# Phylogeny of western Mediterranean Leptodirini, with an emphasis on genital characters (Coleoptera: Leiodidae: Cholevinae)

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**Abstract.** The tribe Leptodirini (Leiodidae: Cholevinae) is one of the largest radiations of Coleoptera in the subterranean environment. Although subjected to systematic and evolutionary studies, the phylogeny remains poorly understood. We assessed the phylogeny of the western Mediterranean lineages (Iberian Peninsula, Pyrenees and Sardinia) based on a cladistic analysis of fourteen characters of external morphology and twenty characters of the male and female genitalia, studied in 182 species belonging to thirty-nine genera. We tested the monophyly of the traditional two main divisions of the group (infraflagellates and supraflagellates), as well as that of some 'phyletic series'. The final matrix contained fifty-eight terminal taxa, twenty-four of which had different character state combinations. The strict consensus of the sixty most parsimonious trees recovered a monophyletic Leptodirini, but not their separation into infraflagellates and supraflagellates. The supraflagellates formed a paraphyletic group with respect to the infraflagellates (corresponding to our sampled '*Speonomus*' series), with *Notidocharis* sister to all other included Leptodirini, and *Speonomidius* sister to Leptodirini excluding *Notidocharis*. The series '*Spelaeochlamys*', including the Sardinian genera but excluding *Pseudochlamys*, was recovered as monophyletic with weak support. The '*Quaestus*' series formed a polytomy with *Pseudochlamys* plus the '*Speonomus*' series (including *Bathysciola*), which was recovered as monophyletic with strong support. *Speonomus*, *Bathysciola*, *Quaestus* and *Troglophyes* were para- or polyphyletic. Our results suggested the respective monophyletic origin of the Leptodirini from the Pyrenees (*Pseudochlamys* plus the '*Speonomus*' series) and the Mediterranean coast plus Sardinia (series '*Spelaeochlamys*'). On the contrary, the Leptodirini of the Atlantic north coast of the Iberian Peninsula (series '*Quaestus*' and '*Speonomidius*') were not monophyletic.

## Introduction

Since the discovery of the first subterranean species of Coleoptera (*Leptodirus hochenwartii* Schmidt, 1832 in the Carniolian region of Slovenia), the morphological modifications of the carvernicoles have fascinated entomologists and evolutionary biologists. An apparent limited number of 'morphotypes' in what are supposed to be independent lineages have been described as a striking example of evolutionary convergence (Decu & Juberthie, 1998; Caccone & Sbordoni, 2001). However, this poses problems for systematists, as many characters traditionally used in the higher taxonomy of other families or subfamilies of Coleoptera are either absent as a result of reduction or apparently highly homoplastic as a result of convergence (for example, Newton, 1998; Giachino *et al.*, 1998).

The tribe Leptodirini (Leiodidae: Cholevinae) is the largest of the Coleopteran groups in which most members have exclusively subterranean habits, with morphological

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modifications assumed to be adaptations to this medium (such as the absence of eyes, reduced or absent wings, and depigmentation; Jeannel, 1924; Vandel, 1964). At present, the tribe has 235 recognized genera and *c*. 1800 species (Perreau, 2000), with a mostly Palaearctic distribution (with one Nearctic genus, see below). The highest diversity is found in the Mediterranean basin, in particular in the north and east: Iberian Peninsula, some Mediterranean islands, southern Alps, Balcanic Peninsula, Romania and southern Russia, the Caucasus, Middle East and Iran (Perreau, 2000, 2004). The monotypic genera *Coreobathyscia* Szynczakowski in South Korea, *Fusi* Perkovsky and *Proleptodirina* Perkovsky in eastern Russia, and *Sinobathyscia* Perreau in the Hubei region in eastern China (Newton, 1998; Perreau, 2000, 2004) are the only leptodirines found east of Iran. The only Nearctic representatives are two species of *Platycholeus* Horn in California, Nevada and Oregon, which are winged and not subterranean (associated with decaying conifer logs; Newton, 1998). Their phylogenetic position is uncertain, although they have been postulated to have many plesiomorphic character states (for example, Newton, 1998). Most genera and species of Leptodirini have very restricted distributions, and thus are suitable for biogeographical studies.

The classification of the leptodirines, and of cholevids in general, has been the subject of much controversy (see Newton, 1998 for a detailed account). Here, we follow Lawrence & Newton (1995), Newton (1998) and Perreau (2004), and consider them to be a tribe within Cholevinae, which would be a subfamily of a more inclusive Leiodidae. Other than questions of rank, there are several alternative classifications. The first author to deal with the group was Reitter (1889), who characterized three morphological 'models' for the general body shape (bathyscioid, pholeuonoid and leptodiroid) and used them to classify the genera within the lineage. The works of Jeannel (1910a, b, 1911, 1924, 1955) established a system of informal groups ('phyletic series') based on male genitalia and certain other external characters, with an explicit phylogenetic intention. This author was the first to consider characters of the internal sac of the aedeagus and the lateral stylus of the tegmen (i.e. the parameres), which he considered to be constant characters with high phylogenetic value, corresponding to '... en quelque sorte à la signature d'une série phylétique' (Jeannel, 1922). In Jeannel (1924), the group was revised as a whole (under the name 'Bathysciinae Horn, 1880') for the first time, and, in Jeannel (1955), what is still considered by some authors to be the main division within the lineage was established, based on the structure of the basal region of the internal sac of the aedeagus: the 'supraflagellates' (with the internal sac of the aedeagus with a basal, dorsal flagellum) and the 'infraflagellates' (with the internal sac of the aedeagus with a basal, Y-shaped ventral piece, the 'Y-piece') (see also Giachino *et al*., 1998). Laneyrie (1967, 1969, 1978) proposed an alternative ordination in four main lineages, also based on the structures of the basal region of the internal sac of the aedeagus. Guéorguiev (1974a, b, 1976) formalized Jeannel's system in

seven subtribes, followed by Newton (1998) (although with the recognition of their non-monophyly), and partly by Perreau (2004), defined on external morphology (insertion of antennae, tarsal formula, number, type and arrangement of the tibial spines). Subsequent workers introduced modifications of the system, leading to discordant phylogenetic scenarios based on alternative, and contradictory, sets of characters, such as Giachino & Vailati (1993) and Giachino *et al*. (1998) based on characters of the internal sac of the male genitalia and the tarsal formula. The latter authors accepted the division of the subterranean Leptodirini in Jeannel's (1955) infraflagellates and supraflagellates. Within these two main clades, the 'phyletic series' of Jeannel (1924) mostly have been assumed to be monophyletic (Giachino *et al*., 1998; Fresneda & Salgado, 2000; Salgado, 2000; Salgado & Fresneda, 2003).

The western Mediterranean fauna studied here includes the Iberian Peninsula (plus adjacent mountain massifs in south France) and Sardinia. It includes *c*. forty genera with some 230 species, all of them endemic (Appendix 1), and mostly strictly troglobiontic (some are endogeous or muscicolous; for example, species of *Bathysciola* or the genus *Notidocharis*). Most of the genera (thirty-three) and species (*c*. 160) are found in the Iberian Peninsula, with only two endemic genera in Sardinia and some species of Pyrenean genera occurring on the Atlantic side. No Leptodirini are known from the Balearic Islands. Corsica has three species currently included in *Speonomus* and five species in *Parabathyscia*, a genus included in the '*Bathysciola*' series *sensu* Perreau (2000), with Alpine and eastern European affinities.

Iberian and Pyrenean Leptodirini were arranged by Bellés *et al*. (1978) according to Guéorguiev (1976) and predecessors, using fundamentally the structure of the internal sac of the aedeagus. In this work, Iberian species were arranged in four 'sections', with mostly allopatric distributions: *Anillochlamys* (Mediterranean coast), *Speocharis* (Cantabrian Mountains), *Speonomus* (Pyrenees and pre-Pyrenees) and *Bathysciola* (with a wide distribution), the last two considered to be closer to each other. In Bellés (1984), a standard terminology was established, allowing their use for the first time at lower taxonomic levels for species separation or description of new genus-level taxa (for example, Bellés, 1983, 1984; Comas, 1983; Comas & Escolà, 1989; Dupré, 1989, 1990, 1991; Fresneda & Hernando, 1994; Fresneda *et al*., 1994). In Dupré (1992), the structure of the internal sac of the aedeagus was revised, and a new system for the classification of the taxa was proposed. More recent work on Iberian Leptodirini includes the systematic revision of the different lineages: supraflagellates of the series '*Quaestus*' and '*Speonomidius*' (Salgado, 1975, 1993, 2000), supraflagellates of the series '*Spelaeochlamys*' (Salgado & Fresneda, 2003) and infraflagellates (Fresneda & Salgado, 2000). Characters of the female genitalia have been considered only recently in the classification of the group, following Perreau (1989) (for example, Fresneda, 1998; Fresneda & Salgado, 2000; Salgado, 2000; Salgado & Fresneda, 2003).

The current systematics of Leptodirini is based mostly on characters of the genital apparatus, supposedly not affected

by the constraints of the subterranean environment, unlike external structures (Jeannel, 1955). The complexity of the internal sac of the male aedeagus provides a potential source of characters, which so far has been used only informally, or for a few taxa (for example, Giachino *et al.*, 1998; Salgado, 2000; Salgado & Fresneda, 2003). The main objective of this study was to conduct the first formal cladistic analysis of the main lineages of western Mediterranean Leptodirini, emphasizing the Iberian fauna and the genitalic characters of both sexes.

Amongst the supraflagellates, Iberian lineages include Jeannel's (1924) series 'Quaestus' and 'Speonomidius' in the Cantabrian area (the 'Speocharis' section of Bellés *et al.*, 1978) and the series 'Spelaeochlamys' in Catalonia, the Mediterranean coast down to Alicante and Sardinia (the 'Anillochlamys' section of Bellés *et al.*, 1978). The two Sardinian genera have been considered as a separate series ('Ovobathysciola') by some authors (for example, Perreau, 2000).

The infraflagellates are distributed throughout most of the northern Mediterranean basin, with a group ('theleomorphes') with a predominantly eastern Mediterranean distribution (not sampled). The remaining infraflagellates include, in the western Mediterranean, the series 'Speonomus' (including the genus *Bathysciola*), distributed in the Pyrenees, Catalonia, Sardinia and the Vasc Country. According to the classification of Guéorguiev (1976), followed by some modifications by Newton (1998) and Perreau (2004) (in which Leptodirini were considered as a tribe, and thus all infra-subfamily categories were downgraded accordingly), of the seven subtribes of Leptodirini, all western Mediterranean genera would be included in the subtribe Pholeuina.

A study of the Iberian, Pyrenean and Sardinian fauna allows the testing of the following: (1) the monophyly of the two main divisions of subterranean Leptodirini according to Jeannel (1955) and Giachino *et al.* (1998) (infraflagellates and supraflagellates); (2) the monophyly of each of the series 'Quaestus', 'Speonomidius', 'Spelaeochlamys' and 'Speonomus' (including the Pyrenean *Bathysciola*) as currently recognized (Fresneda, 1998; Fresneda & Salgado, 2000; Salgado, 2000; Salgado & Fresneda, 2003; Fresneda & Salgado, 2006); and (3) the recognition of possible monophyletic lineages within each of these series. As our ingroup sampling corresponds to the subtribe Pholeouina, we cannot test the monophyly of the subtribes of Leptodirini as arranged in Newton (1998) and Perreau (2000, 2004).

## Materials and methods

### Taxa studied

We studied most genera, subgenera and species groups of Leptodirini of the Iberian Peninsula, Pyrenees and Sardinia (see Appendix 1). Following Salgado (2000) and earlier

suggestions (for example, Bellés *et al.*, 1978), we included the Pyrenean species of *Bathysciola* in the series 'Speonomus', which had been considered previously as an isolated lineage by Jeannel (1910a, 1914) and Zoia & Rampini (1994), also followed by Perreau (2000) in his catalogue. The genera *Breulites* and *Cantabrogeus* were included in the series 'Quaestus', following Salgado (2000).

Included in the analyses were 182 species in thirty-nine genera. We did not study the genera *Bathysciella*, *Eskualdunella*, *Gesciella* and *Trocharanis*, and some groups within *Speonomus* (subgenera *Machaeroscelis*, *Metaspeonomus* and 'ehlersi' and 'zophosinus' groups) (see Appendix 1 for their distributions and number of species).

The tree was rooted using two examples of different tribes of Cholevinae: Cholevini (*Catops* sp.) and Anemadini (*Speonemadus* sp.) (Appendix 1).

### Morphological analyses

All studied material was collected by JF and JMS, with the exception of a few specimens obtained from colleagues or institutions (see 'Acknowledgements'). Fresh material was preserved in 70% ethanol or mounted dry.

For the study of the genitalia, the following procedure was employed: (1) dissection of the last three abdominal sternites under the microscope; (2) maceration in 10% KOH for 2 h; (3) dehydration for several minutes in a series of increasing ethanol concentration (70–96%); (4) 2-h immersion in xylol; (5) inclusion in Canada balsam on a transparent acetate label, pinned together with the dry specimen. Drawings were outlined from digital microscopic photographs (Olympus C5060WZ, Tokyo, Japan).

Voucher specimens of all studied species are deposited in the authors' collections and in the collections Giachino (Torino), Museu de Zoologia (Barcelona), Museo Nacional de Ciencias Naturales (Madrid) and Muséum National d'Histoire Naturelle (Paris).

### Selection of characters

This first formal cladistic analysis of the species of Leptodirini follows many informal phylogenetic hypotheses proposed for the group. We used all characters considered relevant in the literature for the deep phylogenetic relationships within Leptodirini, plus others newly described here. Many had been observed previously in only a few taxa, and we provide here a wide survey of their occurrence in a comprehensive representation of the species of Leptodirini. A detailed analysis of the structures of the internal sac is conducted here for the first time in a broad phylogenetic context, although they have been used extensively in the taxonomy of the group, and the main structures (for example, the Y-piece and the presence of a stylus) were known to Jeannel (1924).

Some characters used habitually for the separation of species or species groups were not included in the analyses,

as their homology across the tribe could not be established readily (see below). All characters were constructed to be binary. Character states present in the outgroups were coded '0' by convention.

The final data matrix included thirty-four characters of external morphology and of male and female genitalia (see 'Results'). Character states absent in a particular taxon, or of doubtful homology, were coded as missing (Appendix 3). Characters of the external morphology of troglobitic species, assumed to be strongly affected by selection, could be highly homoplastic and introduce artefacts in the analyses (for example, Marquès & Gnaspini, 2001). Characters of the male and female genitalia, in contrast, would be less affected by habitat constraints and provide a more reliable phylogenetic signal (Jeannel, 1955). We considered all characters equally in our analyses (Grandcolas *et al.*, 2001; Desutter-Grandcolas *et al.*, 2003), and tested the congruence between external and internal characters with the partition homogeneity test (PHT) (Swofford, 2002), which is the implementation of the incongruence length difference (ILD) test (Mickevich & Farris, 1981 Farris *et al.*, 1994) using 1000 iterations of fifty replicas each, not saving multiple trees. The utility of the ILD test as a measure of incongruence has been criticized recently, mostly in the context of molecular data (see, for example, Barker & Lutzoni, 2002), and its interpretation is questionable. However, the character number is too small for any alternative procedure (for example, measures of topological congruence or partitioned Bremer support; Baker & DeSalle, 1997).

### Phylogenetic analyses

The final data matrix was analysed with PAUP 4.0b10 (Swofford, 2002) using parsimony. Shortest trees were heuristically searched with 10 000 tree bisection–reconnection (TBR) replicas, swapping on all multiple starting trees and saving all of them. Branches were collapsed when the branch length was zero. Node support was measured with non-parametric bootstrap (Felsenstein, 1985) using 1000 iterations of thirty TBR each, with the 'save multiple trees' option not enforced, and with Bremer support values (Bremer, 1994). To obtain a higher resolution, data were reweighted successively according to the rescaled consistency index (Swofford, 2002), and a heuristic search was conducted on the initial set of shortest trees. Character transformation was mapped using MACCLADE 4.0 (Maddison & Maddison, 2000), employing the strict consensus of the trees obtained after reweighting the characters and mapping only unambiguous changes.

To test alternative hypotheses of monophyly (see 'Results'), nodes were constrained using MACCLADE, and PAUP searches (using the strategy outlined above) were conducted enforcing the constrained nodes as a backbone tree. The shortest trees obtained with the backbone constrained were compared with the shortest unconstrained tree via the non-parametric Wilcoxon signed-rank test of Templeton (1983).

## Results

### Excluded characters

*External morphology*. The length of the antenna, relative length of the antennomeres, shape of the pronotum and tip of the elytra (used by, for example, Salgado, 1994, 2000; Salgado & Tizado, 2005) were excluded as too difficult to categorize.

*Genital characters*. The shape of the median lobe of the aedeagus has been used traditionally to distinguish between the series '*Spelaeochlamys*' (including *Pseudochlamys*) (supposedly with the apex strongly sinuate in lateral view) and the rest (regularly curved in lateral view) (Salgado & Fresneda, 2003). However, these differences are unclear and difficult to categorize: see, for example, the aedeagus of *Naspunius eseranus* (Lagar) (fig. 42 in Fresneda, 1998), supposedly with a regular curvature of the median lobe, or the aedeagus of *Paranillochlamys catalonicus* (Jeannel) (fig. 32 in Salgado & Fresneda, 2003), supposedly with a sinuated apex. Other quantitative measures of the median lobe of the aedeagus and the spermatheca (size of the relative parts, thickness of the sclerotized parts) were also excluded (used at species-level separation in, for example, Fresneda, 1998).

Some genera or species groups of the series '*Speonomus*' have complex small sclerotized pieces in the internal sac of the aedeagus, useful for species separation (for example, Dupré, 1989, 1990, 1998; Fresneda, 1998). However, these pieces are not present in most species of Leptodirini, and thus their usefulness for deep-level phylogenies within the tribe is very limited, and they were not considered here.
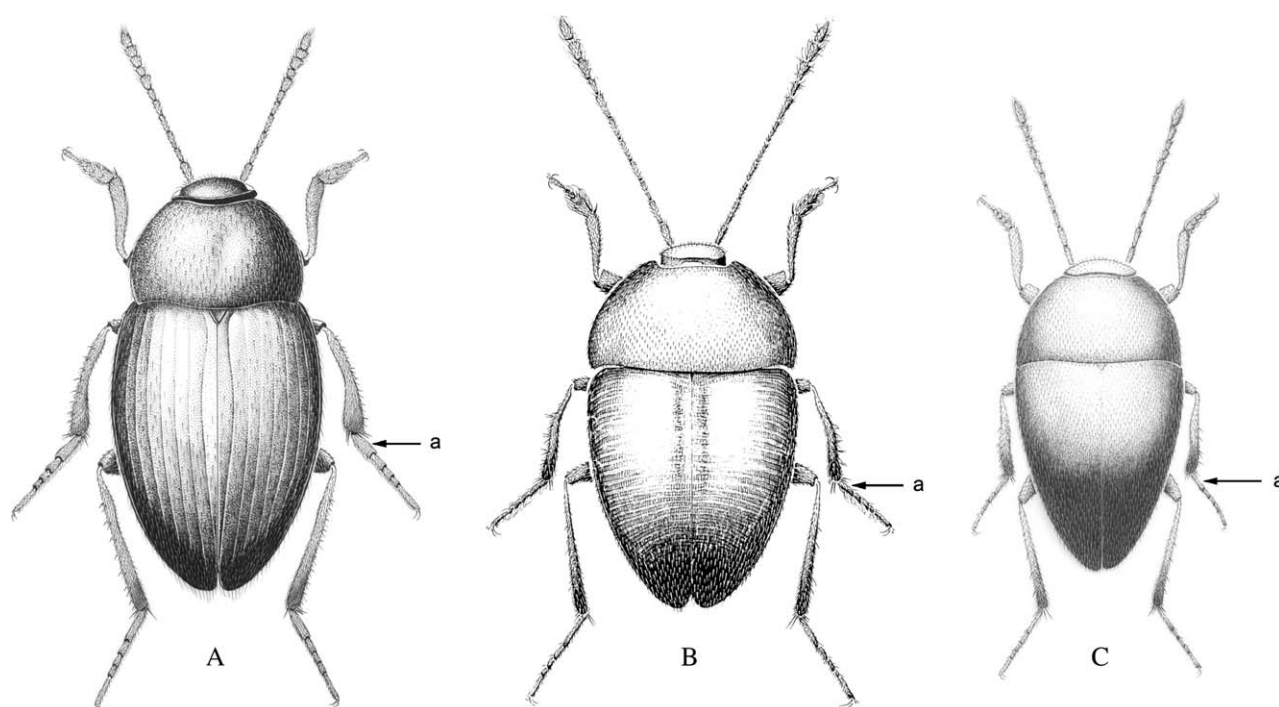
### Character list

For each character, the number of reconstructed transitions in the strict consensus of the trees, obtained after reweighting the characters (see below and Fig. 15), is given. When the consensus tree is unresolved, we give the range of possible changes.

#### Head.

1. *Eyes*: present (even if reduced to a small number of ommatidia) (0) (Fig. 1A); absent (1) (Fig. 1B, C).

Although amongst all subfamilies of Leiodidae some species lack eyes (Jeannel, 1924; Salgado & Tizado, 2005), both outgroups were coded as 'eyes present'. Within the studied Leptodirini, only the species of the genus *Notidocharis* have vestigial eyes (Salgado, 2000), all the others being anophthalmous. Amongst other Leptodirini not included in our study, pigmented eyes are present in the Nearctic species, in three species of *Bathysciola* from the Caucasus and the eastern Palaearctic (Jeannel, 1924) (although greatly reduced) and in *Adelopsella bosnica* (Reitter). However, as a result of the uncertain systematics regarding both

**Fig. 1.** Male habitus: A, *Catops nigricans* Spence, with dilated first segment of the mesotarsi (character 3 = 0); B, *Phacomorphus (Phacomorphus) fratyi* (Dupré), with non-dilated first segment of the mesotarsi (character 3 = 1) and transverse strioles on the elytra (character 6 = 1); C, *Quaestus (Asturianella) incognitus* Salgado & Fresneda, with irregular punctation on the elytra (character 6 = 0). a, first mesotarsomere.

members of *Bathysciola* (see 'Discussion') and *Adelopsella* (with males with dilated mesotarsi, see below), it is not possible to discuss the evolution of this character other than within the included species. One change.

*Legs.*

2. *Number of female protarsomeres*: five (0) (Fig. 2A); four (1) (Fig. 2B).

All tribes of Cholevinae, except Leptodirini, have five protarsomeres in both sexes. All females of the studied Leptodirini have four protarsomeres (including males of 'theleomorphes', not sampled here) (Casale *et al.*, 1991; Giachino *et al.*, 1998). One change.

3. *Tarsomeres of the male mesotarsi*: some tarsomeres dilated (0) (Fig. 1A); non-dilated (1) (Fig. 1B, C).

All studied species of Leptodirini have non-dilated male mesotarsomeres. The variation in the number of dilated tarsomeres in Cholevinae has been interpreted as a 'transformation series' (Giachino *et al.*, 1998), with the non-dilated condition of Leptodirini as plesiomorphic within the subfamily. One change.

4. *Internal tibial spurs*: simple (0) (Fig. 3A, B); pectinate or polytoothed (in the sense of Vailati, 1988) (1) (Fig. 3C–F).

All studied species of Leptodirini have pectinate or polytoothed internal tibial spurs. One change.

5. *Tarsal empodium*: with two articulated setae or small lobes (in some species one is greatly reduced) (0); with one non-articulated seta (split into two in the studied species) (1).

All studied species of Leptodirini have one non-articulated seta between the claws in the oniquium, by contrast with other tribes of Cholevinae (Newton, 1998). One change.

*Dorsal sculpture.*

6. *Dorsal sculpture*: surface of elytra with transverse strioles, which could disappear towards the apex (0) (Fig. 1B); elytra covered with an irregular punctation (1) (Fig. 1A, C).

Several authors have considered the presence of transverse strioles in the elytra to be a plesiomorphy of the subfamily Cholevinae (for example, Blas, 1981; Giachino & Vailati, 1993). From a hypothetical striolated ancestor, the elytral sculpture would have derived towards two states: (1) a reduction in the strioles, as observed in Cholevini and some Leptodirini; (2) a further development of the strioles, with a more complex design (i.e. longitudinal strioles), as in, for example, the genera *Anemadus* and *Speonemadus* (Anemadinae). The ancestral condition of the character in
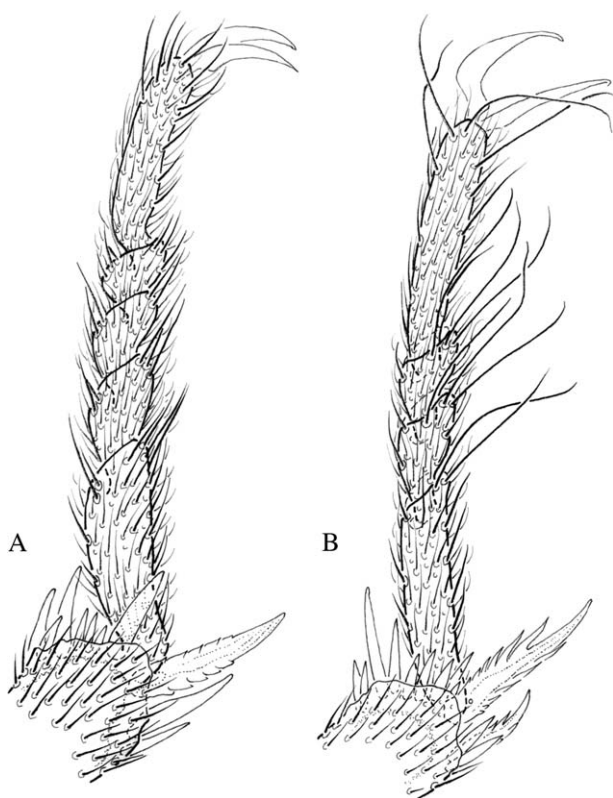
**Fig. 2.** Female protarsi: A, *Catops fuliginosus* Erichson, with five segments (character 2 = 0); B, *Trapezodirus gimenezi* (Fresneda, Hernando & Lagar) with four segments (character 2 = 1).

Leptodirini, as reconstructed in the preferred tree (see Fig. 15), is that of elytra covered with transverse strioles, as in *Speonemadus*. According to the same tree, the irregular punctation of the elytra is independently developed in *Catops* and in the origin of the clade grouping the series 'Spelaeochlamys', 'Quaestus' and 'Speonomus'. There is a reversal to the transverse strioles in the series 'Speonomus' (excluding part of *Bathysciola*). Three changes (one reversal).

*Thorax.*

7. *Metathoracic wings*: present (0); absent (1).

All Leptodirini included in the study are apterous. One change.

8. *Mesocoxal cavities*: fused (0) (Fig. 4A); separated by an extension of the mesoventrite, with a keel (1) (Fig. 4B–D).

All studied Leptodirini have mesocoxal cavities separated by a more or less developed keel, whereas Cholevini and Anemadini have confluent mesocoxal cavities. However, other subfamilies or Leiodidae, not included as outgroups, also have non-confluent mesocoxal cavities (Newton, 1998). One change.

9. *Extension of the mesoventrite*: with a well-defined suture between the mesoventrite and the anteromedian process of the metaventrite (i.e. 'metasternal suture' of authors, cf. Beutel & Lawrence, 2005) (0) (Fig. 4B, C); totally fused with the metaventrite (1) (Fig. 4D).

Most species of the studied Leptodirini have a well-defined lateral suture between the extension of the mesoventrite and the anteromedian process of the metaventrite (the 'metasternal process'). In the genera of the series 'Spelaeochlamys' (except *Patriziella*), the mesoventrite is fused with the anteromedian process of the metaventrite, so that there is no visible suture on the ventral side (Fig. 4D). Viewed laterally, there are indications of a suture starting from the mesocoxal cavities, but not reaching the ventral surface. Species with mesocoxal cavities fused (character 8 = 0) are coded as missing data. This character was mentioned by Laneyrie (1967) for some eastern Mediterranean species of Leptodirini. One change.

10. *Mesosternal keel*: never extending over the metaventrite (0) (Fig. 4C); extending over the metaventrite (1) (Fig. 4B).

The mesosternal keel is well developed, extending over the metaventrite, in the series 'Quaestus' and in *Bathysciola ovata* Kiesenwetter. In the other species, it may have a very variable size, but never reaches the metaventrite. Species with mesocoxal cavities fused (character 8 = 0) are coded as missing data. Three changes (one reversal).

Salgado (1996) described the different types of the mesosternal keel occurring in the series 'Quaestus' and the genus *Notidocharis*. It is present in most of the species of Leptodirini, but not in Cholevini. However, some Leptodirini do not have it, as in, for example, the genus *Troglocharinus*, in which there is a whole series of development from fully absent (*T. espanoli* Jeannel) to a well-developed keel with a denticulated vertex (*T. ferreri* Reitter) (although never reaching the metaventrite).

11. *Metacoxae*: contiguous (0) (Fig. 4A); separated by a metaventrite apophysis (1) (Fig. 4B–D).

All studied Leptodirini have an apophysis in the metaventrite separating the metacoxa (in some species bifid), whereas the Cholevini and Anemadini do not (Giachino *et al.*, 1998). One change.

12. *Suture between the mesoventrite and the anteromedian process of the metaventrite (i.e. 'metasternal suture')*: a single suture between the mesoventrite and the anteromedian process of the metaventrite (likely to be the fusion of the two), running parallel and close to the coxal cavities, delimiting an extremely reduced area (0) (Fig. 5B); two well-defined sutures between the mesoventrite and the anteromedian process of the metaventrite, delimiting an internal area (usually triangular, but
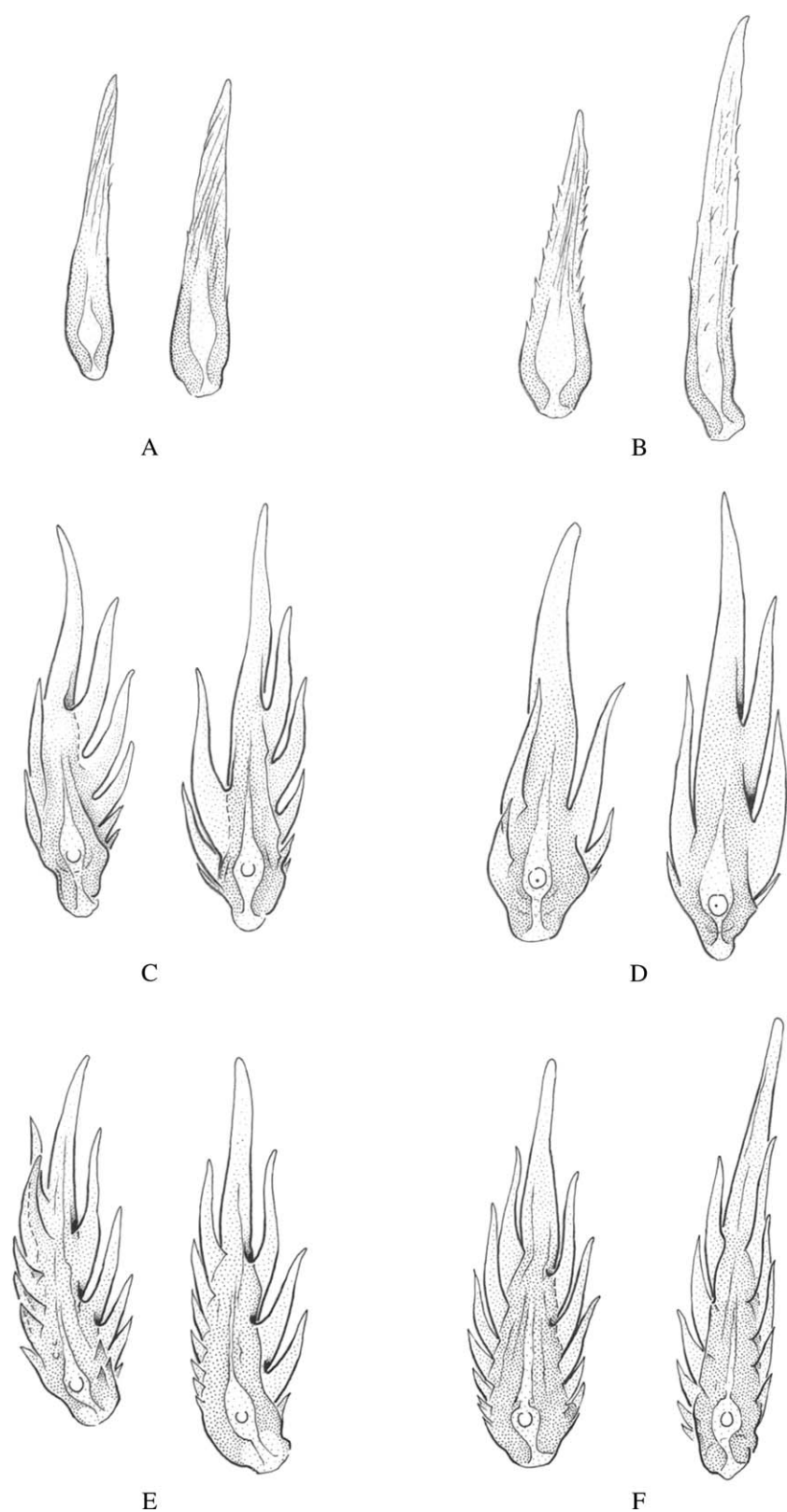
**Fig. 3.** Internal meso- and metatibial spurs: A, B, *Catops fuliginosus*, non-pectinated (character 4 = 0); C, D, *Espanoliella jeanneli* (Bolívar), pectinated, with a reduced number of spines; E, F, *Stygiophyes akarsticus* (Escolà), pectinated, with numerous spines (character 4 = 1 for the last two species).
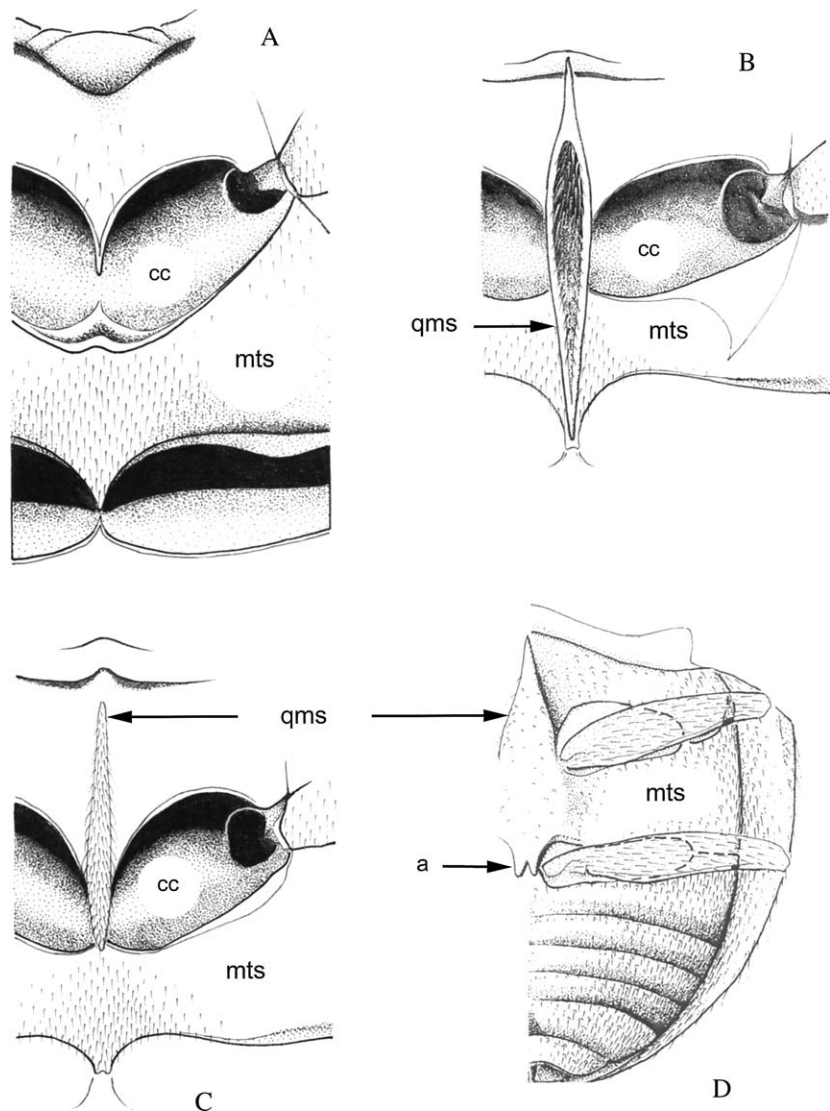
**Fig. 4.** Meso- and metaventrite: A, *Catops fuliginosus*, with mesocoxal cavities fused (character 8 = 0); B, *Quaestus (Quaesticulus) noltei*, with mesocoxal cavities separated by a mesosternal keel (character 8 = 1) which extends over the metaventrite (character 10 = 1); C, *Stygiophyes puncticollis* (Jeannel), with mesocoxal cavities separated by a mesosternal keel (character 8 = 1) which does not extend over the metaventrite (character 10 = 0); D, *Paranillochlamys urgellesi* (Español), with metacoxae separated by a bifid metaventrite apophysis (character 11 = 1). a, bifid metasternal apophysis; cc, coxal cavity; mts, metaventrite; qms, mesosternal keel.

could be reduced forming a more or less narrow string) (1) (Fig. 5A).

The two sutures are well defined, delimiting a triangular area in all supraflagellates with the only exception of *Spelaeochlamys*. Three changes (two reversals).

Some Cholevinae have two well-developed metasternal keels, as in *Sciaphyes sibiricus* Reitter (Sciaphyini) (Perreau, 2000). In the studied species, these are not well developed, and have the appearance of a suture. In the species with clearly visible sutures, the external suture starts in the posterior part of the contact area between the mesepimeron and the mesocoxal cavity, and extends to the posterior margin of the metaventrite, at the edge of the metacoxal cavity. The internal suture starts on the other side of the mesocoxal cavity, in contact with the mesosternal keel, and runs parallel to the mesocoxal cavity first and then goes towards the metacoxal cavity, joining the external suture.

The two sutures and the margin of the coxal cavity form a triangular area, similar to that described for *Sciaphyes* by Perreau (2000). Alternatively, the two sutures may run together crossing the mesocoxal cavity.

There are different degrees of differentiation of these sutures, likely to influence the overall flexibility and resistance of the ventral structures, and thus likely to be of adaptive value in subterranean or endogeous species in close contact with a hard substratum, as with many species of Leptodirini. The character described above ranges from the extreme reduction of the two sutures, with almost complete obliteration of the triangular internal area (for example, the genera of the 'Speonomus' series and the genus *Spelaeochlamys*) to a fusion of the sutures with a strong reduction of the internal area (for example, *Leonesiella* and *Anillochlamys*) and the presence of typical sutures with a well-defined triangular internal area. An intuitive progression (from well-defined sutures to extreme reduction) is contradicted by
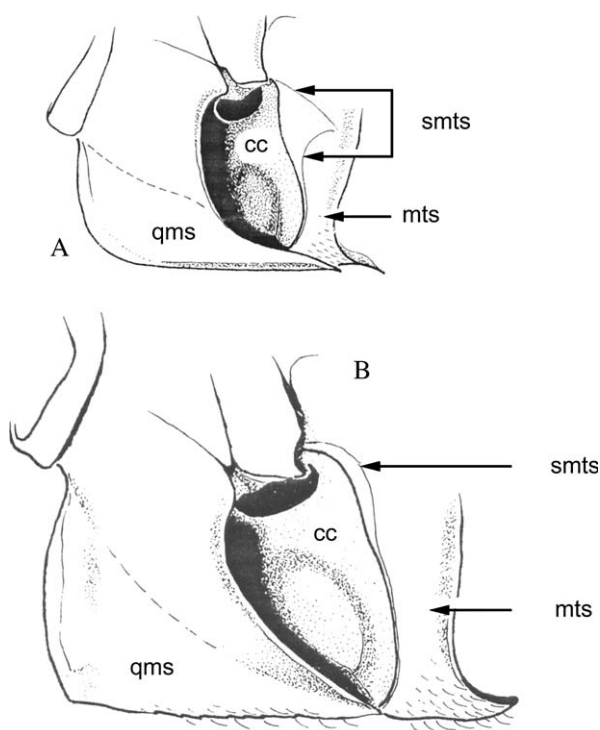
**Fig. 5.** Mesosternal keel: A, *Espanoliella jeanneli*, extending over the metaventrite (character 10 = 1) and with suture between mesoventrite and anteromedian process of metaventrite well defined (character 12 = 1); B, *Aranzadiella leizaolai*, not extending over the metaventrite (character 10 = 0) and with sutures between mesoventrite and anteromedian process of metaventrite reduced (character 12 = 0). cc, coxal cavity; mts, metaventrite; qms, mesosternal keel; smts, suture between mesoventrite and anteromedian process of metaventrite.

species with character states that are considered to be rather plesiomorphic within the group, such as, for example, the genus *Espanoliella*, with almost completely reduced sutures but with an internal sac resembling those of Cholevini and other 'primitive' Cholevinae. This character has never been studied systematically in Leptodirini.

*Genital segment.*

13. *Male ninth sternite (urosternite)*: reduced, either partially, forming an open receptacle for the reception of the apex of the aedeagus, or completely, forming a chitinous ring (0) (Fig. 6A, B); fully developed, forming a closed receptacle for the aedeagus (1) (Fig. 6C).

The ninth urosternite of males is fully developed only in *Speonemadus*. It is partially reduced in *Catops*, and fully reduced in the studied Leptodirini. According to Giachino *et al.* (1998), the plesiomorphic state of the subfamily is the non-reduced chitinous ring. One change.

14. *Female eighth sternite (urosternite)*: smooth (0) (Fig. 7 B); with a depression (1) (Fig. 7A).

The female eighth urosternite has a depression in *Catops*. One change.

*Aedeagus.*

15. *Apical reinforcement bands of the internal sac of the aedeagus*: absent (0) (Fig. 12B); present (1) (Figs 10A, B; 12A).

All studied Leptodirini, with the exception of the series 'Speonomidius', have reinforcement bands in the internal sac of the aedeagus. One change.

16. *Internal sac of the aedeagus*: inerm, without copulating armature (0) (Fig. 12A–C); with an internal armature formed by three sclerotized pieces, allowing the differentiation of three regions (basal, medial and apical) (1) (Figs 10A, B; 11A).

The evolution of the internal sac of the aedeagus in the Cholevinae is complex, with examples of all degrees of development, from inerm and simple to very complex with numerous well-differentiated structures (Dupré, 1989, 1990, 1992, 1998). Amongst the studied taxa, all infraflagellates plus *Pseudochlamys* have a complex internal sac of the aedeagus. One change.

17. *Y-piece in the basal area of the internal sac of the aedeagus*: absent (0) (Fig. 12A–C); present (1) (Figs 10B; 11A).

All studied infraflagellates have a piece with a 'Y' shape in the internal sac of the aedeagus (the Y-piece). One change.

In the original description of the genera *Bellesia* and *Josettekia* (Fresneda & Hernando, 1994 and Bellés & Deliot, 1983, respectively), an 'atypical' Y-piece was described for both of them, which was considered subsequently to be non-homologous to the standard Y-piece by Fresneda (1998) and Salgado (2000), in which the 'section *Josettekia*' was defined as including species otherwise close to the *Speonomus* section, but without a typical Y-piece. Re-examination of the aedeagus of these genera in a wider comparative study shows no reason to consider their 'atypical' Y-piece as non-homologous to the Y-piece of the rest of the species of the *Speonomus* section (see below and figs 1 and 3 in Fresneda & Hernando, 1994 and figs 5 and 1 in Dupré, 1991 and Fresneda, 1999, respectively).

18. *Ventral–basal complex (VBC)*: absent (0) (Figs 11A, B; 12A–C); present (1) (Fig. 10A, B).

In the species with VBC, the internal sac of the aedeagus has a flagellum originating in the union of the two branches of the Y-piece, surrounding the base of the sac, and extending to the apex. The basal part of this flagellum could be enlarged, and it is inserted in a sclerotized tube. All studied infraflagellates have VBC. One change.
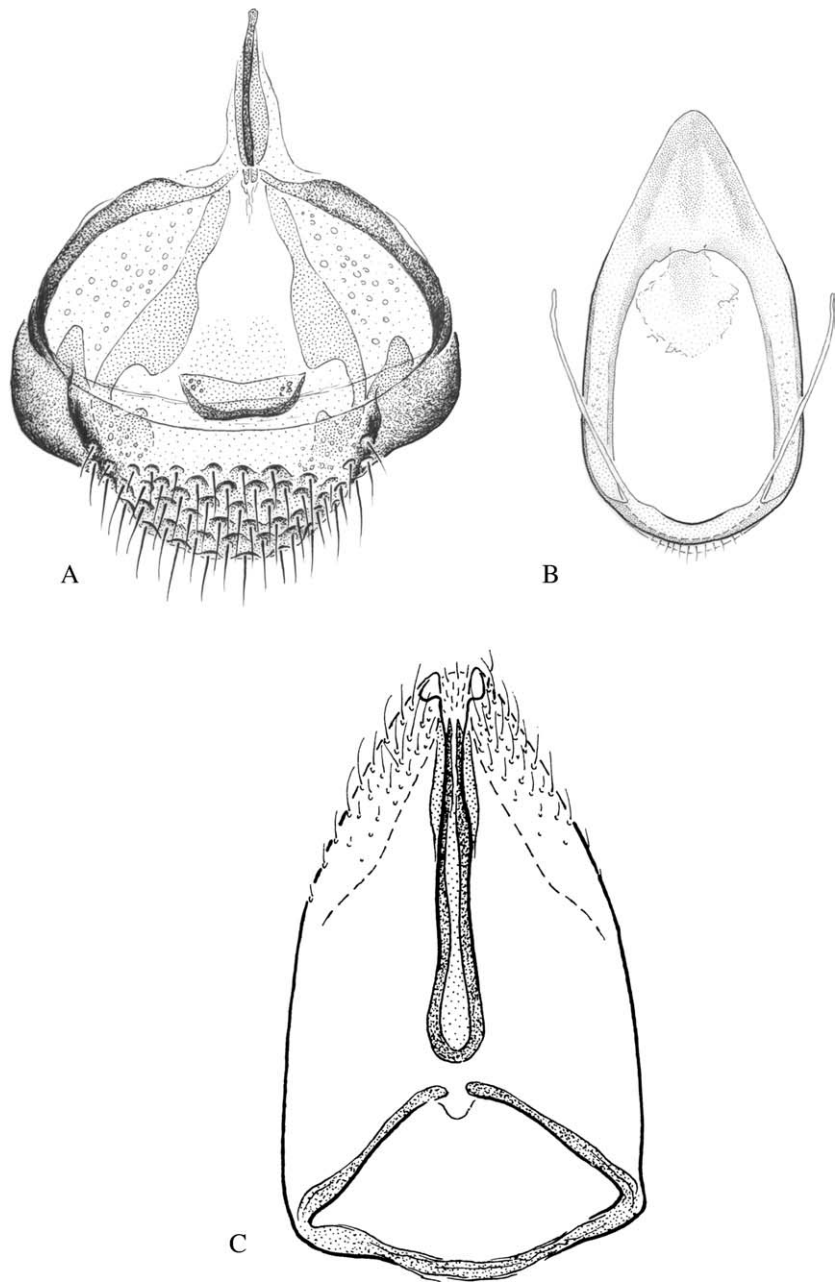
**Fig. 6.** Male ninth abdominal urosternite: A, *Catops fuliginosus*, open and partially reduced (character 12 = 0); B, *Josettekia angelinae* Bellés & Deliot, completely reduced (character 12 = 0); C, *Speonemadus angusticollis* (Kraatz), fully developed, forming a closed receptacle for the aedeagus (character 12 = 1).

19. *Basic structure of the internal sac of the aedeagus*: formed by groups of spines (0) (Figs 11B; 12C); without groups of spines (1) (Figs 10A, B; 11A, B; 12A, B).

The internal sac of the aedeagus of all studied species of Leptodirini does not have spines as a basic structural component, with the exception of the genera *Breuilia*, *Espanoliella* and the species *Quaestus* (*Amphogeus*) *escalerai* Jeannel (which has spines plus a stylus). The genus *Cantabrogeus* has two rows of spines in the basal region of the internal sac of the aedeagus (in addition to other structures), considered to be homologous to similar, very small structures present in the same region in other species of the genus

*Quaestus*, but not to the spines of *Espanoliella*, *Breuilia* and *Q. escalerai*, which are more distal (Fig. 12C). Three changes (two reversals).

20. *Stylus of the internal sac of the aedeagus*: absent (0) (Figs 10A, B; 11A, B; 12C); present (1) (Figs 8A, B; 12A, B).

In all studied species of supraflagellates, with the exception of *Pseudochlamys*, *Breuilia*, *Espanoliella* and *Breuilites*, the internal sac of the aedeagus has a stylus of variable length, from a small dent with a complex shape to a straight
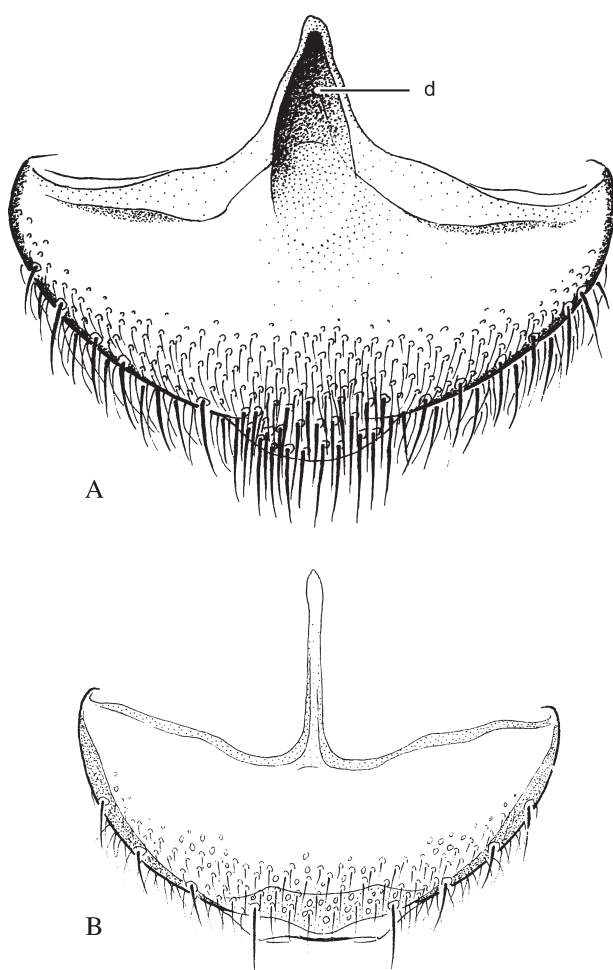
**Fig. 7.** Female eighth abdominal urosternite: A, *Catops fuliginosus*, with a depression (character 14 = 1); B, *Espanoliella jeanneli*, smooth (character 14 = 0). d, depression.

stylus as long as the full sac. Three to four changes (two to three reversals).

21. *Stylus of the internal sac of the aedeagus (II)*: well developed (0) (Fig. 12A, B); reduced to a small dent (1) (Fig. 8A, B).

Species without a stylus (character 20 = 0) are coded as missing data. The stylus of the internal sac of the aedeagus is reduced to a small dent in the series '*Spelaeochlamys*', with the exception of *Paranillochlamys*. Two changes (one reversal).

22. *Basal region of the internal sac of the aedeagus*: without membranous conduct (0) (Figs 8A–C; 10A, B; 11A, B; 12B, C); with a membranous conduct covered with scales or spicules, including the stylus (when present) (1) (Fig. 12A).

In some species, the stylus in the internal sac of the aedeagus is placed inside a membranous conduct, sometimes

with different kinds of sclerotized structures in the surface (scales, spicules, spines). All species of the series '*Quaestus*' with a stylus have character state 1, with the exception of *Oresigenus*. The genus *Breuilites*, without a stylus, has character state 1. One change.

23. *Symmetrical feather-like structures at both sides of the medial region of the internal sac*: absent (0) (Figs 8A; 11B; 12A–C); present (1) (Figs 10A, B; 11A).

All studied infraflagellates plus *Pseudochlamys* have feather-like structures in the internal sac of the aedeagus. One change.

24. *Axial piece (ap) and armature of the axial piece (aap)*: absent (0) (Figs 8A; 11A, B; 12A–C); present (1) (Fig. 10A, B).

All studied infraflagellates have a tubule (ap) with a more sclerotized piece at the apex (aap) in the medial part of the internal sac of the aedeagus. One change.

25. *Base of the apical reinforcing bands of the internal sac of the aedeagus*: inerm (0) (Figs 8A; 11A, B; 12A–C); with well-defined structures (1) (Fig. 10A, B).

All studied infraflagellates have well-defined structures (for example, sclerotized nodules, membranous ligulae) in the apical reinforcing bands of the internal sac of the aedeagus. One change.

26. *Apex of the lateral stylus of the aedeagus*: without a penicillum (0) (Fig. 9A, C, E, F); with a penicillum (1) (Fig. 9B, D).

Some genera within the series '*Speonomus*' have a more or less developed tuft of setae (the 'penicillum'; Fresneda, 1998) on the apex of the lateral stylus of the aedeagus. One to three changes.

27. *Apex of the lateral stylus of the aedeagus (II)*: without lateral excavation (0) (Fig. 9A–C, E, F); with a well-defined lateral excavation (1) (Fig. 9D) (Fresneda, 1998).

Some genera within the series '*Speonomus'* have a well-defined, spoon-shaped lateral excavation in the apex of the lateral stylus of the aedeagus. The genus *Bellesia* has a funnel-shaped cavity, which was not considered to be homologous with this structure. One change.

28. *Apex of the lateral stylus of the aedeagus (III)*: with two setae (0) (Fig. 9A); with more than two setae (1) (Fig. 9B–F).

All studied Leptodirini have more than two setae in the apex of the lateral stylus of the aedeagus. One change.
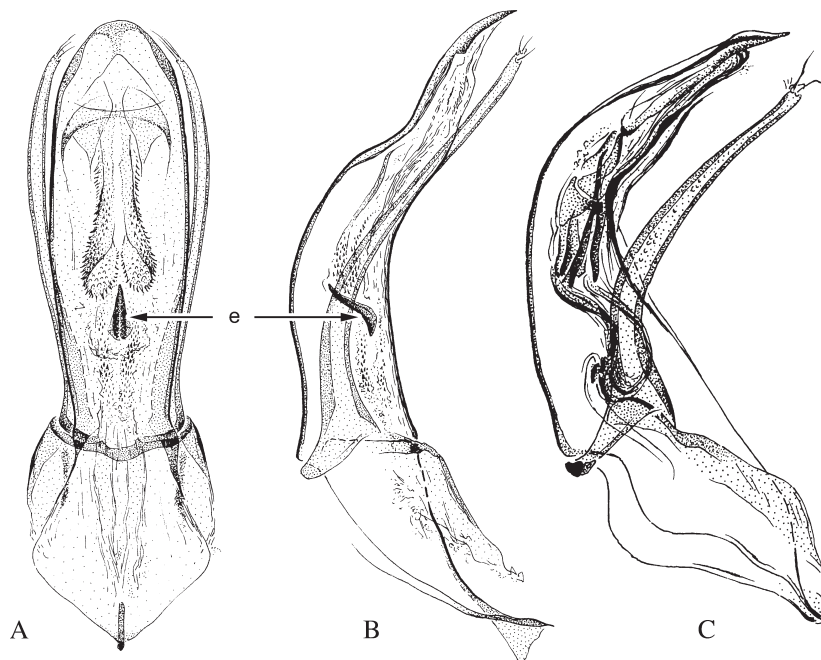
**Fig. 8.** Aedeagus: A, *Anillochlamys bueni* Jeannel, dorsal view, with the stylus of the internal sac of the aedeagus reduced to a small dent (character 21 = 1); B, *Anillochlamys bueni*, lateral view; C, *Stygiophyes sanctigervasi* (Jeannel), lateral view, without stylus (character 20 = 0). e, stylus (see Fig. 12A, B for the state 0 of character 21).

29. *Apex of the lateral stylus of the aedeagus (IV)*: with four setae or more (not a multiple of three) (0); with three setae, or a multiple of three (1) (Fig. 9B–F).

Species with two setae (character 28 = 0) are coded as missing data. In most of the taxa of the series 'Quaestus', 'Spelaeochlamys' and 'Speonomus', the apex of the lateral stylus of the aedeagus has three setae, or a multiple of three. Four changes (three reversals).

30. *Apex of the lateral stylus of the aedeagus (V)*: without membranous appendage, or, if a membranous lamina is present, without a clearly defined insertion suture (0) (Fig. 9A, D–F); with a membranous appendage with a well-defined insertion suture (1) (Fig. 9B, C).

The apex of the lateral stylus of the aedeagus has a membranous appendage with a clearly defined insertion suture in two groups of genera within the series 'Speonomus'. One to two changes.

31. *Apex of the lateral stylus of the aedeagus (VI)*: with a well-defined contour (0) (Fig. 9A–D, F); extended forming a membranous lamina (1) (Fig. 9E).

Although species with character 30 = 1 have a (presumably) modified sensilia forming a membranous lamina with a clear insertion suture, species with character 31 = 1 have the apex of the lateral stylus of the aedeagus modified, with an extension forming a lamina. The two conditions are not considered to be homologous, and thus are coded as different characters. The apex of the lateral stylus of the

aedeagus is extended into a membranous lamina in two genera of the series 'Spelaeochlamys' (*Anillochlamys* and *Paranillochlamys*) and in the *Speocharidius* group of genera. Two changes.

*Spermathecal complex.*

32. *Spermatheca*: fully membranous, without any particular structure in the insertion of the spermatic duct ('atypical spermatheca' of Perreau, 1989) (0) (Fig. 13C); with basal and apical lobes sclerotized to some degree and the medial part membranous, with a well-defined insertion point of the spermatic duct ('type 1' spermatheca according to Perreau, 1989) (1) (Fig. 13A, B).

The 'atypical' spermatheca is present in *Catops* and *Speonemadus*. All studied species of Leptodirini have a spermatheca of 'type 1' of Perreau (1989). One change.

33. *Spermatheca*: short, with a reduced medial region (0) (Fig. 13A); long, with a well-developed medial region (1) (Fig. 13B).

Species with fully membranous spermatheca ('atypical spermatheca' of Perreau, 1989; character 32 = 0) are coded as missing data. All studied species of infraflagellates have a long spermatheca, with a well-developed medial region. One change.

34. *Connection between the bursa copulatrix and the spermatic duct*: direct, without any defined structure (0) (Fig. 13A); through a sclerotized piece (1) (Fig. 13B).
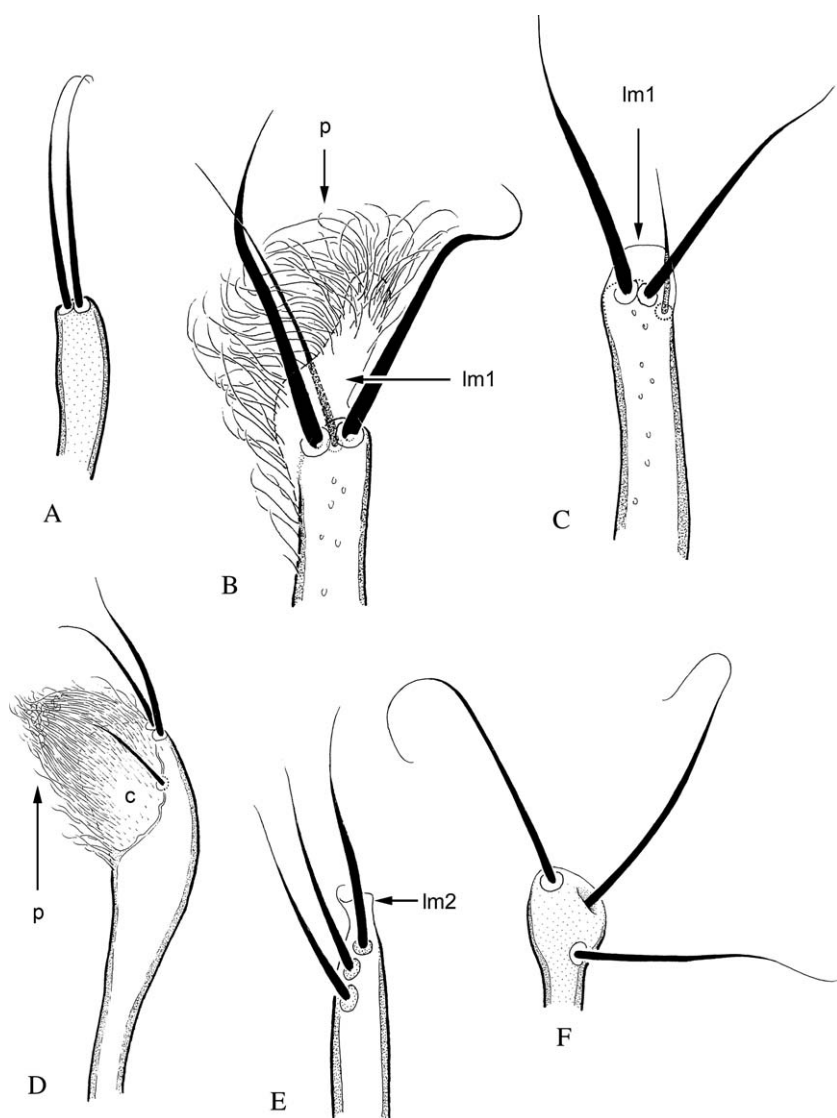
**Fig. 9.** Apex of the lateral stylus of the aedeagus: A, *Catops fuliginosus*, with only two setae (character 28 = 0); B, *Pallaresiella pallaresana* (Jeannel), with a developed penicillum (character 26 = 1), no excavation (character 27 = 0), three setae (character 28 = 1; character 29 = 1) and a membranous lamina with a clearly defined insertion suture (character 30 = 1); C, *T.* (*Anthrocharidius*) *orcinus* (Jeannel), with a membranous lamina with a clearly defined insertion suture (character 30 = 1), without a penicillum (character 26 = 0); D, *Parvospeonomus urgellesi* (Español), with a well-defined lateral excavation (character 27 = 1) and a penicillum; E, *Anillochlamys subtruncatus* Jeannel, with a membranous lamina without a clearly defined insertion suture (character 30 = 0), apex of the lateral stylus extended in a membranous lamina (character 31 = 1); F, *Quaestus* (*Quaesticulus*) *nadali* (Salgado), with three setae and without a penicillum. c, lateral excavation; lm1, membranous lamina with a clear insertion suture; lm2, membranous lamina which is an extension of the apex of the stylus; p, penicillum.

Species with fully membranous spermatheca (character 32 = 0) are coded as missing data. Three groups of the studied Leptodirini have a sclerotized piece connecting the bursa copulatrix and the spermatic duct: the infraflagellates, the series 'Speonomidius' and the Sardinian genera *Ovobathysciola* and *Patriziella*. Three changes (one reversal). This character was described by Giachino & Guéorguiev (1989) in *Gesciella*, but has not been studied in other genera of Leptodirini previously.

*Character variation*

All recognized less inclusive taxonomic groups included in Appendix 1 were found to be homogeneous in the character states of all the included species, with only two exceptions: (1) the genus *Troglophyes*, in which one species with two subspecies (*T. aubryi aubryi* Coiffait and *T. aubryi*

*vallierensis* Coiffait) differed from the rest in that the apex of the lateral stylus of the aedeagus lacked a penicillum (character 26); and (2) the subgenus *Quaestus* (*Amphogeus*), in which one of the two species had spines in the internal sac of the aedeagus (character 19). The species within these taxa consequently were considered separately in the data matrix. The number of terminal taxa in the analyses was fifty-eight (see Appendices and Fig. 14).

Nine groups of terminal taxa had identical character states (Figs 14, 15; Appendix 2). These included: (1) a group of genera including *Quaestus* (partim), *Cantabrogeus* and *Leonesiella*; (2) *Speocharinus* plus the subgenus *Asturianella* of *Quaestus*; (3) the *Bathysciola* section IV of Jeannel (1924); and (4–9) six groups of genera within the 'Speonomus' series. The total number of terminal taxa with distinct character states was twenty-four (plus two outgroups).

The final matrix included fourteen characters of the external morphology (characters 1–14) and twenty characters
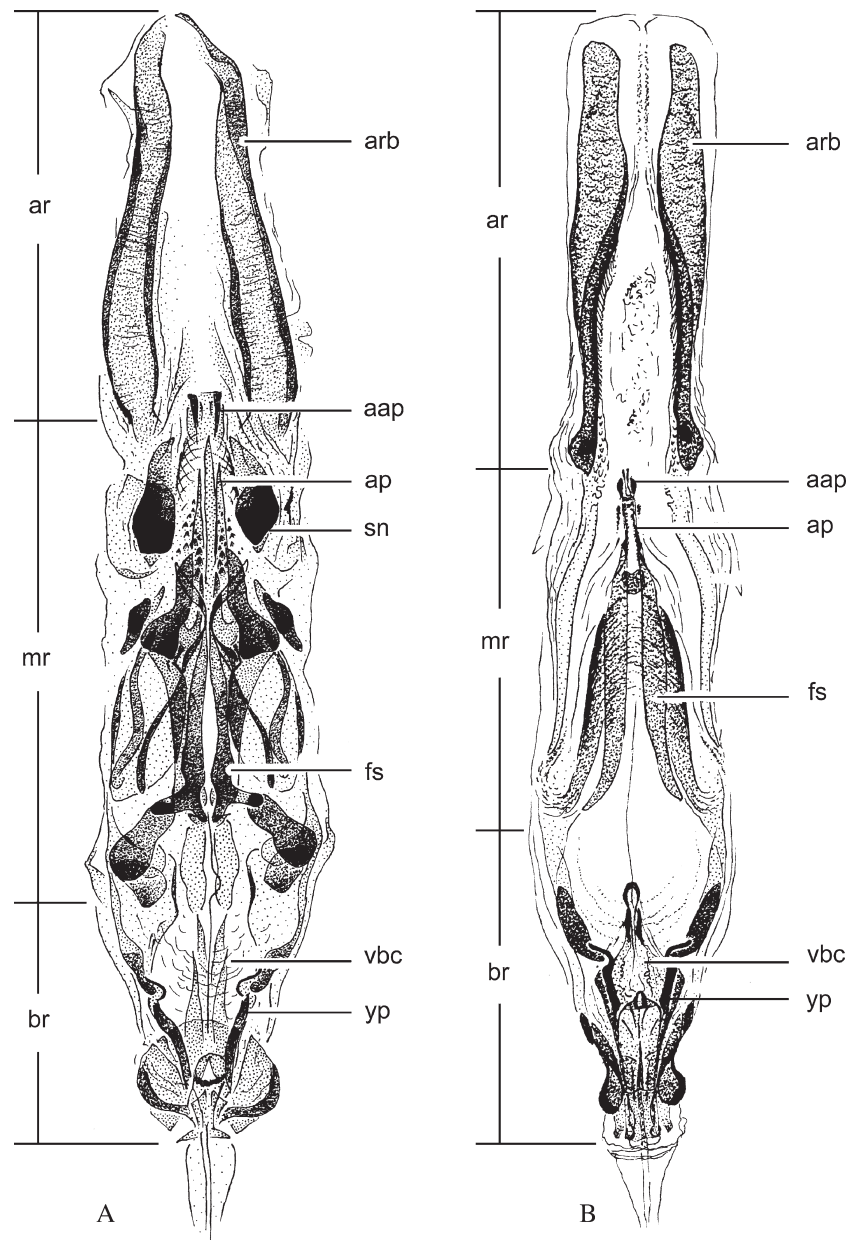
**Fig. 10.** Internal sac of the aedeagus (characters 15–25): A, *Speonomites antemi* (Escolà); B, *Parvospeonomus delarouzeei* (Fairmaire). ap, aap, axial piece and armature of the axial piece (character 24 = 1); ar, apical region; arb, apical reinforcement bands of the internal sac of the aedeagus (character 15 = 1); br, basal region (character 16 = 1); fs, feather-like structures (character 23 = 1); mr, medial region; sn, sclerotized nodules at the base of the reinforcing bands of the internal sac (character 25 = 1); vbc, ventral basal complex (character 18 = 1); yp, Y-piece (character 17 = 1). See 'Results' for the definition of the characters, and Appendix 3 for the character states in the different species.

of the internal genitalia, seventeen of the male (characters 15–31) and three of the female (characters 32–34). A PHT implemented in PAUP for both types of character was not significant (*P* = 0.98).

*Phylogenetic analyses*

Of the thirty-four studied characters, thirty-two were parsimony informative. Characters 13 and 14 were non-informative with our taxon sampling (autapomorphies of the outgroup genera *Speonemadus* and *Catops*, respectively).

The heuristic search on the matrix (as described in 'Materials and methods') resulted in 1254 trees of fifty-two steps, with a consistency index (CI) of 0.65 and a retention index of 0.95. The strict consensus of the 1254 trees had relatively low resolution, with weak support in most nodes, with the exception of Leptodirini (100% bootstrap/Bremer support value 11) and the infraflagellates (corresponding to the series '*Speonomus*'; Appendix 1) (99%/6) (Fig. 14). The genus *Pseudochlamys*, usually included within the series '*Spelaeochlamys*' (with the supra-flagellates) (for example, Bellés *et al*., 1978; Salgado & Fresneda, 2003), was placed as sister to the series '*Speonomus*' (infraflagellates), although with only moderate support (69%/1). Another traditionally recognized group recovered as monophyletic, although with weak support, was the series '*Spelaeochlamys*' (with the exclusion of

**Fig. 11.** Internal sac of the aedeagus (characters 15–25): A, *Pseudochlamys raholai*; B, *Catops fuliginosus*. sp., spines of the internal sac of the aedeagus (character 19 = 1). See 'Results' for the definition of the characters, and Appendix 3 for the character states in the different species.
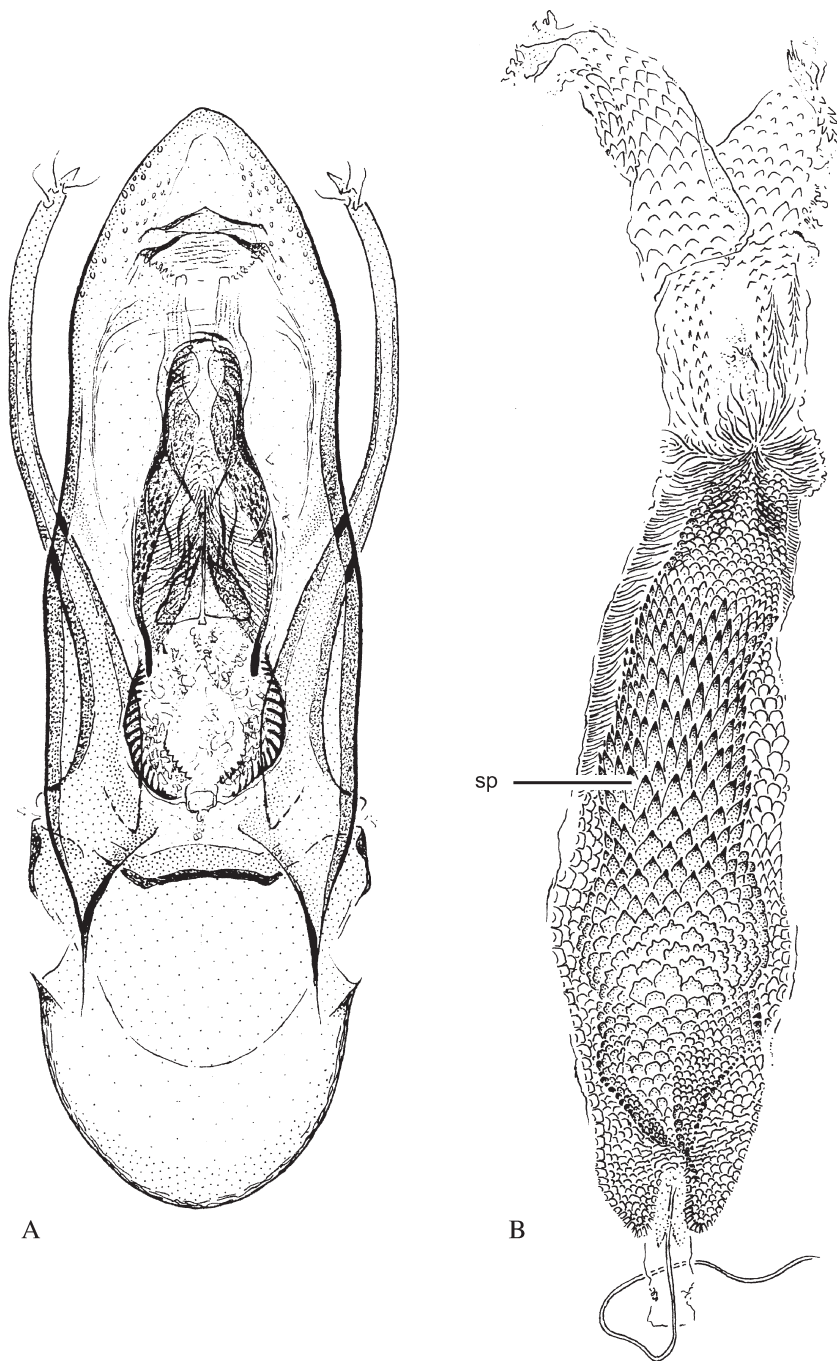
*Pseudochlamys* and *Patriziella*) (< 50%/1). The members of the series '*Quaestus*' and '*Speonomidius*' formed a polytomy (Fig. 14). In the series '*Speonomus*', two of the Pyrenean species groups of the genus *Bathysciola* (*sensu* Fresneda & Salgado, 2006) were sister to the remaining taxa, divided into three main groups: the *Speonomus*, *Speocharidius* and *Anthrocharis* groups of genera (Appendix 2). The genera *Bathysciola*, *Speonomus*, *Quaestus* and *Troglophyes* were found to be paraphyletic (Fig. 14).

When the characters were reweighted according to the rescaled consistency index, the heuristic search on the 1254 starting trees resulted in sixty shortest trees, stable after three reweighting cycles (resulting in 336, 108 and sixty trees, respectively). The strict consensus of the reweighted trees differed from that of the equally weighted trees only in the presence of several new nodes resolving the base of the tree (the 'supraflagellates'). The series '*Quaestus*' was found to be monophyletic (64% bootstrap) with the exclusion of
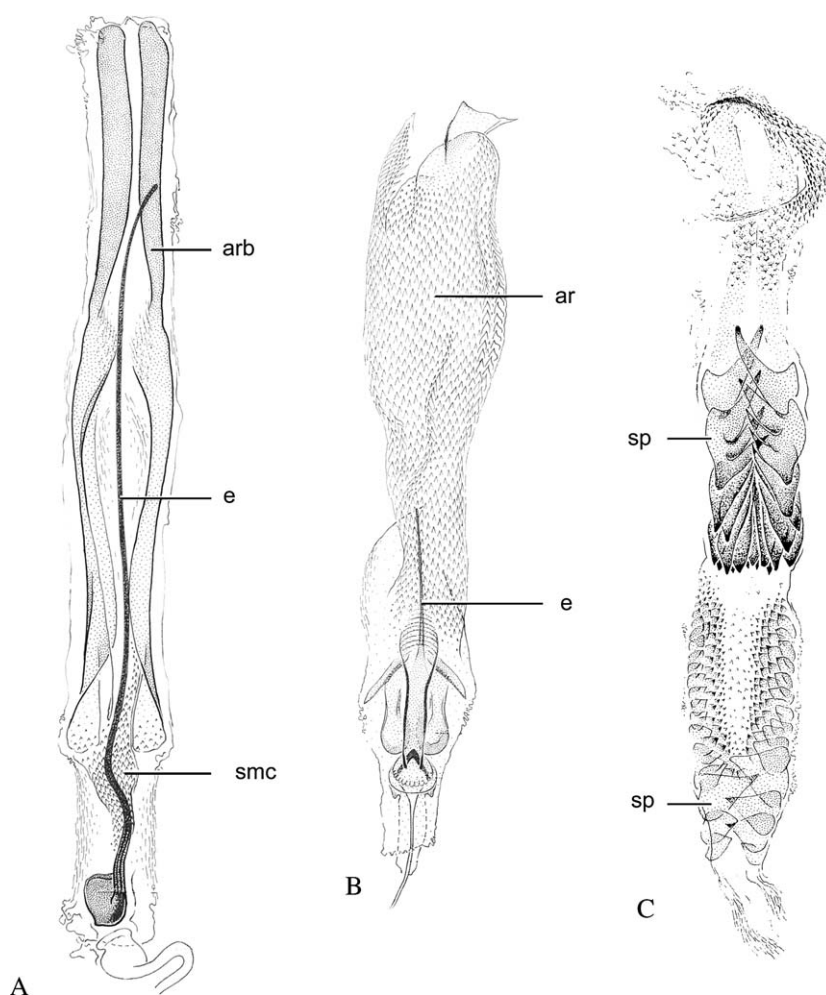
**Fig. 12.** Internal sac of the aedeagus (characters 15–25): A, *Quaestus (Speogeus) nietoi* (Salgado); B, *Speonomidius crotchi aitzquirrensis* (Bolívar); C, *Espanoliella jeanneli*. arb, apical reinforcement bands of the internal sac of the aedeagus (character 15 = 1); ar, apical region; e, stylus of the internal sac of the aedeagus (character 20 = 1); smc, squamous membranous conduct (character 22 = 1); sp, spines of the internal sac of the aedeagus (character 19 = 1). See 'Results' for the definition of the characters, and Appendix 3 for the character states in the different species.

*Oresigenus* and a clade formed by *Breuilia* and *Espanoliella* (< 50% bootstrap), whose relationships appeared unresolved (Fig. 14). *Patriziella* was sister to the rest of the 'Spelaeochlamys' series (excluding *Pseudochlamys*), which was, in turn, sister to the series 'Quaestus' (unresolved) plus *Pseudochlamys* and the series 'Speonomus' (although with bootstrap support lower than 50%). The series 'Speonomidius' was paraphyletic, with *Notidocharis* sister to all remaining included Leptodirini (60% bootstrap), and *Speonomidius* sister to the Leptodirini excluding *Notidocharis* (81% bootstrap) (Fig. 14).

Most characters had a CI of unity, with a single, unambiguous transformation (between characters 25 and 27, two of them parsimoniously non-informative). In the consensus tree (Fig. 15), and considering the minimum number of changes in those characters affected by ambiguities in the topology, one character was reconstructed as having experienced two changes (character 21) (CI = 0.5), six characters had a minimum of three changes (characters 6, 10, 12, 19, 20 and 34) (CI < 0.33), with most character changes being reversals, and one character had four changes, three of them reversals (character 29).

*Character support for the main nodes*

*Leptodirini.* The monophyly of the studied Leptodirini was strongly supported by nine unique synapomorphies, mostly characters of the external morphology (characters 2–5, 7, 8 and 11), but also of male (character 28) and female (character 32) genitalia. Three other homoplastic characters supported the clade, with some reversals: character 12, presence of two well-defined sutures between the mesoventrite and the anteromedian process of the metaventrite (secondarily absent in the series 'Speonomus' and in Spelaeochlamys); character 19, absence of spines in the internal sac of the aedeagus (with a reversal in the group of genera formed by *Breuilia* and *Espanoliella*, and in the species *Quaestus escalerai*) (Fig. 15); and character 20, presence of a stylus in the internal sac of the aedeagus (secondarily absent in infraflagellates plus *Pseudochlamys*, and in some genera of the 'Quaestus' series).

*Leptodirini excluding Notidocharis.* The monophyly of Leptodirinae with the exclusion of *Notidocharis* is supported by one synapomorphy: the absence of eyes (character 1 = 1).
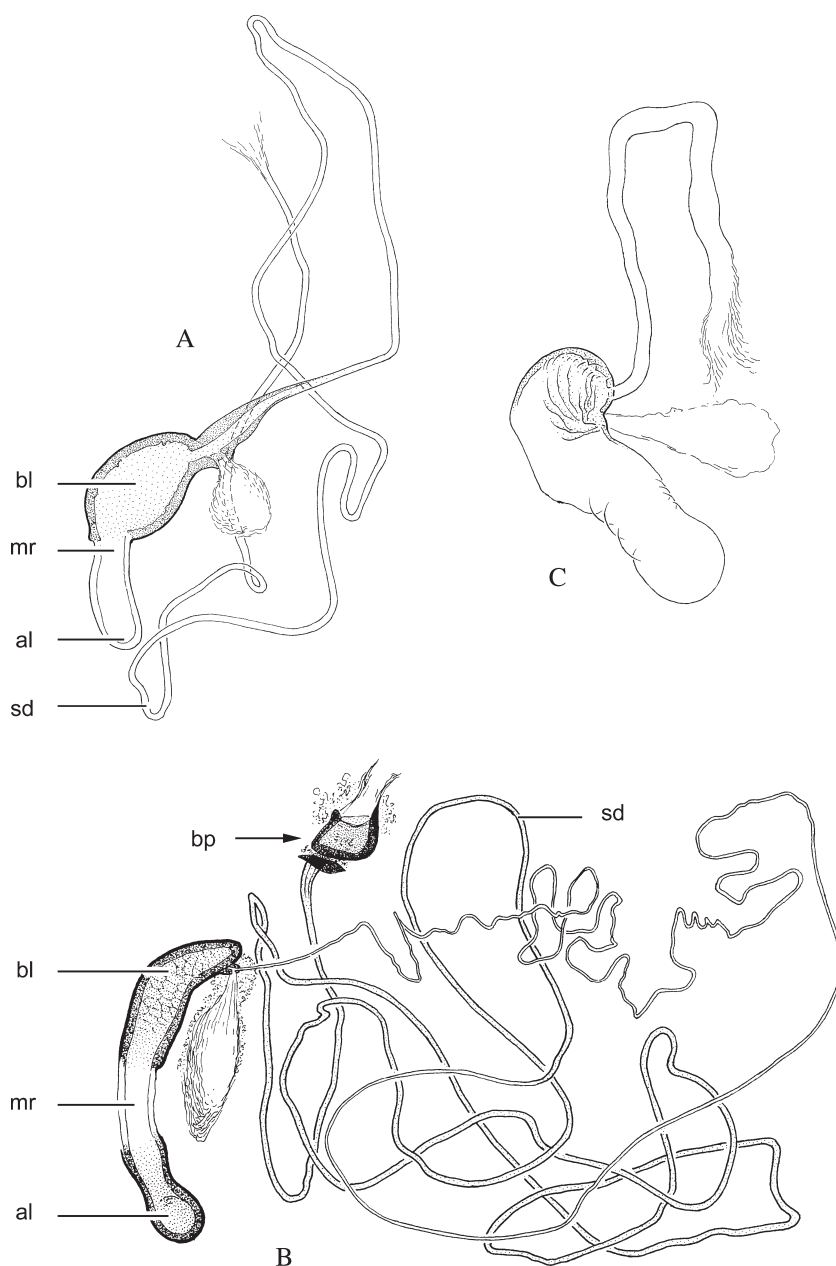
**Fig. 13.** Spermatheca: A, *Quaestus (Quaestus) jeannei* (Coiffait), 'type 1' of Perreau (1989), with basal and apical lobes sclerotized (character 32 = 1), medial region reduced (character 33 = 0), and connection between the bursa copulatrix and the spermatic duct direct (character 34 = 0); B, *Troglocharinus (Troglocharinus) ferreri*, 'type 1' of Perreau (1989) (character 32 = 1), medial region long, well developed (character 33 = 1), and connection between the bursa copulatrix and the spermatic duct through a sclerotized piece (character 34 = 1); C, *Catops fuliginosus*, 'atypical spermatheca' of Perreau (1989), fully membranous (character 32 = 0). al, apical lobe; bl, basal lobe; bp, basal process; mr, medial region; sd, spermatic duct.

*Leptodirini excluding series 'Speonomidius'*. The Leptodirini with the exclusion of the series 'Speonomidius' (i.e. the series 'Spelaeochlamys', 'Quaestus' and 'Speonomus') is supported by one unique synapomorphy, the presence of apical reinforcement bands in the internal sac of the aedeagus (character 15 = 1), and two homoplastic characters: the presence of an irregular punctation in the elytra (character 6 = 1, with one reversal within the series 'Speonomus') and the presence of three (or a multiple of three) setae in the apex of the lateral stylus of the aedeagus (character 29 = 1, with several reversals).

*Series 'Spelaeochlamys'*. The series 'Spelaeochlamys' (including the series 'Ovobathysciola', see 'Discussion') is supported by only one homoplastic character: the presence of a stylus reduced to a small dent in the internal sac of the aedeagus (character 21 = 1). The genus *Paranillochlamys* (included within the series 'Spelaeochlamys') has the plesiomorphic state of the character (stylus well developed).

*Series 'Spelaeochlamys' excluding Patriziella*. The series 'Spelaeochlamys' excluding *Patriziella* is supported by one unique synapomorphy: the fusion of the meso- with the metaventrite, without any visible ventral suture (character 9 = 1).

*Series 'Quaestus' plus 'Speonomus'*. The monophyly of the two series is supported by two homoplastic characters:
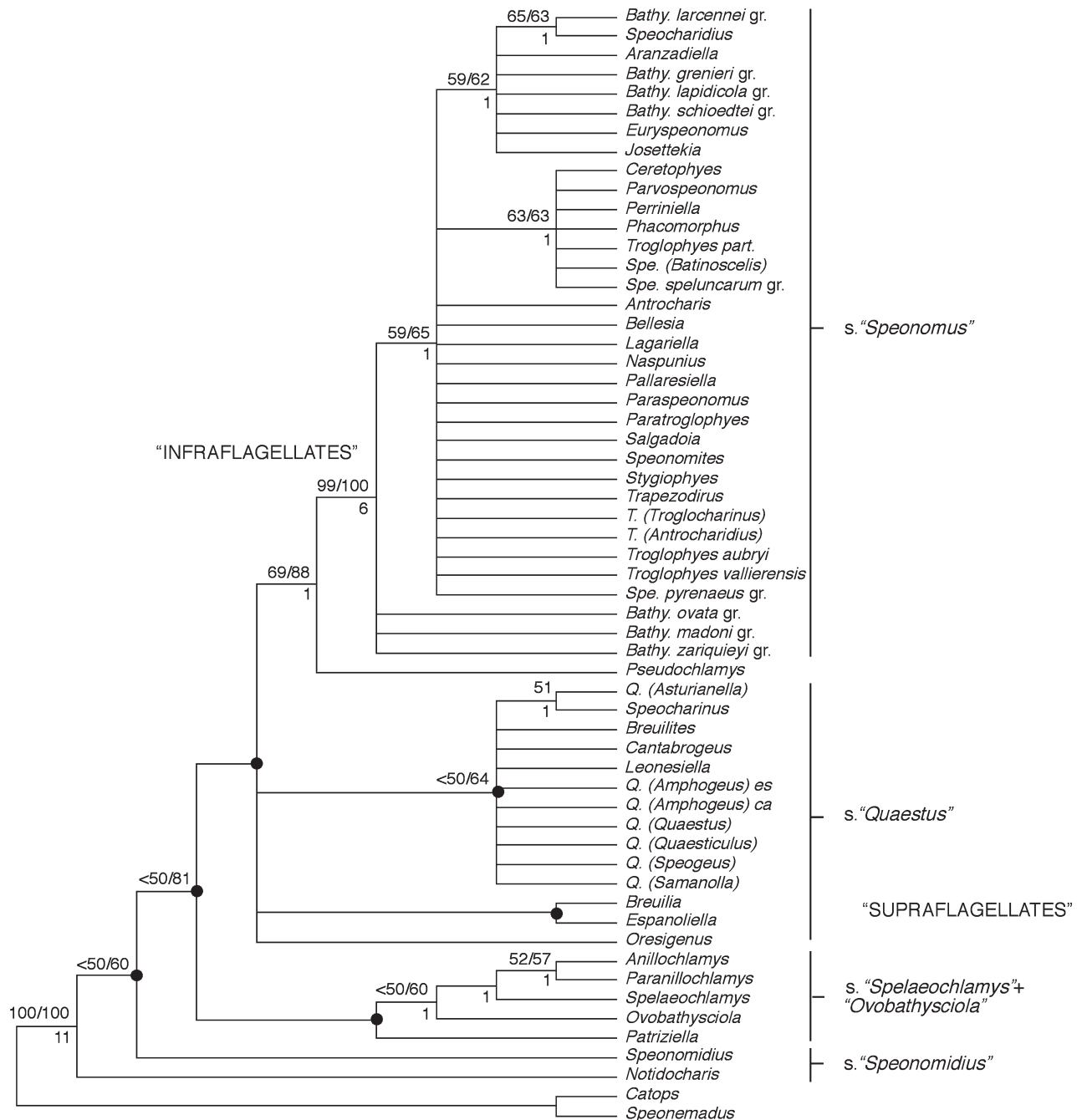
**Fig. 14.** Strict consensus of the sixty trees obtained after successive reweighting of the characters. Numbers above the nodes, bootstrap support values of the equally weighted consensus tree/reweighted consensus tree; numbers below the nodes, Bremer support values of the equally weighted tree. Filled circles, nodes collapsed in the strict consensus of the trees obtained with equally weighted characters.

a well-developed mesosternal keel, extending over the metaventrite (character 10 = 1, with a reversal in *Pseudochlamys* plus the '*Speonomus*' series); and the lack of structure of the connection between the bursa copulatrix and the spermatic duct (character 34 = 0, with a reversal in the '*Speonomus*' series). The interpretation of both characters depends on the resolution of the polytomy at the base of the

'*Quaestus*' series (Fig. 14), and could become synapomorphies if it is resolved as monophyletic.

*Series '*Quaestus*' excluding Oresigenus, Breuilia and Espanoliella.* The monophyly of the genus *Quaestus* plus *Cantabrogeus*, *Leonesiella*, *Speocharinus* and *Breuilites* is supported by a unique synapomorphy: the presence of

31 *Speocharidius* gr.

29

*Euryspeonomus* gr.

26 30 *Spe.pyrenaeus* gr.

27 26 *Spe.speluncarum* gr.

6

26 *Paraspeonomus* gr.

*Antrocharis* gr.

"INFRAFLAGELLATES"

30 *T. (Antrocharidius)*

17 18 24 25 33 12 34

*Bathysciola* IV

16 23 10 20

10 *Bathy. ovata* gr.

*Pseudochlamys*

s. *"Speonomus"*

29 *Speocharinus* gr.

10 34 22 *Quaestus* gr.

20 *Breuilites*

19 *Q. (Amphogeus) es*

29 *Breuilia*

19 20 *Espanoliella*

15 6 29

*Oresigenus*

s. *"Quaestus"*

"SUPRAFLAGELLATES"

31 *Anillochlamys*

34 21 *Paranillochlamys*

9 12 *Spelaeochlamys*

21 *Ovobathysciola*

1

*Patriziella*

s. *"Spelaeochlamys"* +
*"Ovobathysciola"*

2 3 4 5 7 8 11 28 32

12 19 20

*Speonomidius*

*Notidocharis*

s. *"Speonomidius"*

14 6

*Catops*

13

*Speonemadus*

— 1 change

a membranous conduct in the basal region of the internal sac of the aedeagus (character 22 = 1).

*Pseudochlamys plus series 'Speonomus'.* Two unique synapomorphies support this clade: the presence of a complex internal sac of the aedeagus, with three well-differentiated regions (character 16 = 1), and the presence of symmetrical feather-like structures at both sides of the medial region of the internal sac of the aedeagus (character 23 = 1). Two other homoplastic characters support the node: the presence of a mesosternal keel never extending over the metaventrite (character 10 = 0) and the lack of a stylus in the internal sac of the aedeagus (character 20 = 0).

*Infraflagellates.* The monophyly of the infraflagellates (which, with our sampling, overlap with the series '*Speonomus*') is strongly supported by characters of the male (characters 17, 18, 24 and 25, all with character state 1) and female (character 33 = 1) genitalia, also unique. Two other homoplastic characters support the node: the presence of a single suture between the mesoventrite and the anteromedian process of the metaventrite (character 12 = 0) and the presence of a sclerotized piece connecting the bursa copulatrix and the spermatic duct (character 34 = 1).

*Speonomus pyrenaeus, S. speluncarum and Paraspeonomus groups.* This large clade within the '*Speonomus*' series (Appendix 2), although forming a polytomy in the strict consensus tree, shares the presence of a penicillum in the apex of the lateral stylus of the aedeagus (character 26 = 1).

*Speonomus speluncarum group.* The taxa included in this group (see Appendix 2) share a unique synapomorphy: the presence of a well-defined lateral excavation in the apex of the lateral stylus of the aedeagus (character 27 = 1).

### Constrained trees

The monophyly of some clades proposed previously in the literature, but not recovered in our trees, was constrained to test for significant differences with the preferred phylogenetic hypothesis, as exemplified in Fig. 15 (see 'Materials and methods'). Constrained taxa included the supraflagellates, with and without the inclusion of *Pseudochlamys*, the series '*Speonomidius*' and the genera *Quaestus* and *Bathysciola*.

The maximum increase in the length of the trees when some of the constraints were enforced was four (to a total of fifty-six steps) when the supraflagellates (including *Pseudochlamys*) were constrained to be monophyletic and sister

**Table 1.** Number of steps of the trees with constrained nodes (see 'Materials and methods').

| Constraint | No. of steps |
|---|---|
| Unconstrained | 52 |
| Supraflagellates | 56 (including *Pseudochlamys*) |
| Supraflagellates | 55 (excluding *Pseudochlamys*) |
| Series '*Speonomidius*' | 53 |
| Series '*Quaestus*' | 53 |
| Genus *Bathysciola* | 53 |

to the infraflagellates (Table 1). This difference is insignificant according to the non-parametric test of Templeton (1983), as implemented in PAUP ($P = 0.3$). Other constraints resulted in lower increases in the number of steps, and thus the differences with respect to the unconstrained trees were not significant (Table 1).

### Discussion

*Character distribution*

The number of informative characters included in the analyses was relatively low (thirty-two) because of the difficulty of finding informative homologous characters across all taxa in the group. The final number of terminal taxa with different state characters (twenty-six, including two outgroups) was also highly reduced compared with the number of studied species (182). The study of all species within accepted genera, subgenera or species groups was necessary because of the uncertainties in the classification of the Iberian and Pyrenean Leptodirini, as reflected in the many recent taxonomic rearrangements (Salgado, 1994, 2000; Fresneda, 1998; Fresneda & Salgado, 2000, 2006; Salgado & Fresneda, 2003). The use of single species as representatives of the higher taxa would have been unacceptable in this case (Bininda-Emonds *et al.*, 1998), as this would have prevented recognition of the para- or polyphyly of some accepted genera or subgenera (*Speonomus*, *Troglocharinus*, *Bathysciola*, *Quaestus*, see below). Although the ratio of characters to terminal taxa was low, some strongly supported clades could be recognized, as well as providing reasonable resolution at lower levels.

The two sources of characters used in the analyses (external morphology and internal genitalia) were congruent as measured by PHT implemented in PAUP, although, as noted in 'Materials and methods', this should not be taken as a strict measure of congruence. In any case, there was no evidence of a higher homoplasy of the characters of

**Fig. 15.** Phylogram of the strict consensus of the sixty trees obtained after successive reweighting of the characters (in the tree, the character weights were reset to unity). Terminal taxa with identical character states were grouped (see Appendix 2). Filled rectangles, unique changes in character states; open rectangles, homoplastic changes in character states; arrows, homoplastic reversals in character states (see 'Results' for the character list, and Appendix 3 for the character matrix). Habitus (from top to bottom): *Aranzadiella leizaolai* (*Euryspeonomus* gr.); *Euryspeonomus* (*Euryspeonomus*) *breuili* (Jeannel); *Salgadoia brieti* (Jeannel) (*Speonomus pyrenaeus* gr.); *Troglocharinus* (*Antrocharidius*) *orcinus*; *Quaestus* (*Asturianella*) *incognitus*; *Spelaeochlamys ehlersi* Dieck; *Catops nigricans* (Spence).

external morphology: eight of the twenty genital characters had more than one change in the reconstructed tree (*c.* 40%), whereas only three of the fourteen (*c.* 21%) external characters were homoplastic. However, there was a clear separation of the level at which the different types of character were phylogenetically informative: seven of the fourteen characters of the external morphology were synapomorphies of the basal node defining the included Leptodirini, i.e. without variation within the ingroup. Of the remaining thirteen resolved nodes of the reweighted consensus tree, in only six did external characters contribute to their support, three of them exclusively (series '*Speonomus*' excluding *Bathysciola* IV and *ovata* groups, character 6; series '*Spelaeochlamys*' excluding *Patriziella*, character 9; and Leptodirini excluding *Notidocharis*, character 1; Fig. 15).

The high homoplasy of some characters of the male genitalia could be a result of uncertain homology. For example, the penicillum on the apex of the lateral stylus of the aedeagus (character 26) could be directly on the stylus (Fig. 9D) or in the membranous lamina (Fig. 9B). Although considered as homologous, and thus sharing the same underlying developmental mechanism (see, for example, Simpson *et al.*, 1999), this remains open to interpretation. Likewise, the number of setae in the apex of the lateral stylus of the aedeagus (characters 28 and 29), although widely used in the taxonomy of the group (for example, Fresneda, 1998; Fresneda & Salgado, 2000), had a weak phylogenetic signal at the level used in this study.

### Systematics of the west Mediterranean Leptodirini

Our results do not support the division of the Leptodirini into two main sister groups: the infraflagellates and the supraflagellates (Jeannel, 1955; Giachino *et al.*, 1998). Although there is strong support for a monophyletic infraflagellates (corresponding to the '*Speonomus*' series with our taxon sampling), the supraflagellates appear to be paraphyletic (Figs 14, 15). The division of the west Mediterranean taxa into 'phyletic series' (Jeannel, 1910a, 1924, 1955) accords more with our results, especially for the '*Speonomus*' and '*Spelaeochlamys*' series (the latter including the series '*Ovovathysciola*' from Sardinia). The series '*Quaestus*' was unresolved in the strict consensus tree, but compatible with monophyly; in this case, a mesosternal keel extending over the mesoventrite (character 10 = 1) could be a potential synapomorphy of the group, with an independent, homoplastic origin in the species of the *Bathysciola ovata* group. Another potential synapomorphy may be the absence of a sclerotized piece connecting the bursa copulatrix with the spermatic duct (character 34 = 0), shared with *Pseudochlamys* and some genera in the '*Spelaeochlamys*' series.

The monophyly of the '*Spelaeochlamys*' plus '*Ovobathysciola*' series is weakly supported, with the only synapomorphy being the reduction of the stylus of the internal sac of the aedeagus to a small dent (character 21 = 1), with

a reversal in *Paranillochlamys*. A unique synapomorphy of the group with the exclusion of *Patriziella* is the lack of a suture between the meta- and mesoventrite (character 9 = 1). This places *Patriziella* as sister to the remaining genera of the series, not supporting the monophyly of the two Sardinian genera, which seems unlikely from a biogeographical point of view (see, for example, Caccone & Sbordoni, 2001). However, the possibility exists that the two genera diverged before the separation of the Corso-Sardinian plate from mainland Europe by the mid-Miocene, as has been suggested for the sister species of the cave groundbeetle genus *Sardaphaenops* (Casale, 2004).

The series '*Spelaeochlamys*' (including *Ovobathysciola*), as reconstructed in our tree, is morphologically very heterogeneous, having been grouped traditionally mostly by geography (Mediterranean coast of the Iberian Peninsula and Sardinia), with poorly defined morphological diagnostic characters (for example, shape and punctation of the elytra, relative size of the lateral stylus of the aedeagus; Jeannel, 1910a).

The paraphyly of the series '*Speonomidius*' is supported by the presence of eyes in *Notidocharis*, which is also the only non-subterranean taxon of the included Leptodirini (Salgado, 2000). No potential unique synapomorphy links the two genera, which share many of the plesiomorphic character states of the tribe Leptodirini (as reconstructed in our phylogenetic tree). When character 1 (presence of eyes) was excluded from the analyses, the heuristic search resulted in 700 shortest trees of fifty steps, the strict consensus of which placed *Notidocharis* sister to *Speonomidius* (i.e. the '*Speonomidius*' series) in a polytomy including *Pseudochlamys* plus the '*Speonomus*' series, the series '*Speochlamys*' excluding *Patriziella*, and all genera of the '*Quaestus*' series with the exception of *Breuilia* and *Espanoliella*, which were sister to the remaining Leptodirini (not shown). After successive reweighting, the strict consensus of the 190 resulting trees placed *Patriziella* as sister to the remainder of the '*Spelaeochlamys*' series, which was sister to the '*Speonomidius*' series. The exclusion of the character 'presence or absence of eyes' thus had the effect of rendering the series '*Speonomidius*' monophyletic (although supported by homoplastic characters), and sister to the '*Spelaeochlamys*' series – not to the geographically closer '*Quaestus*' series (Fig. 16), which was paraphyletic. An additional effect would be the assumption of a redevelopment of eyes in *Notidocharis* from an eyeless ancestor, unless the lack of eyes had occurred independently between five and ten times amongst the taxa studied, without any variability in the degree of reduction and without any eyed extant relative.

One of the most remarkable results of our analysis was the sister relationship between *Pseudochlamys raholai* (Zariquiey) and the series '*Speonomus*'. The species was described originally as a member of *Anillochlamys* (Zariquiey, 1922a, 1922b), included subsequently in a monotypic subgenus of *Anillochlamys* by Comas (1977), and upgraded to genus by Bellés *et al.* (1978). These authors and others (for example, Jeannel, 1924; Comas, 1977; Salgado & Fresneda, 2003) recognized some similarities between this

species and the genera of the 'Spelaeochlamys' series (including *Anillochlamys*): the absence of a Y-piece in the internal sac of the aedeagus (character 17 = 0), the irregular punctation of the elytra (character 6 = 1) and the presence of three setae in the apex of the lateral stylus of the aedeagus (character 29 = 1). However, all of these character states are reconstructed as plesiomorphic for the included Leptodirini in our preferred tree, and some are highly homoplastic. They share with some genera within the series 'Spelaeochlamys' a strongly sinuated apical aedeagus, but this highly variable character is difficult to characterize, and is not considered in our analysis (see 'Materials and methods').

By contrast, two unique synapomorphies link *Pseudochlamys* with the series 'Speonomus': a complex internal sac of the aedeagus with an internal armature and three well-differentiated regions (character 16 = 1), and symmetrical feather-like structures at both sides of the medial region of the aedeagus (character 23 = 1). These structures are hyaline but clearly present (Fig. 11A; see also fig. 48 in Salgado & Fresneda, 2003). *Pseudochlamys* also lacks a stylus in the internal sac of the aedeagus (character 20 = 0), by contrast with all supraflagellates, including all remaining genera of the *Spelaeochlamys* section. According to our phylogenetic hypothesis, the genera *Breuilia*, *Breuilites* and *Espanoliella* have secondarily lost the stylus, but other male and female genitalic characters relate these species clearly with the 'Quaestus' series (Salgado, 2000).

The geographical distribution of *P. raholai* is also more congruent with a relationship to the 'Speonomus' series: it is found in several caves on the Mediterranean coast of the north of the province of Girona, on the eastern edge of the Pyrenean chain (Salgado & Fresneda, 2003). The 'Speonomus' series has a predominantly Pyrenean distribution, whereas the genera of the 'Spelaeochlamys' series are distributed along the Mediterranean coast south of Tarragona (Fig. 16; see below and Bellés, 1987).

According to our tree, four currently recognized genera are not monophyletic: *Bathysciola*, *Quaestus*, *Speonomus* and *Troglophyes* (the relationships of the subgenera of *Troglocharinus* are unresolved, but compatible with the monophyly of the genus). The genus *Bathysciola* has c. 100 species and is widely distributed in the northern Mediterranean region, east to the Caucasus and Iran (Perreau, 2000, 2004). Many species live in forest litter or are muscicolous (some of them with eyes) and, of the subterranean species, most are found in deep soil (endogeous) or in interstitial habitats ('milieu suterrain superficiel'; Juberthie *et al.*, 1981). The series 'Bathysciola' (*sensu* Zoia & Rampini, 1994) includes four genera: *Bathysciola* itself plus *Besuchetiola* (one species), *Parabathyscia* (forty-one species) and *Sengletiola* (one species) (Perreau, 2000). Originally, Jeannel (1910a, 1914, 1924) established the 'phyletic series *Bathysciola*' and the sections within the genus according to the external morphology, structure of the aedeagus and geographical distribution. The species included in the genus *Bathysciola* were considered to be 'primitive', although Jeannel (1924) noted that their closest relatives should be sought amongst genera inhabiting the
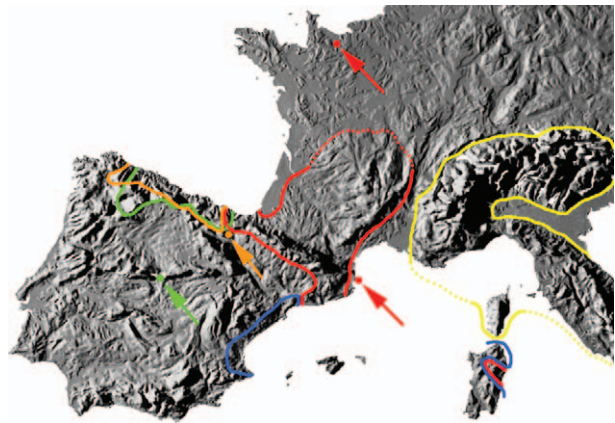


**Fig. 16.** Distribution in the Iberian Peninsula and the Pyrenees of the main lineages of Leptodirini. Red outline, 'Speonomus' series, including some species groups of *Bathysciola* (in Sardinia, *Speonomus* subgenus *Batinoscelis*); red arrows: Normandie, *Bathysciola fauveli* Jeannel; eastern Pyrenees, *Pseudochlamys raholai*. Blue outline, 'Spelaeochlamys' series (in Sardinia, *Ovobathysciola* plus *Patriziella*). Green outline, 'Quaestus' series; green arrow: *Quaestus vasconicus cisnerosi* (Pérez Arcas). Orange outline, *Notidocharis*; orange arrow: *Speonomidius*. Yellow outline, Leptodirini not included in our study.

same geographical areas, and thus the heterogeneity already was apparent. This view of the heterogeneous condition of *Bathysciola* prevails (for example, Giachino, 1988; Vailati, 1988; Newton, 1998; Fresneda & Salgado, 2000, 2006; Perreau, 2000), with the exception of Zoia & Rampini (1994), who redefined the series based on the presence of an apophysis extending the metatergal apparatus and reaching the apex of the unfused elytra (although the same authors recognized the lack of study in most species of Leptodirini). All studied species in the former series 'Bathysciola' have the synapomorphies of the series 'Speonomus', some already recognized by Jeannel (1924), and could be said to be present in all the species he considered within the section (for example, the complex internal sac of the aedeagus and the presence of a Y-piece in the internal sac of the aedeagus; characters 16 and 17). Some characters have been described only recently, and their state in most of the species of the section is unknown, such as the presence of symmetrical feather-like structures in the internal sac of the aedeagus or the structure of the spermatheca (characters 23, 32–34) (Salgado, 2000).

The Iberian species of *Bathysciola* fall within the sections IV, VI and VII of Jeannel (1924) (Fresneda & Salgado, 2006). The Iberian species of section VI seem to be closely related to the genera of the *Antrocharis* group, sharing all studied character states. These genera are also distributed in the same geographical area (Jeannel, 1924; Fresneda & Salgado, 2006), showing again (as with *Pseudochlamys*) the highly phylogenetically conserved geographical relationships between the lineages of Leptodirini. The revision of the non-Iberian species of these sections, as well as the species in

other, unrevised sections, certainly will lead to the recognition of separate evolutionary lineages in what is presently known as *Bathysciola*.

The genus *Quaestus* was never recovered as monophyletic in our analyses: *Quaestus* (*Asturianella*) differs from the remaining species in not having three (or a multiple of three) setae in the apex of the lateral stylus of the aedeagus (character 29 = 0), although this character seems to be highly homoplastic. *Quaestus* (*Amphogeus*) *escalerai* shares with *Espanoliella* and *Breuilia* the presence of spines in the internal sac of the aedeagus (character 19 = 0, a reversal to the hypothesized plesiomorphic state of Leiodidae from the derived condition of the absence of spines in Leptodirini; Dupré, 1992), but this is reconstructed as a homoplasy in our tree. The second species of the subgenus, *Quaestus* (*Amphogeus*) *cantabricus* (Uhagón), shares identical character states with all other species of the *Quaestus* group (Appendix 2).

Most species of *Troglophyes* share all character states with the species of the *Speonomus speluncarum* group (Appendix 2), and are distributed in the same area (northern side of the oriental Pyrenees) (Fresneda, 1998). However, one species (*T. aubryi*, with two subspecies *T. aubryi aubryi* and *T. aubryi vallierensis*) shares all character states with the species of the *Antrocharis* group (Appendix 2), and all are distributed in the central Pyrenees (Fresneda, 1998). The genera *Antrocharis*, *Paratroglophyes* and *Troglophyes aubryi* share a very similar model of the internal sac of the aedeagus, and their generic status should be revised.

The genus *Speonomus* includes a series of 'orphan' taxa for which the predominantly plesiomorphic morphological characters do not allow the recognition of closer relationships, but they probably do not constitute a monophyletic taxon. Successive partial revisions of the genus have resulted in the segregation of several new genera (for example, *Phacomorphus*, Dupré, 1989, 1990; *Salgadoia*, *Stygiophyes*, *Trapezodirus*, *Pallaresiella* and *Parvospeonomus*, Fresneda, 1998; *Speonomidius*, Salgado, 2000). The taxonomic revision of the remaining sections of *Speonomus* will most probably result in the segregation of new genera, and the recognition of monophyletic groups.

*Geographical distribution of the main Iberian lineages*

The distribution of the main lineages of Iberian Leptodirini has a well-defined geographical pattern, which has been used traditionally to hypothesize their palaeobiogeographical history (for example, Jeannel, 1908, 1919, 1947; Salgado, 1976; Español, 1981; Bellés & Escolà, 1986; Bellés, 1986, 1987; Giachino *et al.*, 1998). Our results introduce several important changes in this geographical pattern, which leads to the re-interpretation of some of the early assumptions.

The geographical location of *Pseudochlamys*, in the extreme east of the Pyrenees, is now more in agreement with its redefined phylogenetic relationship as sister to the '*Speonomus*' series (distributed along the Pyrenees; Fig. 16). The Pyrenean Leptodirini thus would be a well-defined monophyletic radiation, including all species from the east of the Vasc Country to the Mediterranean coast, to north of the Ebro river (Fig. 16). Only some members of *Bathysciola* are found north of the Pyrenees (section VI of Jeannel, 1924 in the west up to Bordeaux, and section IV east to the oriental Pyrenees, the Alps and north Italy; Jeannel, 1924; Fresneda & Salgado, 2006), although the taxonomic status of the species of this genus is most uncertain and in need of detailed revision (see above).

In the extreme west of the distribution of the '*Speonomus*' series, there are the only localities known in which species of this group coexist with species of the '*Quaestus*' series: two caves at both sides of the Deva river, in the Vasc Country, in which *Aranzadiella leizaolai* Español (the only species of the genus; Appendix 1) is found together with *Quaestus noltei* (Coiffait) (Fresneda & Salgado, 2000). The species of the series '*Quaestus*' are distributed from these localities west of the Vasc Country through the Cantabrian Mountains to Galicia, with only one known locality in the north of the Sistema Central (Fig. 16; Salgado, 2000). This area of distribution overlaps almost perfectly with that of the '*Speonomidius*' series (Fig. 16; Salgado, 2000). The relationships of the two series are poorly supported in our trees, but imply that the Leptodirini of the Cantabrian region do not form a monophyletic radiation comparable with that of the Pyrenees.

The Mediterranean Leptodirini, from north of the Ebro river to Alicante, are included in the '*Spelaeochlamys*' series, traditionally linked to the '*Ovobathysciola*' series of Sardinia (Giachino *et al.*, 1998; Caccone & Sbordoni, 2001). In our tree, the two series form a monophyletic group, although with weak support, and with the two Sardinian genera forming a paraphyletic series with respect to the Iberian genera.

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Accepted 13 August 2006

## Appendix 1

Checklist of the genera, subgenera and species groups of western Mediterranean Leptodirinae (Iberian Peninsula, Pyrenees and south France, Sardinia), with their distribution and the studied material. Classification follows Lawrence & Newton (1995) and Perreau (2004) down to tribe level. Biospeleological areas are defined following Bellés (1987): CAN, Cantabrian; CAT, Catalonian; MED, Mediterranean coast; PYRN, northern Pyrenees; PYRS, southern Pyrenees; VAS, Vasc Country, including a small region in south-west France and overlapping to some extent with the Cantabrian area in the west (Fig. 16).

Unless otherwise stated, all species in each genera/species group have been studied (see Bellés *et al.*, 1978; Fresneda, 1998; Fresneda & Salgado, 2000; Salgado, 2000 for a complete checklist of species). Genera/species groups for which no material could be studied are in square brackets.

### Family Leiodidae
*Subfamily Cholevinae*
*Tribe Cholevini*
*Catops* sp.

*Tribe Anemadini*
*Speonemadus* sp.

*Tribe Leptodirini*
**1. Series *"Quaestus"* Perreau, 2000**
*Breuilia* Jeannel, 1910 (CAN, 1 sp.).
*Breuilites* Salgado, 1980 (CAN, 1 sp.).
*Cantabrogeus* Salgado, 2000 (CAN, 1 sp.).
*Espanoliella* Guéorguiev, 1976 (CAN, 5 spp.).
*Leonesiella* Salgado, 1996 (CAN, 1 sp.).
*Oresigenus* Jeannel, 1948 (CAN, 1 sp.).

*Quaestus* Schaufuss, 1861 (CAN-VAS, 48 taxa); including six subgenera:
1. *Amphogeus* Salgado, 2000 (the two species of the subgenus, *Q. (A.) escalerai* (Jeannel, 1909) and *Q. (A.) cantabricus* (Uhagón, 1881), were found to differ in some character states and thus were considered separately in the data matrix).
2. *Asturianella* Salgado & Fresneda (2004) (studied species: *Q. (A.) incognitus* Salgado & Fresneda, 2004).
3. *Quaesticulus* Schaufuss 1861.
4. *Quaestus* Schaufuss 1861.
5. *Samanolla* Salgado, 2000.
6. *Speogeus* Salgado, 1985.
*Speocharinus* Español & Escolà, 1977 (CAN, 1 sp.).
**2. Series *"Spelaeochlamys"* Jeannel, 1910a**
*Anillochlamys* Jeannel, 1909 (MED, 6 spp.).
*Ovobathysciola* Jeannel, 1924 (Sardinia, 3 spp.).
*Paranillochlamys* Zariquiey, 1940 (CAT-MED, 3 spp.).
*Patriziella* Jeannel, 1956 (Sardinia, 3 spp.).
*Pseudochlamys* Comas, 1977 (PYRS, 1 sp.).
*Spelaeochlamys* Dieck, 1870 (MED, 3 spp.).
**3. Series *"Speonomidius"* Salgado, 2000**
*Notidocharis* Jeannel, 1956 (CAN, 7 spp.).
*Speonomidius* Jeannel, 1924 (CAN-VAS, 1 sp. with 4 ssp.).
**4. Series *"Speonomus"* Jeannel, 1910a**
*Antrocharis* Abeille de Perrin, 1878 (PYRN, 2 taxa).
*Aranzadiella* Español, 1972 (CAN-VAS, 1 sp.).
[*Bathysciella* Jeannel, 1906 (PYRN, 1 sp.)].
*Bathysciola* Jeannel, 1910 (Fresneda & Salgado, 2006).
  Section IV Jeannel, 1924.
    Group *'madoni'* (PYRN-PYRS, 2 spp.).
    Group 'zariquieyi' (CAT, 2 taxa).
  Section VI Jeannel, 1924.
    Group *'grenieri'* (PYRN, 3 taxa).
    Group *'lapidicola'* (PYRN, 4 taxa).
    Group *'larcennei'* (PYRN, 2 taxa).
    Group *'schiodtei'* (PYRN-PYRS-VAS, 9 taxa) Perreau, 2000.
  Section VII Jeannel, 1924.
    Group *'ovata'* (PYRN-PYRS, 8 taxa) Perreau, 2000.
*Bellesia* Fresneda & Hernando, 1994 (PYRS, 1 sp.).
*Ceretophyes* Comas & Escolà, 1989 (PYRS, 2 spp.).
[*Eskualdunella* Coiffait, 1950 (PYRN, 1 sp.)].
*Euryspeonomus* Jeannel, 1919 (VAS, 5 taxa); with two subgenera:
1. *Euryspeonomus* Jeannel, 1919.
2. *Urbasolus* Español, 1948.
[*Gesciella* Giachino & Guéorguiev, 1989 (PYRN, 1 sp.)].
*Josettekia* Bellés & Deliot, 1983 (VAS, 2 spp.).
*Lagariella* Fresneda, 2000 (PYRS, 2 spp.).
*Naspunius* Fresneda, Hernando & Lagar, 1994 (PYRS, 1 sp.).
*Pallaresiella* Fresneda, 1998 (PYRS, 1 sp.).
*Paraspeonomus* Coiffait, 1952 (PYRN, 1 sp.).
*Paratroglophyes* Fourès, 1954 (PYRN, 2 sp.).
*Parvospeonomus* Bellés & Escolà, 1977 (PYRN, PYRS, 5 spp.).
*Perriniella* Jeannel, 1910 (PYRN-PYRS, 3 spp.).
*Phacomorphus* Jeannel, 1908 (PYRN-VAS, 12 taxa); with two subgenera:

1. *Phacomorphus* Jeannel, 1908.
2. *Phacomorphoides* Dupré, 1989.

*Salgadoia* Fresneda, 1998 (PYRS, 1 sp.).

*Speocharidius* Jeannel, 1919 (VAS, 4 spp.); with two subgenera:

1. *Speocharidius* Jeannel, 1919.
2. *Kobiella* Español & Bellés, 1980.

*Speonomites* Jeannel, 1910 (PYRS, 11 spp.).

*Speonomus* Jeannel, 1908 (PYRN-PYRNS-VAS-Sardinia, 76 taxa); with four subgenera:

1. *Speonomus* Jeannel, 1908: groups ['*ehlersi*'], ['*zophosinus*'], '*speluncarum*' and '*pyrenaeus*.
2. *Batinoscelis* Jeannel, 1924.
3. [*Machaeroscelis* Jeannel, 1924].
4. [*Metaspeonomus* Coiffait, 1959].

*Stygiophyes* Fresneda, 1998 (PYRS, 12 taxa).

*Trapezodirus* Jeannel, 1924 (PYRS, 9 taxa).

[*Trocharanis* Reitter, 1885 (PYRN, 1 sp.)].

*Troglocharinus* Reitter, 1908 (PYRS-CAT, 32 taxa); with two subgenera:

1. *Troglocharinus* Reitter, 1908.
2. *Antrocharidius* Jeannel, 1910.

*Troglophyes* Abeille de Perrin, 1894 (PYRN, 7 taxa) (*T. aubryi aubryi* Coiffait, 1953 and *T. aubryi vallierensis* Coiffait, 1953 differ from the rest of the species of the genus and were considered separately in the data matrix).

## Appendix 2

Groups of terminal taxa with identical character states (see Figs 14, 15).

1. *Quaestus* gr.: *Q. (Amphogeus) cantabricus*, *Q. (Quaestus)*, *Q. (Quaesticulus)*, *Q. (Speogeus)*, *Q. (Samanolla)*, *Cantabrogeus*, *Leonesiella*.
2. *Speocharinus* gr.: *Speocharinus*, *Quaestus (Asturianella)*.
3. *Bathysciola* series IV: *B. madoni* gr., *B. zariquieyi* gr.
4. *Antrocharis* gr.: *Antrocharis*, *Paratroglophyes*, *Troglophyes a. aubryi*, *Troglophyes a. vallierensis*.
5. *Euryspeonomus* gr.: *Aranzadiella*, *Bathysciola grenieri* gr., *B. lapidicola* gr., *B. schiodtei* gr., *Euryspeonomus*, *Josettekia*.
6. *Speocharidius* gr.: *Speocharidius*, *Bathysciola larcennei* gr.
7. *Paraspeonomus* gr.: *Bellesia*, *Lagariella*, *Paraspeonomus*.
8. *Speonomus pyrenaeus* gr.: *Naspunius*, *Pallaresiella*, *Salgadoia*, *Speonomites*, *Stygiophyes*, *Trapezodirus*, *Troglocharinus (Troglocharinus)*, *Speonomus pyrenaeus* species gr.
9. *Speonomus speluncarum* gr.: *Ceretophyes*, *Parvospeonomus*, *Perriniella*, *Phacomorphus*, *Speonomus (Batinoscelis)*, *Speonomus speluncarum* species gr., *Troglophyes* partim.

## Appendix 3

### Data matrix

Terminal taxa with identical character states were pooled (see Appendix 2 and Figs 14, 15).

| Character no. | 01 | 02 | 03 | 04 | 05 | 06 | 07 | 08 | 09 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Spe. pyrenaeus* gr. | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | ? | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |
| *Spe. speluncarum* gr. | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | ? | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 |
| *Paraspeonomus* gr. | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | ? | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 |
| *Speocharidius* gr. | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | ? | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 |
| *Euryspeonomus* gr. | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | ? | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 |
| *Antrocharis* gr. | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | ? | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 |
| *Bathysciola* IV | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | ? | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 |
| *Speocharinus* gr. | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 |
| *Quaestus* gr. | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 |
| *Catops* | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | ? | 0 | ? | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | ? | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? | 0 | 0 | 0 | ? | ? |
| *Speonemadus* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? | 0 | ? | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | ? | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? | 0 | 0 | 0 | ? | ? |
| *Breuilia* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | ? | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 |
| *Breuilites* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | ? | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 |
| *Espanoliella* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | ? | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 |
| *Oresigenus* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 |
| *Q. (Amphogeus) escalerai* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 |
| *Anillochlamys* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 |
| *Ovobathysciola* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 1 |
| *Paranillochlamys* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 |
| *Pseudochlamys* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | ? | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 |
| *Spelaeochlamys* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | ? | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| *Patriziella* | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 |
| *Notidocharis* | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | ? | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 |
| *Speonomidius* | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 1 |
| *Bathysciola ovata* gr. | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | ? | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 |
| *T. (Antrocharidius)* | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | ? | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 |