; 2 seta antero-
distally on merus; carpus elongate, palm bearing 4 regularly placed setae
(sensory hairs with transparent tip) and 2 longer setae at the palmar
corner on both sides of a spine, also submarginally a broad cuticular
pointed process and a small spine; propodus slender with 2 spinules on
inner margin and a row of fine setules in between, comblike; dactylus
with 3 teeth on inner margin. Second gnathopod (Figs. 2d,e) with 1 seta
anterodistally on basis, ischium and merus; carpus in left and right
gnathopod with the following differences: left with 1 heavy spine on

Fig. 2 - *Ingolfiella (Tyrhenidiella) cf catalanensis*, male 2.1 mm.
palmar corner and, submarginally, another smaller spine, 7 sharp denticles on margin and some setae along this serrate margin. Right without a heavy spine – very probably broken off – and with less pronounced serration of the carpal margin. Propodus with 4 setae; dactylus with 3 spines on inner margin.

Pereopods 3-7 (Figs. 2f-j) slender. Pereopod 5 shorter than P4 and with small protuberances 5 on ischium and carpus, pereopods 6 and 7 with long distal spines on carpus. All dactyli with bifid claws, in P7 the distal inner spine on the dactylus is lacking.

Pleopods (Fig. 3f). Only pleopod 1 present, elongate. This indicates that the animal probably is a male.

First uropod (Fig. 3d) biramous; peduncle with 1 posterodistal seta; exopodite pointed, without setae; endopodite much longer than exopodite, medial surface with 15 long setae, apex with toothed process. Second uropod (Fig. 3h) with peduncle carrying 8 rows of spines and a
sub-basal hookshaped process; rami equal in length, 3 spines on exopodite, 1 on endopodite. Third uropod (Fig. 3g) very small, 2-segmented, basis with two distal setae, apex with 1 long distal seta. Telson (Fig. 3g) with 2 spatulate setae. At the base of each seta two small punctures present in cuticula.

**Locality** - The well (indicated in Notenboom et al., 1995, as number 3) had a diameter of 1 m, a total depth of 6 m, and is located 2.1 km from the sea shore. The well was normally closed and frequently used for low scale water supply. The well water was fresh (chloride content: ca. 90 mg/l) and the physicochemical characteristics indicate, with exception of nitrate (ca. 107 mg/l), little incidence of pollution. The well harboured a relatively rich fauna consisting of Cyclopoida, Harpacticoidea, nauplii larvae, Ostracoda, Syncarida, Isopoda (Microparasellidae), Amphipoda (Ingolfiellidae, Bogidiellidae, Salentinellidae), insect larvae, Acari, Oligochaeta, Nematoda, and Rotifera. The total amount of organisms was 107 per m$^3$ of extracted water.

**Discussion**

Because of the absence of pleopods 2-3 in the male and the elongated, digitiform shape of pleopod 1, the Spanish specimen most likely belongs to the subgenus *Tyrrhenidiella* Ruffo & Vigna-Taglianti, 1989.
The subgenus comprises three species, all in land waters around the western Mediterranean.

Comparison of the Spanish specimen with descriptions of *Tyrrhenidiella* species from the literature is hampered, in particular by the incomplete description of *I. catalanensis*. Moreover, the variability of *Tyrrhenidiella* species within and between populations was not treated in the original descriptions because only very little material was available (max. 8 specimens in *I. cottarellii*). Whereas *I. thibaudi* is described from three localities, the other two species are described from one single locality.

With *I. catalanensis* the Spanish specimen shares some important features: strong dentated palmar margin of gnathopod 2; dactyli of pereiopods 3-7 with an elongated claw, bifid at the tip; uropod 1 exopodite less than half as long as endopodite; and uropod 2 with hammershaped baso-facial spine. The Spanish specimen differs from *I. catalanensis*, as far as can be judged from its description (see Coineau, 1968), by the absence of a differentiated element on the carpus, “allongé terminé en biseau”; uropod 1 endopodite, with numerous long setae, and provided with an apical bifid spine; and peduncle of uropod 2 with 8 rows of setae. The Spanish specimen appears less similar to *I. thibaudi* and *I. cottarellii* mainly because these species have the claw of pereiopods 3-7 simple and no baso facial spine on uropod 2. For the moment we decided to designate the Spanish specimen, with some reservations, to *I. catalanensis*. More material from Spain and from the type locality of *I. catalanensis* is desired in order to study more appropriately sexual differences and character variation. This is necessary for a final judgement of the status of the Spanish *Ingolfiella*.

The discovery along the Mediterranean coast in Spain of an *Ingolfiella* belonging to the subgenus *Tyrrhenidiella* confirms the existence of a western Mediterranean facies of ingolfiellids.

**Acknowledgements**

We like to acknowledge the good correspondence with Professor Ruffo over the years, concerning the systematics of ingolfiellids and other stygobitic Amphipoda, in particular of the Mediterranean region.

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LITERATURE


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INGOLFIELLA BEATRICIS, NEW SPECIES (AMPHIPODA: INGOLFIELLIDAE) FROM SUBTERRANEAN WATERS OF SLOVENIA

Sandro Ruffo and Ronald Vonk

A new species of ingolfiellid Amphipoda, Ingolfiella beatricis, collected from groundwater in a cave near Ljubljana in Slovenia is described. It is the first ingolfiellid, possessing developed “ocular lobes”, found in inland fresh groundwater and is therefore of biogeographic interest. Its morphological characters suggest a marine origin, a supposition that is strengthened by the marine relationships of accompanying fauna.

Although based only on one specimen, we have decided to describe this species because it is the first record of Ingolfiella in the Alpine region and the first ingolfiellid with well-developed “ocular lobes” to be found in underground freshwater.

MATERIALS AND METHODS

The specimen examined was collected in a small stream in the cave of Pajsarjeva Jama, Pajsar, Vrhnika, Slovenia (45°59'51"N, 14°16'15"E). Collection was accomplished by upturning the sand and lime sediment upstream of a handheld net which was to filter out the animals carried downstream by the flowing water. The temperature of the water was 9.9°C and the conductivity microSiemens 240/cm. Accompanying fauna consisted of Oligochaeta (Tubificidae Phalldrilinae gen. sp., Parvildrilidae gen. sp.); Gastropoda (Graziana pupula); Copepoda Harpacticoida (Bryocamptus (Limocamptus) daceus s.l., Bryocamptus (Rheocamptus) baldanicus, Ceuthonecetes serbicis, Elaphoidella cvetkei, Elaphoidella jeanneli, Parastenocaris gertrudae); Copepoda Cyclopoida (Diacyclops belicus); Amphipoda (Gammarius sp., Niphargus cfs. minor, Niphargus stygius, Synurella ambulans), and some unidentified Rotifera and Nematoda.

The specimen was first immersed in glycerin and drawn in toto, then dissected and permanently mounted in Faure’s medium on glass slides. Drawings of the separate appendages were made with a camera lucida attached to a phase contrast compound microscope.

SYSTEMATICS

Family Ingolfiellidae

Ingolfiella beatricis, new species

Material Examined.—Holotype (MVRCr 387), probably female (2 mm) collected 16 May 1998 in Pajsarjeva Jama, Pajsar, Vrhnika, Slovenia. The dissected holotype (slides 4225, 4226) is deposited in the Museum of Natural History, Verona, Italy.
RUFFO AND YONK: A NEW SPECIES OF *INGOLFIELLA*

Fig. 1. *Ingolfiella beatricis* n. sp. (?female), Pajsarjeva Jama (Slovenia). A, habitus; B, mandible; C, D, maxilla 1, 2; E, maxilliped; F, head with antennae 1, 2. (Scales in mm.)
Fig. 2. *Ingolfiella beatricis* n. sp. (?female), Pajsarjeva Jama (Slovenia). A, B, gnathopods 1, 2; C, D, pereiopods 3, 4; E–G, pereiopods 5–7. (Scale, all figures 0.1 mm.)
**Diagnosis.**—Ingolfiellidae, ocular lobes developed. Maxilla 1, outer plate with 5 spines. Oostegites not observed. Gnathopods 1, 2 with smooth palm, dactyli with 4 elongate spiniform processes. Pereiopods 3–7 with dactyli similar, with proximal part ending in an interior acutely pointed spur, claw slender, finely bifid distally. Pleopods 1–3 present, subtrapezoidal, similar. Uropod 1 with outer ramus about ¾ length of inner ramus, uropod 2 peduncle long, with 3 diagonal rows of short distally bifid spines.

**Description.**—Female (?) 2 mm. Body very elongate, all segments laterally compressed (Fig. 1A). Head with weakly protruding rostrum, apically rounded, lateral margin rounded, “ocular lobes” developed, suboval. Pereionite 1 shorter than head, pereionites 2–7 increasing in length, pereionites 5–7 markedly longer than deep; pleonites 1–3 and urosomites 1, 2 subtrapezoidal, urosomite 3 subcylindrical, markedly longer than deep.

Antenna 1: peduncular (Fig. 1F) article 1 longer than articles 2+3; flagellum 4-articulate, shorter than peduncle, articles 2–4 with 1 aesthetasc, accessory flagellum 3-articulate, shorter than flagellar articles 1+2. Antenna 2 (Fig. 1F) subequal in length to antenna 1, flagellum 5-articulate, article 5 with 1 apical aesthetasc.
Mandible (Fig. 1B) without palp, incisor with teeth, lacinia mobilis distally denticulate, spine row with 3 denticulate spines, molar formed as long, pointed, partially bifid process. Maxilla 1 (Fig. 1C) palp 2-articulate, subequal in length to outer plate, with 2 distal setae; outer plate with 5 spines, inner spine strongly curved, distally denticulate, second strong, short and bifid, third to fifth spines elongate, with subapical denticile; inner plate rounded quadrangular, with 1 distal seta. Maxilla 2 (Fig. 1D) with 3 distal spiniform setae on every plate. Maxilliped (Fig. 1E) basally fused, only basal lobes present, with 2 distal setae; palp with 5 articles, article 1 with 2 elongate setae, articles 2-4 each with single elongate seta, dactylus with long, falcate claw.

Coxal plates 1-4 small (Fig. 2A-D), subquadrate, distally rounded, with 1 anterior seta; coxal plates 5-7 lobed, posterior lobe shorter than anterior one, with 1 posterior seta. Gills on pleon segments 3-5. Oostegites not observed (absent?).

Gnathopod 1 (Fig. 2A) carposubchelate, carpus strong, ovato-elongate, palm feebly convex, smooth, with 3 setae finely bifid apically, and 1 submedial spine on inner face, palm having 1 strong spine and 1 short triangular process on inner face, dactylus with 4 slender spiniform processes, claw slender, elongate. Gnathopod 2 (Fig. 2B) carposubchelate, dissimilar from gnathopod 1, carpus subpiriform, stronger than in gnathopod 1, palm oblique, distally concave, smooth, with 3 setae finely bifid apically and 1 submedial spine on inner face, palm defined by 1 strong spine, dactylus stronger than in gnathopod 1, with 4 elongate spiniform processes, claw elongate, falciform; c/p index (see Stock, 1977) = 1.9.

Pereiopods 3, 4 (Fig. 2C, D) similar, dactylus longer than ½ propodus, basal part ending in acutely pointed internal spur, claw slender, as long as basal part of dactylus, finely bifid distally. Pereiopods 5-7 (Fig. 2E-G) progressively longer. Pereiopods 5, 6 (Fig. 2E, F) basis piriform-elongate, merus, carpus, and propodus with 1 distoposterior curved spine, merus and carpus inflated, dactylus similar to those of pereiopods 3, 4, but relatively shorter, claw bifid distally, shorter than basal part of dactylus. Pereiopod 7 distinctly longer than pereiopods 5, 6, basis elongate, linear, merus and carpus not inflated, dactylus similar to those of pereiopods 5, 6, but longer, claw bifid distally, as long as basal part of dactylus.

Pleopods 1-3 (Fig. 3A) subtrapezoidal, with disto-anterior corner finely pointed, without setae, similar, but pleopod 3 slightly narrower than pleopods 1-2.

Uropod 1 (Fig. 3B) peduncle longer than inner ramus, with 1 subdiscal ventral seta; outer ramus very short, about ¼ length of inner ramus, without setae; inner ramus subdistally emarginate, distally truncated, with 3 distal spines and row of 6 long setae on inner face. Uropod 2 (Fig. 3C) as long as urosomites 3, peduncle length about twice the rami, with 1 long distoventral seta and with 3 diagonal rows of strong spines, truncated and shortly bifid, on inner face, every row preceded and finished by 1 normal elongate spine; outer ramus slightly shorter than inner, rami distally constricted and apically pointed. Uropod 3 (Fig. 3D) very short, peduncle with 1 distal seta, ramus shorter than peduncle, with 1 long distal seta. Telson fleshy, subglobular, apparently without setae.

Etymology.—The new species is named after Dr. Beatrice Sambugar, one of the collectors of the specimen during her research on subterranean Oligochaetes.

Remarks.—Ingolfiella beatricis is thought to have a marine origin. It was reported by B. Sambugar that in the same cave where I. beatricis was found, two species of Tubificidae Phalodrilinae occurred, whose relatives are in the marine environment (Sambugar et al., 1999). It seems that the aquatic fauna of the Pajarjeva Jama is a mixture of freshwater and marine elements. This may be confirmed by the observation that I. beatricis is similar to a group of species with oligohalynete occurrence, e.g., the subgenus Antillella Ruffo and Vigna Taglianti, 1989, that is present in the Atlantic, the Antilles, Canary Islands, and Madeira Island, with a probably tethian distribution (see Discussion).

DISCUSSION

Ruffo and Vigna Taglianti (1989) divided the genus Ingolfiella Hansen, 1903, into seven subgenera. Three of them (Ingolfiella s. str.; Hansenliella Stock, 1981; Tethydiella Ruffo and Vigna Taglianti, 1989), containing 16 true marine species, all with developed "oculare lobes", are contrasting with three other sub-
Table 1. Characters of *Ingolfiella* (Antillella) species (from *putealis* to *unguiculata*) and *Ingolfiella* (Tethydiella) species (from *xarfae* to *longipes*).

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<th>femoralia</th>
<th>rubularis</th>
<th>margaritae</th>
<th>similis</th>
<th>unguiculata</th>
<th>beatrix</th>
<th>xarfae</th>
<th>kapuri</th>
<th>grandipina</th>
<th>quadrella</th>
<th>canariensis</th>
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</table>
genera (Gevgeliella S. Karaman, 1959; Balcanella S. Karaman, 1959; Tyrhenidiella Ruffo and Vigna Taglianti, 1989) containing 9 freshwater subterranean species, without "ocular lobes." The subgenus Antilleella Ruffo and Vigna Taglianti, 1989, has an intermediate position between the two groups described above and is composed by 6 anchialalyte species which live in fresh- or brackish water, but always in close vicinity to marine coasts. These species have their "ocular lobes" more or less reduced, sometimes vestigial or lacking.

Ingolfiella beatricis is similar to all species of the subgenus Antilleella (see Table 1), especially in the pereiopods 3–7 dactyli, which are all equal, with one distointerior spur and a thin bident claw, furthermore for the presence of pleopods 1–3 (but because the male is unknown, it is impossible to say if the pleopod 1 male has 2 distal setae, like all the species of Antilleella). From all these species I. beatricis is clearly different by: 1) presence of developed "ocular lobes" (reduced or probably quite absent in the Antilleella species; 2) gnathopods 1 and 2 dactyli with 4 inner teeth (always 3 in the Antilleella species); gnathopod 2 palmar margin smooth (always serrated in the Antilleella species); 3) outer ramus of uropod 1 very short, \( 1/4 \) length of inner ramus (\( 1/2 \) in Antilleella, except I. unguiculata \( 1/4 \) of inner ramus); 4) gnathopod 2 female c/p index (for definition see Stock, 1977) = 1.9, while it is more than 2 in species of Antilleella except I. putealis with c/p = 1.8–1.9.

Ingolfiella beatricis has some characters that are similar to species of the subgenus Tethydiella (see Table 1): 1) "ocular lobes" developed as in all Tethydiella species; 2) gnathopods 1 and 2 dactyli with 4 inner teeth (as in Tethydiella species except I. grandispina with 3 teeth and I. xarifaie with 3 teeth in gnathopod 1 and 4 teeth in gnathopod 2); 3) c/p index = 1.9, as in all Tethydiella species (1.9–2). Ingolfiella beatricis, however, is different from Tethydiella by the similar dactyli of pereiopods 3–7 (like Antilleella), with a distointernal spur (absent in Tethydiella, except I. xarifaie and I. grandispina with distointernal spur only in pereiopods 3 and 4, and I. quadridentata with distointernal spur present only in pereiopod 7).

We did not include in this discussion the mouthparts, as they are not always, and then often insufficiently, described for the difficulty of their preparation. But what is known does not indicate significant differences. For I. beatricis we want to underline the structure of mandibular molar, which is elongate, pointed, and bifid (as in I. (Antilleella) similis Rondé-Broekhuizen and Stock, 1987), the spination of maxilla 1 inner plate with 5 spines (as in I. (Antilleella) unguiculata Stock, 1992, the inner strongly curved as in some species of Tethydiella). Also we did not take into consideration the characters of sexual dimorphism, especially for gnathopod 2, pleopods 1–3, uropod 2, because I. beatricis is represented by only a single female specimen.

In conclusion it appears that Ingolfiella beatricis has an intermediate position between the true marine species (especially Tethydiella) and those anchialalyne ones, which are inhabiting fresh- interstitial or phreatic waters, in close vicinity to marine coasts, especially the subgenus Antilleella, to which our species has its closest affinities. The discovery of this species in subterranean freshwater, some 50 km inland from the Adriatic coast seems therefore quite significant for the evolution and biogeography of the genus Ingolfiella.

The subdivision in subgenera (Stock, 1976; Ruffo and Vigna Taglianti, 1989) which has been already discussed (Dojiri and Sieg, 1987; Lowry and Poore, 1989) should be revised with more complete character sets, as soon as one will have further material of the known species and hopefully also additional new discoveries in the future.

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**LITERATURE CITED**


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