
Phylogeny and biogeography of the genus *Pseudectinosoma*, and description of *P. janineae* sp. n. (Crustacea, Copepoda, Ectinosomatidae)

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Recent stygobiological investigations carried out in the circum-Mediterranean area resulted in the discovery of a new species of the genus *Pseudectinosoma* Kunz, described herein as *P. janineae* sp. n. The taxonomic status of the genus is reviewed in a phylogenetic perspective. The origin of the present-day distribution of the genus *Pseudectinosoma* is reconstructed both from patterns of relationships among the species and from palaeogeographical data.

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Introduction

The genus *Pseudectinosoma* Kunz, 1935 belongs to the family Ectinosomatidae Sars, 1903, which primarily inhabits marine and brackish water environments. The family is rarely represented in continental freshwater bodies, with a few exceptions known in the genera *Halectinosoma* Lang, 1944, *Arenosetella* C. B. Wilson 1932 and *Pseudectinosoma*, all of which, however, relate to continental groundwater. The genus *Pseudectinosoma* is a poorly diversified genus of marine origin, although mainly recorded from continental groundwater localities. It was established to accommodate the type-species *P. minor* Kunz, 1935, which was based on material from the detritus-rich environment of the Kiel Bay (Germany). The following year, Schäfer (1936) recorded the same species from the Baltic Proper, and synonymized the genus *Pseudectinosoma* with *Sigmatidium* Giesbrecht, 1881. Later the genus was re-instated by Kunz (1974) and subsequently emended by Galassi (1997). *P. minor* has been recorded from salt marshes in New Jersey, USA (Coull 1977), but this record is doubtful and would require confirmation. The species has also been cited from the North Sea by Heip *et al.* (1983) and by Huys *et al.* (1996), however, this record most probably represents a misidentification of an as yet undescribed *Pseudectinosoma* species (Huys, personal communication). Rouch (1969)

described the second species of the genus, *P. vandeli*, from groundwater habitats of the Cent-Fons karstic system (Hérault, France). Recently, two new species were discovered and described from Italian groundwater, namely *P. kunzi* Galassi, 1997 and *P. reductum* Galassi & De Laurentiis, 1997. Another unidentified *Pseudectinosoma* sp. has also been reported by Gourbault & Lescher-Moutoué (1979) from Mallorca. Ongoing investigation of the interstitial communities in the Rhône River (France) since 1977 (Dole-Olivier *et al.* 1994), at different spatio-temporal scales, revealed the presence of a new species of *Pseudectinosoma*, described herein as *P. janineae* sp. n.

The phylogenetic relationships among the species of *Pseudectinosoma* are analysed using cladistics and a plausible palaeogeographical landscape is also drawn to explain the present-day distribution of the genus.

Materials and methods

Samples were taken from wells using a 80- μ m mesh net (Bou-Rouch method) through which water of phreatic origin was collected and filtered. Quantitative replicate samples were taken from the interstitial habitat with a Bou-Rouch pump and filtered through a 160–300 μ m mesh net. Specimens were preserved in 7% formalin solution. Dissected specimens were mounted in polyvinyl

lactophenol. Drawing and measurements were made using a camera lucida on a Leitz Laborlux phase contrast microscope. Since body length measurements are often affected by the telescoping action of the body somites, the alternative method for measuring the total length of the specimens was also used, according to Clément & Moore (1995). The terminology proposed by Huys & Boxshall (1991) is adopted.

Abbreviations used

ae, aesthetasc
P1-P6, first to sixth thoracopods
exp, exopod
enp, endopod
benp, baseoendopod
pl, plesiomorphic character state
ap, apomorphic character state

Order HARPACTICOIDA Sars 1903

Family ECTINOSOMATIDAE Sars 1903

Genus *Pseudectinosoma* Kunz, 1935

Pseudectinosoma janineae sp. n. (Figs 1,2,3, Table 1)

Holotype. ♀, completely dissected and mounted in polyvinyl lactophenol on a single slide; deposited in the Natural History Museum, London.

Type locality. River Rhône (France), downstream from the confluence of the River Rhône and the River Ain; coordinates: 44°44' 57"N, 5°13' 14"E; altitude: 191 m a.s.l.; interstitial habitat; 5 December 1975; coll. M.-J. Dole-Olivier.

Paratypes. 2 ♀♀, 2 ♂♂, dissected and mounted as above, River Rhône, Miribel Canal, 186 m a.s.l.; interstitial biotope; 5 December 1975, 11 October 1983; coll. Marie-José Dole-Olivier. 4 ♀♀, 4 ♂♂, dissected and mounted as above, phreatic waters (well), N of Lyon, Villeurbanne, campus of the University Claude Bernard, Lyon I (France); coordinates: 44°44'56"N, 5°13'14"E; altitude: 168 m a.s.l.; 11 November 1993; coll. Dominique Martin.

Other material. 3 ♀♀, 2 ♂♂, 9 copepodites from localities of paratypes. One ♂ paratype in the Natural History Museum, London. Remaining paratypes and other material in personal collection of Dr Galassi, Dipartimento di Scienze Ambientali, University of L'Aquila.

Etymology. It is a pleasure to name this new species in honour of Janine Gibert (University of Lyon, France), in recognition of her important contribution to the field of groundwater ecology.

Description.

Female. Length, excluding caudal setae, from 290 µm to 370 µm ($n = 7$) (300 µm, holotype); sum of individual somite lengths from 320 µm to 400 µm (390 µm, holo-

type). Body fusiform and colourless. Rostrum hyaline, reaching to proximal third of second segment of antennule on frontal side. Cephalothorax irregularly pitted (Fig. 1a); 12 pairs of sensillae recognizable on cephalic shield, the anteriormost at the basis of rostrum. Posterior dorsal margins of thoracic somites, excluding the fifth thoracic somite, smooth; thoracic somites with 2 pairs of sensillae on dorsal surface and 1 pair of sensillae on ventral surface, the latter lacking on the fifth thoracic somite. Abdominal somites, excluding anal somite, with denticulate hyaline frill dorsally; anal somite with 2 sensillae distally on dorsal surface. Ventral margins of abdominal somites armed with semi-incised subulate hyaline frill (Fig. 2a). Both genital and first abdominal somites completely fused forming genital double-somite, without cuticular ornamentation marking original segmentation. Genital field as in Fig. 1e. Irregular distribution of pores on both ventral and dorsal surfaces of body somites, with the exception of both anal and genital somites, the latter lacking pitting only around the genital field.

Caudal rami (Fig. 1b,c) wider than long, hyaline frill of anal somite fully incised subulate. Armature as follows: seta I lacking, setae II and III slender and bare, of about the same lengths, setae IV and V well developed, seta VI very long, seta VII on dorsal inner knob. Posterior margins of each caudal ramus prolonged into triangular lappets, both dorsally and ventrally; dorsal lappet strong, ventral lappet spiniform.

Antennule (Fig. 2b): short, 4-segmented; segment 1 with 1 laterodistal seta, segment 2 with 2 plumose and 3 slender and bare outer setae, 4 setae on frontal ridge and 2 lateral surface setae, 2 apical long setae plus 1 aesthetasc, segment 3 short, with 1 outer seta, segment 4 with 1 outer seta, 2 setae inserted on the middle of segment; 2 apical and slender setae, 1 hyaline seta and 1 aesthetasc distally.

Antenna (Fig. 2c): basis unarmed; exopod 2-segmented, segment 1 bearing 1 lateral plumose seta, segment 2 with 2 long apical plumose setae; endopod 2-segmented, segment 1 unarmed, segment 2 with some spinules and 2 stout and short setae along inner margin, 1 apical slender seta, 2 apical unispinulose setae, 1 geniculate seta and 1 subapical slender seta.

Mandible (Fig. 2d): cutting edge of coxa with 2 strong teeth, 2 smaller teeth fused to the crenulate chitinous lamella; 1 seta at dorsal corner. Mandibular palp: basis with 3 medial setae; exopod bearing 1 subapical and 2 apical setae of different lengths, the longest plumose; endopod bearing 8 setae, 3 of which along medial margin.

Maxillule (Fig. 2e): praecoxal arthrite bearing 3 setae and 1 strong spine, not defined at base, and 2 slender anterior surface setae; basis apparently fused with endopod into a laminar plate bearing 10 setae; exopod 1-segmented, with 2 apical plumose setae.

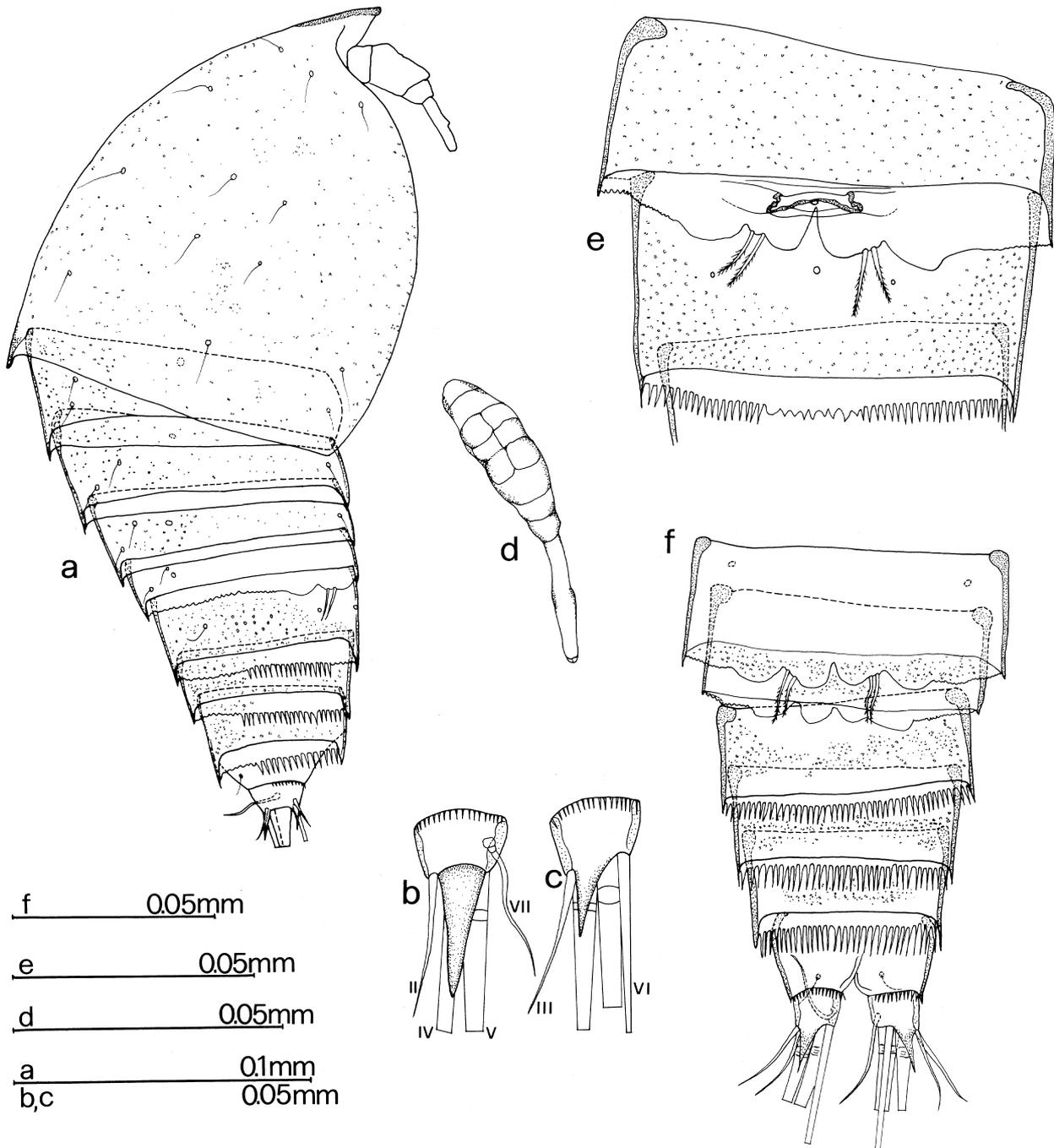


Fig. 1 a–f. *Pseudectinosoma janineae* sp. n. — a. Habitus (♀, paratype). — b. Caudal ramus, dorsal side (♀, paratype). — c. Caudal ramus, ventral side (♀, paratype). — d. Spermatophore. — e. Genital field (♀, paratype). — f. Abdomen and caudal rami (♂, paratype).

Maxilla (Fig. 2f): praecoxa and coxa fused into syncoxa bearing 3 endites; first (proximal) forming single bilobed protuberance bearing 1 plumose seta on each lobe, both setae not defined at base; second (middle) endite rudimentary, with 2 long setae; third (distal) endite well developed, with 3 plumose apical setae. Basis and proximal segments

of endopod fused; free endopod apparently 3-segmented, but only segment 3 clearly defined, bearing 3 slender setae; 2 strong geniculate setae and 2 naked setae on free distal margin of endopodal segment 1, 3 slender setae on distal margin of basis.

Maxilliped (Fig. 2g) slender, not prehensile, 3-segmen-

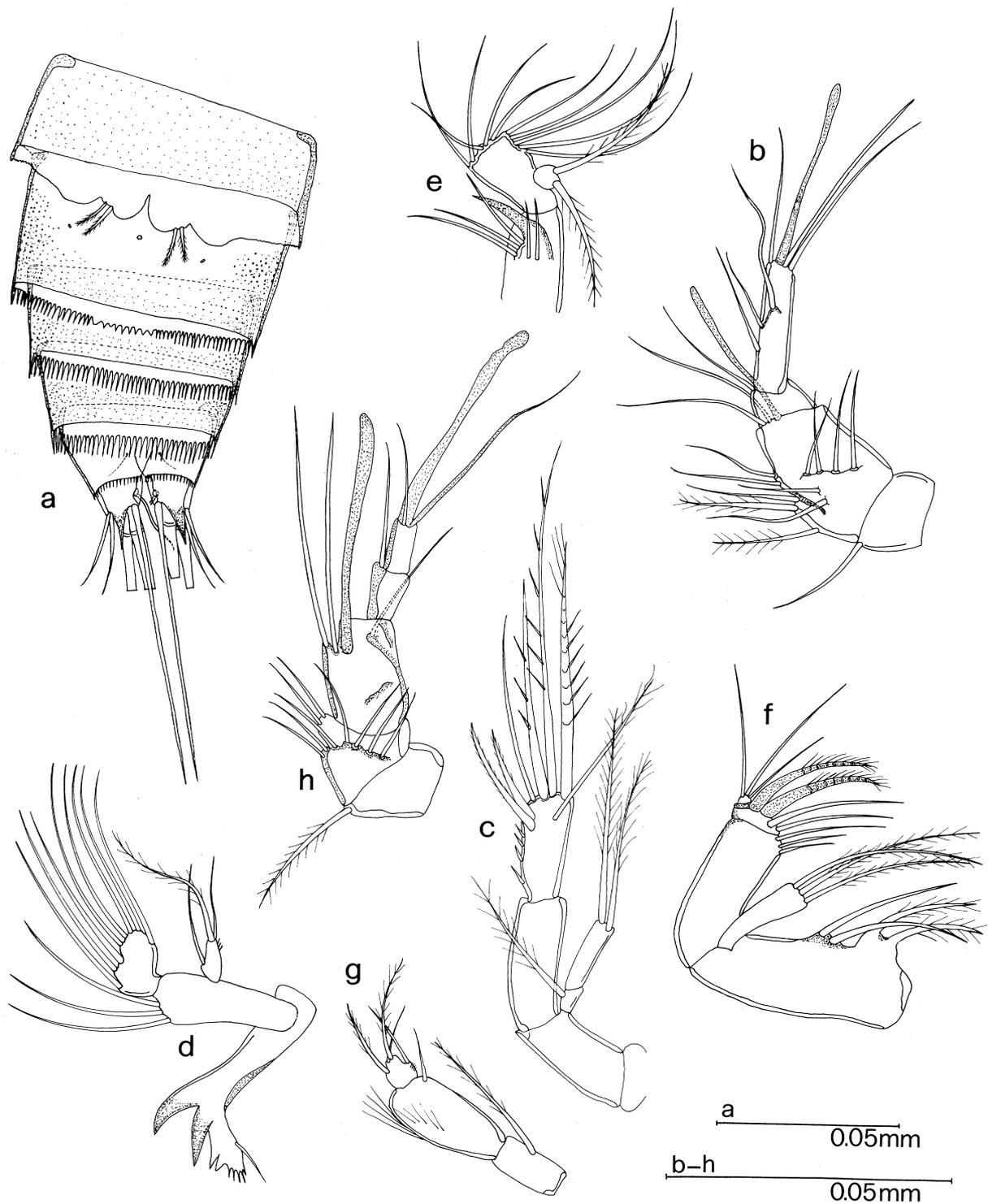


Fig. 2 a–b. *Pseudectinosoma janineae* sp. n. — a. Abdomen and caudal rami (♀, holotype). — b. Antennule (♀, paratype). — c. Antenna (♀, paratype). — d. Mandible (♀, paratype). — e. Maxillule (♀, paratype). — f. Maxilla (♀, paratype). — g. Maxilliped (♀, paratype). — h. Antennule (♂, paratype).

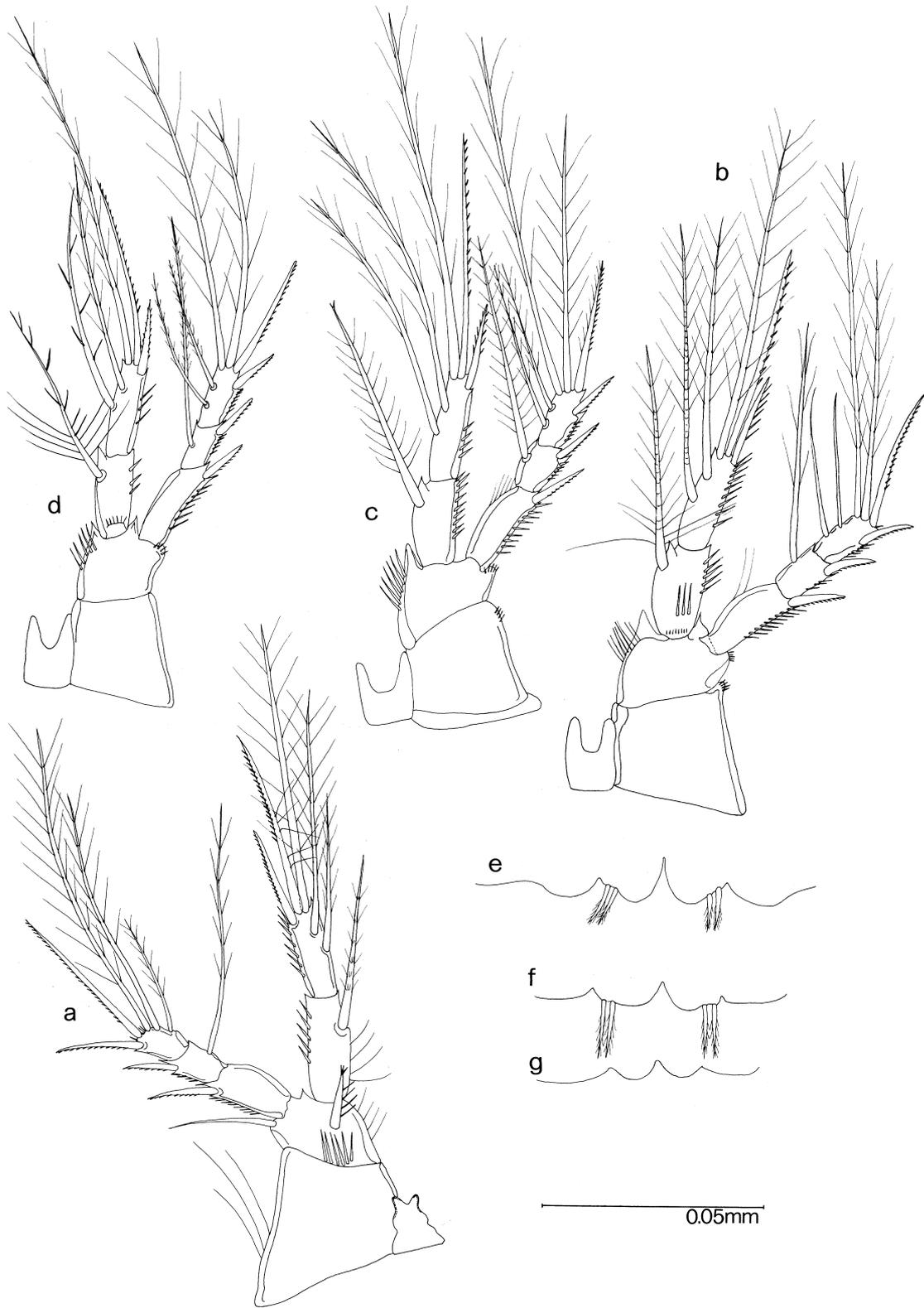


Fig. 3 a–g. *Pseudectinosoma janineae* sp. n. — a. P1 (♀, holotype). — b. P2 (♀, holotype). — c. P3 (♀, holotype). — d. P4 (♀, holotype). — e. P5 (♀, holotype). — f. P5 (♂, paratype). — g. P6 (♂, paratype).

Table 1 Setation formula of P1–P4 in *Sigmatidium* and *Pseudectinosoma* species.

TAXA	P1		P2		P3		P4	
	exp	enp	exp	enp	exp	enp	exp	enp
<i>S. difficile</i>	0.1.122	1.321	1.1.222	1.1.221	1.1.322	1.1.221	1.1.322	1.1.221
<i>S. rouchi</i>	0.1.122	1.111	?	?	0.1.122	?1.221	0.1.122	1.0.121
<i>S. noodti</i>	1.1.122	1.221	1.1.122	1.1.121	0.1.122	1.1.121	0.1.122	1.1.121
<i>S. parvulum</i>	0.1.122	1.121	0.1.122	1.1.220	0.1.122	1.1.220	1.1.122	1.0.220
<i>S. triarticulatum</i>	0.1.122	1.0.221	1.1.122	1.1.221	1.1.122	1.1.221	1.1.122	1.0.221
<i>S. kunzi</i>	0.1.122	1.221	1.1.122	1.1.221	1.1.122	1.1.221	1.1.222	1.1.221
<i>P. minor</i>	0.1.122	1.221	0.1.222	1.221	0.1.322	1.221	0.1.322	1.221
<i>P. vandeli</i>	0.1.122	1.121	0.1.122	1.121	0.1.122	1.121	0.1.122	1.121
<i>P. kunzi</i>	0.1.022	1.121	0.1.122	1.121	0.1.122	1.121	0.1.122	1.121
<i>P. reductum</i>	0.1.022	1.121	0.1.122	1.121	0.1.122	1.121	0.1.122	1.121
<i>P. janineae</i> sp. n.	0.1.122	1.221	0.1.222	1.221	0.1.222	1.221	0.1.222	1.221

ted; syncoxa with a long plumose seta, basis with minor ornamentation consisting of 1 short setule and some hair-like elements on laterodistal corner; endopod with 2 subapical setae and 2 apical setae, two of which plumose.

P1–P4 with 3-segmented exopods and 2-segmented endopods. Intercoxal sclerites without ornamentation.

P1 (Fig. 3a): coxa with 3 thin long setules along outer margin. Basis with inner medial unipinnate spine and a long outer seta. Exopod: segment 1 with 1 outer spine, segment 2 with 1 outer spine and 1 long plumose inner seta, segment 3 with 2 outer spines, the distal one in subapical position, 2 apical and 1 plumose inner setae. Endopod: segment 1 with a long, strong inner seta, segment 2 with 2 inner and 1 apical plumose setae, 1 apical asymmetrical spine (setulose on inner margin, spinulose on outer margin) and 1 outer unipinnate spine.

P2 (Fig. 3b): basis unarmed, with spinules of different lengths along both inner and outer margins. Exopod: segment 1 with 1 outer spine, an outer row of spinules and some setules along inner margin, segment 2 with 1 outer spine, an outer row of spinules and 1 long, plumose inner seta, segment 3 with 2 outer spines, the distal one in subapical position, and an outer row of spinules, 2 plumose apical setae and 2 bare inner setae. Endopod: segment 1 with 1 long, plumose inner seta and 2 rows of spinules along outer margin and on surface, segment 2 with 2 inner and 1 apical plumose setae, 1 apical and 1 outer unipinnate spine, of different lengths.

P3 (Fig. 3c): basis unarmed, minor ornamentation as in *P2*. Exopod: segment 1 with 1 outer spine; segment 2 with 1 outer spine and 1 long, plumose inner seta; segment 3 with 2 outer spines, the distal one in subapical position, 2 plumose apical setae and 2 shorter plumose inner setae. Endopod: segment 1 with 1 long, plumose inner seta and an outer row of spinules; segment 2 with 2 inner and 1 apical plumose setae, 1 apical unipinnate and 1 outer bipinnate spines. Minor ornamentation as in *P2*.

P4 (Fig. 3d): basis armed and ornamented as in *P2–P3*. Exopod: segment 1 with 1 outer spine; segment 2 with 1 outer spine and 1 long inner seta; segment 3 with 2 outer spines, the distal one in subapical position, 2 plumose apical setae and 2 pinnate inner setae. Endopod: segment 1 with 1 inner seta, proximal third of which with long setules, strong spinules sparsely on the remaining 2/3 of length, segment 2 with 1 spinulose inner seta, 1 inner and 1 apical plumose setae, 1 unipinnate apical spine and 1 bipinnate outer spine. Minor ornamentation as in *P2–P3*.

P5 (Fig. 3e): fifth pair of legs medially confluent, not protruding beyond free ventral edge of *P5*-bearing somite; overlying genital field. Baseoendopod and exopod completely fused and reduced to a lamellar plate bearing 2 plumose setae, of about the same length. Outer basal seta lacking.

Male. Body length, excluding caudal setae, from 260 μm to 380 μm ($n = 6$); sum of individual somite lengths from 290 μm to 380 μm . Hyaline frills, ornamentation of body somites and caudal rami (Fig. 1f) as in female.

Antennule (Fig. 2h): 5-segmented, haplocer, geniculation between segments 3 and 4; segment 2 shorter than in female; segment 3 longest, longer than in female, with weak sclerotization, segments 4 and 5 clearly distinct, segment 4 weakly sclerotized. Armature as follows: segment 1 with 1 outer plumose seta; segment 2 with 4 bare outer setae and 5 setae on frontal ridge; segment 3 with 2 long lateral setae and a well developed aesthetasc on the middle part of segment, and 1 surface seta; segment 4 with 1 lateral seta, segment 5 with acrothek (1 long hyaline seta + 1ae) and 1 slender seta distally.

P1–P4 as in female.

P5 (Fig. 3f): fifth pair of legs medially confluent, armed with 2 setae; incision between right and left legs less marked in comparison with female.

P6 (Fig. 3g) reduced to a chitinous rounded lamellar plate, unarmed. Spermatophore as in Fig. 1d.

Remarks. At present, the genus *Pseudectinosoma* includes 5 species: *P. janineae* sp. n., *P. minor*, *P. vandeli*, *P. kunzi* and *P. reductum*. Within this genus, *P. janineae* sp. n. is easily distinguishable by the unique chaetotaxy of P3-P4 exopods with 6 elements on segment 3 (Table 1). In regard to the elements topology on P1-P4 exopodal segment 3, while *P. minor* is described by Schäfer (1936) with 1 outer spine on P1 exopodal segment 3 and with 2 outer spines on P2-P4 exopodal segment 3, the remaining species of *Pseudectinosoma* were described with 1 outer and 1 apical spine (Rouch 1969; Galassi 1997; Galassi & De Laurentiis 1997). A rigorous comparison with *Sigmatidium* species, which are always reported with 2 outer spines, revealed the same situation in both *Sigmatidium* and *Pseudectinosoma* species for all legs. In particular, the distalmost outer spine shows an apical position, with independent insertion surface in respect to the remaining two apical elements (2 setae or 1 seta plus 1 spine), which, on the contrary, are coupled on the apical rim of the segment. This evidence supports homology between the previously reported 'apical spine' of *Pseudectinosoma* and the distalmost outer spine of *Sigmatidium*. The misinterpretation of the real topology of this spine in *Pseudectinosoma* is due to the close adjacency of elements on the apical rim of P1-P4 exopodal segment 3, which made unresolved the precise insertion of the spine. Consequently, the setation formula of P1-P4 in *Pseudectinosoma* species is emended as in Table 1.

Pseudectinosoma janineae sp. n. weakly resembles both *P. minor* and *P. vandeli* in different morphological characters. With *P. minor*, as redescribed and figured by Schäfer (1936) and adopted by Kunz (1974), it shares the same armature of P1-P2 exopods and the identical chaetotaxy of P1-P4 endopods, markedly differing from this species by the chaetotaxy of P3-P4 exopods (bearing 7 elements in *P. minor* vs. 6 in *P. janineae* sp. n.), by the morphology and armature of P5 in both male and female (4 baseopodal setae and the outer basal seta present in *P. minor* vs. 2 baseopodal setae and the outer basal seta absent in *P. janineae* sp. n.), and by the sexual dimorphism in the segmental pattern of antennules (4-segmented in female and 5-segmented in male of *P. janineae* sp. n. vs. 5-segmented in both male and female in *P. minor*). With *P. vandeli* it shares the segmental pattern of the male antennule, the same armature of P1 exopod and of P5, differing by the antennular segmental pattern in the female (4-segmented in *P. janineae* sp. n. vs. 5-segmented in *P. vandeli*), the armature of the P1 endopod and of both rami of P2-P4. Comparison of the mouthparts and caudal rami proved uninformative due to the lack of detail in the original drawings and descriptions given by Kunz (1935) and Schäfer (1936) for *P. minor*, and by Rouch (1969) for *P. vandeli*.

Ecology. About 50 stations in the alluvial floodplain of the Rhône and Ain rivers (Jons sector) have been investigated since 1983 (Marmonier 1988; Marmonier *et al.* 1992; Dole-Olivier *et al.* 1993), however, *P. janineae* sp. n. was found only at seven of them. This is probably due to sampling bias since the mesh size used was almost certainly too large (160 μm , and more frequently 300 μm) in comparison with the small size of the species. The fact that the more numerous populations were found at stations that had been sampled with a 80- μm mesh net is indicative for this (Gibert, unpublished data). Nevertheless, at present, the distribution of *P. janineae* sp. n. seems to be restricted to the floodplain margins (oxbow lakes and backwaters situated far from the active channel), or to deep zones in the sediments (1 m, 2 m, 2.5 m or 7 m below the surface of the substrate) and/or to upwelling areas of the main channel. These observations suggest that *P. janineae* sp. n. is probably related to stable environmental conditions, indicated by the low frequency of seasonal flooding in these areas and by the steady supply of groundwater. Moreover, the low variability in physico-chemical conditions of the aquifer was previously described in several studies (Dole 1983a; Marmonier 1988). Particularly, low amplitudes in annual variations were mentioned for temperature in an upwelling area in the main channel, i.e. 2.6 °C at 2 m below the surface of the substrate vs. 14.4 °C in the surface water (Dole-Olivier *et al.* 1997), or in the Grand-Gravier oxbow lake, with a mean annual variation of 2.5 °C at 0.5 m below the bottom (Dole 1983b). Many studies, dealing with the tridimensional distribution of interstitial fauna in this alluvial site (longitudinally along reaches, transversally across the floodplain, and vertically below several meters of sediment), revealed the presence of some stygobiont species (*Salentinella juberitiae* Coineau, 1968, *Microcharon regineae* Dole & Coineau, 1987, *Bathynella* sp.). These species were defined as phreatobiont by Dole-Olivier & Marmonier (1992) and Dole-Olivier *et al.* (1993), and show the same fine-scale distribution and ecological requirements as *P. janineae* sp. n. According to the above observations, *P. janineae* sp. n. could be considered a phreatobiont species *sensu* Gibert *et al.* (1994).

Phylogeny of the genus *Pseudectinosoma*

Although the taxonomy of the '*Sigmatidium*-related genera' (Kunz 1974) is in urgent need of revision, comparisons of the morphology and the armature of the mouthparts support a close affinity between the more primitive genus *Sigmatidium*, still living in the marine environment (plesiotypic habitat) and the most derived genus *Pseudectinosoma*, which seems to have abandoned this ancestral environment. Within this group, the marine genera *Noodtiella*

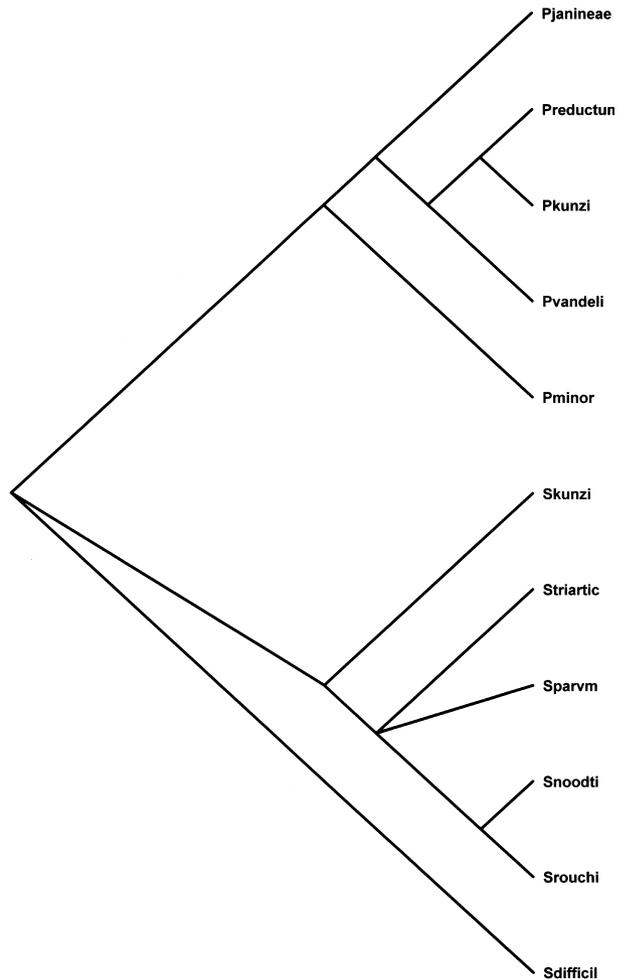


Fig. 4 Strict consensus tree from 9 trees generated by Phylip (Branch-and-Bound option on ordered characters). This tree is identical with the Nelson consensus tree.

Wells, 1965 and *Lineosoma* Wells, 1965 appear to be only distantly related to the *Sigmatidium*-*Pseudectinosoma* lineage, since many differences are found in the structure of the antennule, maxillule, maxilla and maxilliped (Kunz 1974; Huys *et al.* 1996).

In our cladistic analysis within the genus *Pseudectinosoma*, the plesiomorphic states of characters 1–16 (Table 2) are inferred from the most closely related outgroup *Sigmatidium*. A binary matrix (Table 3) was constructed, in which the plesiomorphic state was coded as '0' and the apomorphic state as '1'. The taxonomy of the *Sigmatidium* and *Pseudectinosoma* species is rather difficult due to the lack of complete descriptions and the loss of type-material of *P. minor*, *P. vandeli*, *S. rouchi* Kunz, 1974 and *S. noodti* Kunz, 1974. Moreover, the male is unknown for some *Sigmatidium* and *Pseudectinosoma* species and the results based on adult females only, must therefore be

considered as preliminary. The polarity of the characters has been verified for all *Sigmatidium* and *Pseudectinosoma* species. In this regard, the setation formula of P1 exopodal segment 3 for *P. minor*, given in Schäfer (1936)'s text description, has been corrected according to his contradicting Fig. 9, and the setation formula of P1-P4 exopodal segment 3 of *P. vandeli*, *P. kunzi* and *P. reductum* emended as previously argued (Table 1). Similarly, the setation formula of P3-P4 endopodal distal segments in *Sigmatidium rouchi* is amended in accordance with Kunz (1974: figs 19,20). Moreover, the re-examination of the type-material of *S. difficile* Giesbrecht, 1881 revealed some mistakes and characters that had been overlooked in the original description and, particularly, a quite different setation formula of the P2-P4 endopods and P1-P4 exopods.

The matrix was processed by Penny Algorithm, employing the Branch-and-Bound option to find all most parsimonious trees, Phylip package, version 3.572c (Felsenstein 1993), using the Camin-Sokal parsimony method, under the assumption that true character reversals are extremely rare in copepods (Huys & Boxshall 1991). Moreover, the polarity of characters we assumed seems to be in line with the global trend in copepod evolution, which has proceeded toward fusion and loss of segments and setation elements. Nine equally parsimonious trees were found (tree length = 22 steps), all showing two, more or less, identical robust clades, the *Sigmatidium* and the *Pseudectinosoma* clades. The trees show a very similar topology, differing in the position of the species *S. difficile*. In three trees the species is placed as the plesiomorphic sister species of the *Sigmatidium* group, in three trees is placed as first offshoot of the *Sigmatidium*-*Pseudectinosoma* branch, and in the remaining ones as offshoot of the *Pseudectinosoma* clade. This instability is due to the high number of characters that the species shows in plesiomorphic state, conferring it a condition similar to the '*Sigmatidium*-like ancestor'. *S. difficile*, apart from the striking similarities with all members of the *Sigmatidium*-*Pseudectinosoma* lineage in the morphology of the mouthparts, is more closely related to the *Sigmatidium* clade on the basis of the following characters: antennular and P2-P4 segmental patterns, and P5 structural plan. These characters appear more informative of phylogenetic affinity in respect to those shared with members of the genus *Pseudectinosoma*. In particular, homology in the segmental pattern of antennules and P2-P4 endopods, highly conservative at generic level, is regarded as more consistent, if compared to the significance of the number of inner elements on P3-P4 exopodal segment 3, on the reasonable assumption that the loss of segments requires a more consistent genetic change than the loss of a single seta. Moreover, the major perti-

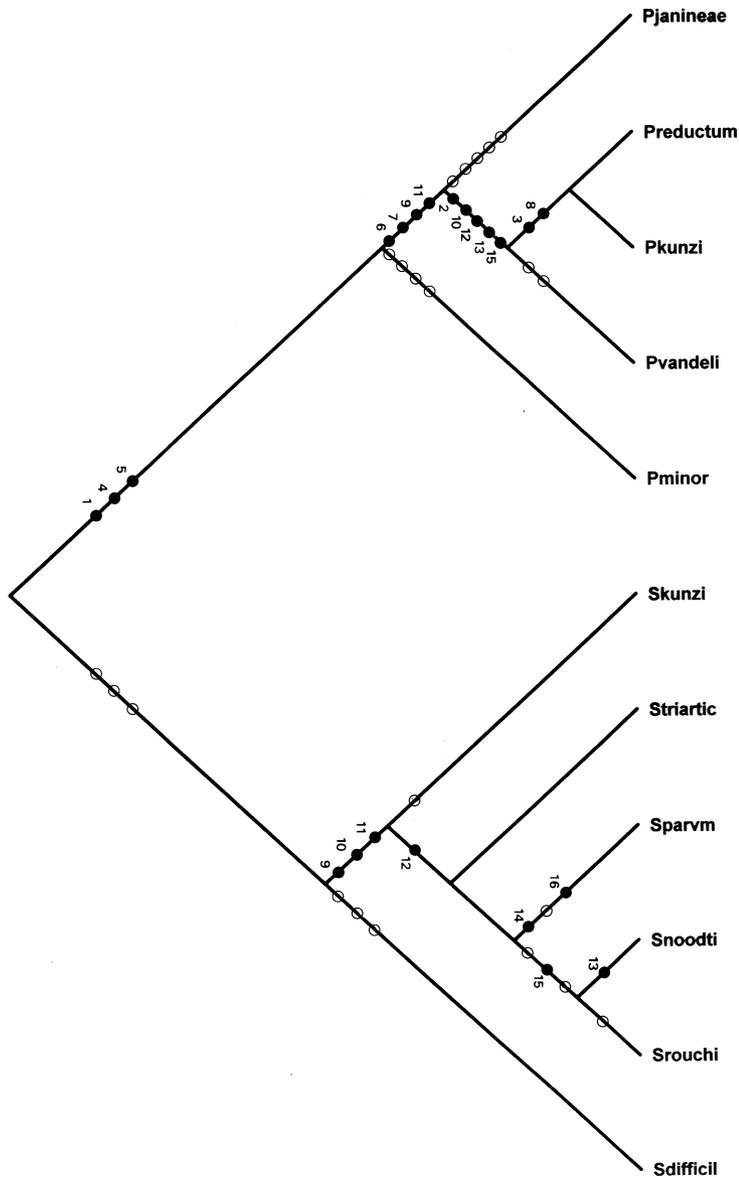


Fig. 5 Cladogram of the hypothetical phylogenetic relationships among species of *Pseudectinosoma* Kunz (○ = plesiomorphic character state; ● = apomorphic character state). This tree is one of the nine trees generated by Phylip (Penny Algorithm, employing the Branch-and-Bound option to find all most parsimonious trees, using Camin-Sokal parsimony method).

nence of this species to the *Sigmatidium* clade is obscured by the fact that some potential characters, proved informative from a phylogenetic point of view, have been omitted in this analysis, due to the lack of complete description for most species. We allude especially to the condition of the P1 endopod segmentation, which is clearly 3-segmented (the plesiomorphic condition in the *Sigmatidium-Pseudectinosoma* lineage) in *S. triarticulatum* Mielke, 1979. Our observations of the type-material of *S. difficile* revealed a rudimentary 3-segmented P1 endopod, but we do not

know explicitly the situation in other *Sigmatidium* species. In the same way, poor information is available in regard to the presence/absence of the surface seta on P5. This seta is present in *S. difficile* (Galassi & De Laurentiis, personal observation), and, most probably, in undescribed species of *Sigmatidium* (Huys *et al.* 1996) but it is apparently absent in the other species of the genus. Additionally, the antennal exopod is reported both 2- and 3-segmented in different *Sigmatidium* species, although some doubts exist on the pedestal nature of the proximal segment (rudimentary in

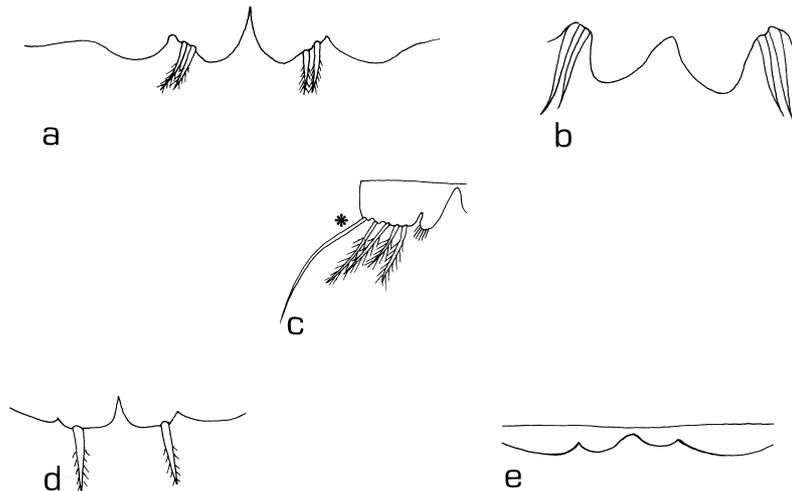


Fig. 6 Comparative morphology of female P5 among species of *Pseudectinosoma* Kunz: — a. *P. janineae* sp. n. — b. *P. vandeli* (after Rouch 1969; redrawn). — c. *P. minor* (after Schäfer 1936, redrawn). — d. *P. kunzi*. — e. *P. reductum* (* indicates the basipodal outer seta).

Pseudectinosoma janineae sp. n.), but once again this character is not verifiable in all species.

The nine tree files were imported into the program Component, version 2.0 (Page 1993) and used as input files for the calculation of consensus trees (Fig. 4) (Strict and Nelson consensus). In both consensus trees, while the position of *S. difficile* is unresolved, all the species of *Pseudectinosoma* form a robust clade. The tree illustrated in Fig. 5 is one of the nine trees generated by Phylip. It represents, at present knowledge, the best compromise between parsimony and homology, under the assumption that, although not quantifiable, certain characters are more significant than others. The monophyletic status of the genus *Pseudectinosoma* is defined by the following synapomorphies: antennular segments 2 and 3 fused (1), P2-P4 with 2-segmented endopods (4), P5 baseoendopod and exopod not recognizable by setation (5). Within the genus, *P. minor* is the sister taxon of a group of species consisting of *P. janineae* sp. n., *P. vandeli*, *P. reductum* and *P. kunzi*. This lineage is defined by the following synapomorphies: incision between P5 baseoendopod and exopod absent (6) and its outer basal seta absent (7) (Fig. 6), P3 and P4 exopodal segment 3 with a maximum of 2 inner setae (9, 11). Within this group, *P. janineae* sp. n. still retains the syncoxal seta on the maxilliped, while it is lacking in *P. vandeli*, *P. kunzi* and *P. reductum* (2). The latter group is also defined by the P3-P4 exopodal segment 3 with 1 inner seta (10, 12) and by the P3-P4 endopodal segment 2 with 1 inner seta (13, 15). The condition of 4 elements on P1 exopodal segment 3 (3), derived by the absence of inner seta on P1 exopodal segment 3 (8) is the synapomorphy of the *P. kunzi*-*P. reductum* group. Some apomorphies (1 inner seta on P3 exopodal segment 3, 1-2 inner setae on P4

exopodal segment 3, and 1 inner seta on P3-P4 endopodal segment 2) may have evolved more than once within the *Sigmatidium*-*Pseudectinosoma* lineage.

As discussed above, comparison of characters shows a general trend towards reduction and/or character losses in the evolutionary history of the *Sigmatidium*-*Pseudectinosoma* complex. Therefore, the regressive nature of characters in stygobiont taxa could also be regarded as adaptative features to life in the groundwater habitat (Parzefall 1986; Botosaneanu & Holsinger 1991; Notenboom 1991; Holsinger 1992; Boutin 1994; Culver *et al.* 1995), raising the question, as summarized by Danielopol & Rouch (1991), whether '... these adaptative features arose before ("adoption") or after ("adaptation") the ancestral populations invaded groundwaters'. Among stygobiont and interstitial copepods, the adaptative significance of many regressive traits is still debatable, if not questionable (Pesce & Galassi 1985, 1986; Reid 1991; Boxshall *et al.* 1993; Reid & Strayer 1994; Jaume & Boxshall 1996a; Galassi 1997; Galassi & De Laurentiis 1997) and for many characters simply unknown. On the basis of the present study, it seems that many regressive traits are already present in the marine epigeal *Sigmatidium* (small body size, short antennule, tendency to fusion between P5 baseoendopod and exopod) and in the surface-waters species *P. minor*, in which further oligomerization of P2-P4 endopods and P5 can be found. In summary, the phenotype of *P. minor* tends to be maintained in the stygobiont relatives, supporting the hypothesis that regressive features shared by groundwater species of *Pseudectinosoma* are not the result of direct selection in groundwater habitats, but could be considered as features already present in the epigeal ancestor. The above observations strengthen the use of the

Table 2 List of characters used in the cladistic analysis. (State '0' represents the plesiomorphic character state.)

1. ANTENNULE: (0) 2th and 3th segments distinct; (1) fused.
2. MAXILLIPED: (0) syncoxa with seta; (1) without seta.
3. P1 EXP-3: (0) with 5 elements; (1) with 4 elements.
4. P2-P4 ENDOPOD SEGMENTATION: (0) 3. 3. 3; (1) 2. 2. 2.
5. P5 BENP AND EXP: (0) recognizable by setation; (1) not recognizable.
6. INCISION BETWEEN P5 BENP- EXP: (0) present; (1) absent.
7. P5 OUTER BASAL SETA: (0) present; (1) absent.
8. P1 EXP-3: (0) with 1 inner seta; (1) without inner seta.
9. P3 EXP-3: (0) with 3 inner setae; (1) with 2 inner setae.
10. P3 EXP-3: (0) with 2 inner setae; (1) with 1 inner seta.
11. P4 EXP-3: (0) with 3 inner setae; (1) with 2 inner setae.
12. P4 EXP-3: (0) with 2 inner setae; (1) with 1 inner seta.
13. P3 ENDOPOD: (0) distal segment with 2 inner setae; (1) with 1 inner seta.
14. P3 ENDOPOD: (0) distal segment with outer spine; (1) without outer spine.
15. P4 ENDOPOD: (0) distal segment with 2 inner setae; (1) with 1 inner seta.
16. P4 ENDOPOD: (0) distal segment with outer spine; (1) without outer spine.

regressive characters in the phylogenetic analysis among marine epigeal and stygobiont taxa of the monophyletic genus *Pseudectinosoma*.

Biogeography of the genus *Pseudectinosoma*

Hypotheses on the descentance of groundwater taxa from marine ancestors are intensively debated, representing one of the major topics of the stygobiology (Stock 1980; Iliffe 1986; Rouch & Danielopol 1987; Boutin & Coineau 1990; Botosaneanu & Holsinger 1991; Notenboom 1991; Coineau & Boutin 1992; Holsinger 1994). Harpacticoids, and copepods in general, are common representatives of the groundwater fauna but only rarely (Sewell 1956; Enckell 1969; Lewis 1986; Rouch 1986; Wells 1986a; Reid 1993) it has been attempted to explain their distribution at different spatial scales in a palaeobiogeographical perspective. General conclusions on the distributional patterns of freshwater (especially groundwater) copepods are still problematic, since a great number of taxa thought to be cosmopolitan in distribution turn out, upon α -taxonomical

re-examination, to assume a much more restricted distribution, as a result of past taxonomic confusion (Reid 1998). Moreover, the existence of sibling-species further compounds the problem. Finally, lack of data obscures the true distributional patterns of many species. A similar situation (Wells 1986b; Huys 1992; Todaro *et al.* 1996) pertains to the meiofauna of coastal marine environments ('meiofauna paradox', Giere 1993). Apart from the above considerations, if some wide distributional patterns of stygobiont copepods could often be explained by a dispersal model, an alternative historical explanation by vicariance (Rosen 1976) appears to be more congruent with the restricted distribution of many stygobiont species, endemic to one site or to a single microhabitat of a given site, owing to their low dispersal abilities.

With regard to the genus *Pseudectinosoma*, with the exception of the 'marine' species *P. minor*, all remaining species show a 'spot' distribution (Fig. 7) and restricted ecological requirements (Table 4), suggesting a vicariant origin for the species. The amphiatlantic distribution of the genus could be related to the opening of the Atlantic Ocean in the Late Cretaceous or Early Tertiary. Moreover, *P. minor*, linked to oligohaline littoral biotopes, could represent an intermediate level in the evolutionary history of the subterranean freshwater species of the genus from a more ancient marine ancestor (a '*Sigmatidium*-like ancestor'). On the other hand, *P. vandeli*, *P. kunzi*, *P. reductum* and *P. janineae* sp. n. are true stygobiont species, endemic to isolated karstic systems or phreatic waters in the Mediterranean area. Probably, the marine littoral ancestor lived along the shore-lines of the Mediterranean, but, in contrast to this fundamental assumption, no representatives of the genus are known from this basin. The branching pattern of the cladogram of the *Pseudectinosoma* species suggests a good correlation between both pattern of relationships among the species and the sequence of epicontinental sea regressions in the Mediterranean Region, during Miocene times. Particularly, both *P. vandeli* and *P. janineae* sp. n. are

Table 3 Character matrix used in the cladistic analysis (1 = apomorphic character state; 0 = plesiomorphic character state).

Characters	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
<i>S. difficile</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>S. rouchi</i>	0	0	0	0	0	0	0	0	1	1	1	1	0	0	1	0
<i>S. noodti</i>	0	0	0	0	0	0	0	0	1	1	1	1	1	0	1	0
<i>S. parvulum</i>	0	0	0	0	0	0	0	0	1	1	1	1	0	1	0	1
<i>S. triarticulatum</i>	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0
<i>S. kunzi</i>	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0
<i>P. minor</i>	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0
<i>P. vandeli</i>	1	1	0	1	1	1	1	0	1	1	1	1	1	0	1	0
<i>P. kunzi</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0
<i>P. reductum</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0
<i>P. janineae</i> sp. n.	1	0	0	1	1	1	1	0	0	0	1	0	0	0	0	0

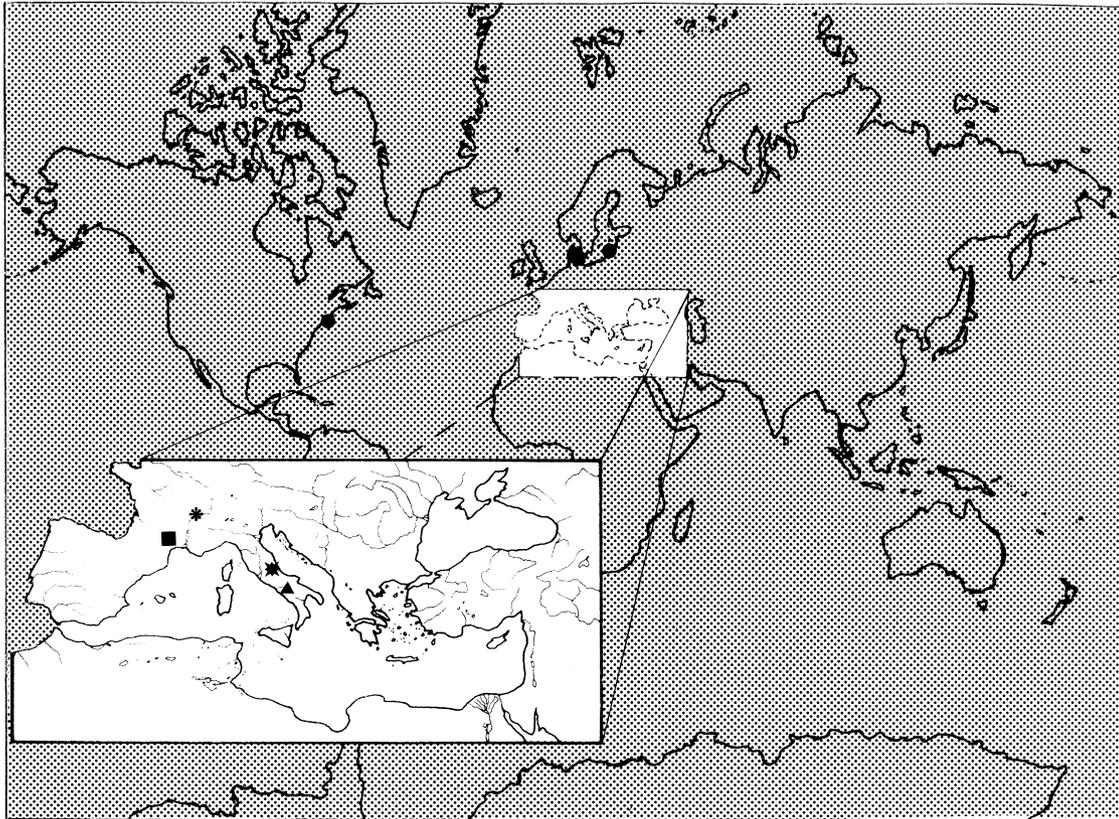


Fig. 7 Distribution of the genus *Pseudectinosoma* Kunz (● = *P. minor* Kunz, ■ = *P. vandeli* (Rouch), ▲ = *P. kunzi* Galassi, ■ = *P. reductum* Galassi & De Laurentiis, * = *P. janineae* sp. n.).

distributed in areas flooded by the Tethys Sea at Burdigalian (Steininger *et al.* 1985; Dercourt *et al.* 1993). The regressions of this continuous sea arm, dated in Late Serravallian for the High Rhône area and in Early Tortonian for the Hérault Massif, might represent the primary events which caused the fragmentation of the marine ancestor's range, leading to an independent evolution of the isolated populations into groundwaters. This palaeogeographical scenario is only little affected by a subsequent peripheral marine-brackish influence, during the basal Pliocene transgression of the Mediterranean Sea, quoted both in the high Rhône area and in the Hérault Massif, some kilometres away from the sampling sites of *Pseudectinosoma* (Demarcq & Barbillat 1971; Bessedik 1985; Clauzon *et al.* 1990; Suc *et al.* 1992). The high number of synapomorphies shared by *P. kunzi* and *P. reductum*, which seem to be the most derived species within the genus, as well as their distribution in Messinian sedimentation areas in Italy (Parea & Ricci-Lucchi 1972; Di Nocera *et al.* 1976, 1981; Aprile *et al.* 1979; Ortolani *et al.* 1979; Torre *et al.* 1988; Bigi *et al.* 1996), corroborate a Messinian isolation of the ancestor's populations into inland groundwaters. As a result, the

hypothetical chronological sequence of the primary vicariance events appears to be in line with the phylogenetic relationships among species, whereas the phylogenetically older *P. janineae* sp. n. is distributed in continental areas

Table 4 Ecology and distribution of the *Pseudectinosoma* species.

<i>Pseudectinosoma</i> species	Ecology	Distribution
<i>P. minor</i>	epipsammic, epiphytic, coastal brackish waters	amphiatlantic, Baltic Sea, Germany; salt marshes of New Jersey, U.S.A.
<i>P. vandeli</i>	stygobiont, karstic spring, not interstitial	endemic, Cent-Fons karstic system, Hérault Massif, France
<i>P. kunzi</i>	stygobiont, phreatic lake, not interstitial	endemic, Castelvivita Cave, Alburni Massif, south Italy
<i>P. reductum</i>	stygobiont, karstic spring, not interstitial	endemic, Presciano spring system, Gran Sasso Massif, central Italy
<i>P. janineae</i> sp. n.	stygobiont, phreatic and hyporheic, upwelling areas, phreatobiont	endemic, alluvial plain of the Rhône River, France

already uplifted since the Early Middle Miocene, and the phylogenetically younger *P. kunzi* and *P. reductum*, in areas covered by seawaters until the Late Miocene.

Combining the assumption based on the ancient Tethyan origin of the genus together with the fact that the stygobiont species of *Pseudectinosoma* appear to be restricted to circum-Mediterranean groundwater, we are led to believe (Galassi *et al.* 1997) that their ancestor invaded groundwaters before or during the Messinian salinity crisis (Hsü *et al.* 1973; Hsü 1978). As a matter of fact, the Upper Tertiary was a period of drastic changes in the Mediterranean marine biota, which may have provided the suitable landscape that is reflected both in the increasing speciation in groundwater and in the extinction of the putative littoral ancestor from the hypersaline Mediterranean biotopes. Moreover, the evaporative Messinian is often quoted by many authors to explain some enigmatic relict distributions, in circum-Mediterranean freshwater and anchihaline stygohabitats, of other invertebrate taxa with marine relatives traceable only outside the Mediterranean Sea (Stock 1980; Holsinger 1988; Danielopol *et al.* 1994; Carola *et al.* 1995; Bartsch 1996; Jaume & Boxshall 1996b,c).

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