



<http://dx.doi.org/10.11646/zootaxa.3821.3.2>

<http://zoobank.org/urn:lsid:zoobank.org:pub:13AD493F-8B71-4F1E-9887-CBDB9A2FAA45>

A new genus of speleophriid copepod (Copepoda: Misophrioida) from a cenote in the Yucatan, Mexico with a phylogenetic analysis at the species level

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Abstract

A new genus and species of speleophriid copepod, *Mexicophria cenoticola* **gen. et sp. nov.**, is described based on material collected from a cenote in the Yucatan Peninsula of Mexico. It is characterised by relatively reduced fifth legs that are located adjacent to the ventral midline in both sexes, by the possession of a bulbous swelling on the first antennular segment in both sexes, and by the reduced setation of the swimming legs. The presence of just one inner margin seta on the second endopodal segment of legs 2 to 4 is a unique feature for the family. A phylogenetic analysis places the new genus on a basal lineage of the family together with its sister taxon, *Boxshallia* Huys, 1988, from Lanzarote in the Canary Islands, and recovers the existing genera as monophyletic units. The zoogeography is discussed at local, regional, ocean basin and global scales.

Key words: descriptive taxonomy, new species, Speleophriidae, phylogeny, anchialine fauna

Introduction

The family Speleophriidae currently comprises eight genera and 19 species: *Speleophria* Boxshall & Iliffe, 1986 (5 species), *Expansophria* Boxshall & Iliffe, 1987 (4 species), *Dimisophria* Boxshall & Iliffe, 1987 (1 species), *Boxshallia* Huys, 1988 (1 species), *Speleophriopsis* Jaume & Boxshall, 1996 (4 species), *Huysia* Jaume, Boxshall & Iliffe, 1998 (1 species), *Protospeleophria* Jaume, Boxshall & Iliffe, 1998 (1 species) and *Archimisophria* Boxshall, 1983 (2 species) (Boxshall & Halsey 2004). Almost all speleophriid species occur in anchialine coastal habitats, the only exceptions being the two species of *Archimisophria*, both of which occur in the deep hyperbenthic community in the tropical Atlantic (Boxshall 1983, Alvarez 1985).

The first cave-dwelling misophrioid to be described, *Speleophria bivexilla* Boxshall & Iliffe, 1986, was reported from Bermuda by Boxshall & Iliffe (1986) and since then new taxa have been found in many anchialine habitats in tropical and subtropical latitudes. As well as Bermuda, *Speleophria* species have been described from the Balearic Islands (Spain), northern Western Australia, Croatia and the Nullarbor region of southern Western Australia (Jaume & Boxshall 1996a, Jaume *et al.* 2001, Kršinić 2008, Karanovic & Eberhard 2009), *Expansophria* species are known from the Galapagos (Ecuador), Sardinia (Italy), Palau, and the Canary Islands (Spain) (Boxshall & Iliffe 1987, 1990, Jaume & Boxshall 1996b), the sole species of both *Dimisophria* and *Boxshallia* are from Lanzarote in the Canary islands (Boxshall & Iliffe 1987, Huys 1988), *Speleophriopsis* species are known from the Balearic and Canary islands, from Palau and from Bermuda (Boxshall & Iliffe 1987, Jaume & Boxshall 1996a), while the sole species of both *Huysia* and *Protospeleophria* are recorded only from the Exuma Cays in the Bahamas (Jaume *et al.* 1998). The three richest genera, comprising four or five species, each display an extreme disjunct distribution with species known from anchialine habitats in at least two ocean basins.

Boxshall & Jaume (2000) analysed the biogeography and attempted to reconstruct the history of colonization of anchialine habitats by the speleophriids, concluding that the extreme disjunct distribution pattern exhibited by members of the family results from a dispersal and colonization episode prior to the closure of the Tethys Sea, and that deep-water forms may represent a secondary colonization. Speleophriids, together with remipedes, thermosbaenaceans, epacteriscid copepods and the myodocopan ostracod genus *Humphreysella* Kornicker & Danielopol, in Kornicker *et al.*, 2006 (see Iglukowska & Boxshall 2013, for revision of *Danielopolina* Kornicker & Sohn, 1976), are typical members of a particular suite of crustacean taxa that comprises the core fauna of anchialine habitats (Wagner 1994, Fosshagen *et al.* 2001, Kornicker *et al.* 2007). Members of this core anchialine faunal suite (referred to as the Remipede Type by Humphreys & Danielopol (2006)), tend to share the same extreme disjunct distributions, and this tendency has been interpreted as evidence of a common biogeographical history (e.g. Kornicker *et al.* 2007). The current working hypothesis interprets these anchialine faunas as Tethyan – relicts of the shallow-water fauna of the Mesozoic Tethys Sea, which were subsequently dispersed by plate tectonic movements (Stock 1993, Boxshall & Jaume 2000, Danielopol *et al.* 2000, Kornicker *et al.* 2007).

The aim of the present paper is to describe a new speleophriid found in an anchialine cave system in the Yucatan Peninsula in Mexico and to include it in a phylogenetic analysis of the family in order to estimate its relationships with other genera. Speleophriid misophrioids have been found and reported from four different cenotes in the Yucatan Peninsula before (Jaume *et al.* 1998), but the material was in too poor condition to permit taxonomic description.

Material and methods

The copepods were collected on 14 April 2004 from Cenote Carwash (also known as Aktun Ha) near Tulum, Quintana Roo, Mexico. They were collected using a plankton net in the downstream drain passage, at or below the halocline at depths of 19–23 m. In addition to ~100 copepods, other collected specimens included ~150–200 nerillid polychaetes, 7 mysids (?*Antromysis cenotensis* Creaser, 1936), 1 remipede (*Speleonectes tulumensis* Yager, 1987), 1 shrimp (*Typhlatya* Creaser, 1936 sp.), and 1 acanthocephalan. This habitat is a type 3 (oblong) open-type cenote (location: 20°16' 26" N; 87° 29' 11"W). It has a length of 50 m and a width of 15 m, with a mean depth of 5 m. It has submerged caves at opposite sides of the cenote vertical walls, one of the entrances is 45 m wide and leads to a large chamber with stalactites and stalagmites. This passage is known to extend at least 3 km and the halocline is found at a depth of 21 m. More information about the cenote is given by Suárez-Morales *et al.* (1996) and its palaeoenvironmental evolution during the Holocene (from ca. 6800 BP) was reconstructed in detail by Gabriel *et al.* (2009). Freshwater copepods collected in the freshwater upper layer above the halocline were: *Arctodiaptomus dorsalis* (Marsh, 1907), *Thermocyclops inversus* (Kiefer, 1936), *Tropocyclops prasinus mexicanus* Kiefer, 1938, and *Mesocyclops yutsil* Reid in Fiers, Reid, Iliffe & Suárez-Morales, 1996.

Entire and dissected specimens were examined on a Leitz Diaplan compound microscope equipped with differential interference contrast. Drawings were made using a drawing tube. The type specimens are deposited in the collections of ECOSUR, Chetumal, Mexico and the Natural History Museum, London.

A Branch and Bound analysis was performed using PAUP version 4.0b10 (Swofford 2003) and all characters were treated as unordered. The matrix comprised 56 characters and 19 taxa, including *Fosshageniella glabra* Jaume & Boxshall, 1997, which was used as the outgroup. *Dimisophria* was excluded because of the uncertainty concerning its state of maturity, and *Expansophria sarda* Jaume & Boxshall, 1996 was omitted because of the absence of data on its cephalosomic limbs. Male and female antennular segmentation patterns vary and are, therefore, treated separately. The characters used in the analysis are as follows:

Female antennule

- | | |
|--|-----------------------------------|
| 1. Articulation between segments I–II: | expressed = 0 / not expressed = 1 |
| 2. Articulation between segments II–III: | expressed = 0 / not expressed = 1 |
| 3. Articulation between segments IX–X: | expressed = 0 / not expressed = 1 |
| 4. Articulation between segments X–XI: | expressed = 0 / not expressed = 1 |
| 5. Articulation between segments XI–XII: | expressed = 0 / not expressed = 1 |

6. Articulation between segments XXVI–XXVII: expressed = 0 / not expressed = 1
7. Proximal segment (I): cylindrical = 0 / inflated dorsally = 1
8. Aesthetasc on antennular segment III: normal = 0 / hypertrophied = 1
9. Aesthetasc on antennular segment VII: normal = 0 / hypertrophied = 1
10. Aesthetasc on antennular segment XI: normal = 0 / hypertrophied = 1
11. Aesthetasc on antennular segment XVI: normal = 0 / hypertrophied = 1
12. Seta on antennular segment XVI: normal = 0 / hypertrophied = 1
- Male antennule
13. Articulation between segments XIX–XX: expressed = 0 / not expressed = 1
14. Articulation between segments XXI–XXII: expressed = 0 / not expressed = 1
15. Articulation between segments XXII–XXIII: expressed = 0 / not expressed = 1
16. Articulation between segments XXV–XXVI: expressed = 0 / not expressed = 1
17. Articulation between segments XXVI–XXVII: expressed = 0 / not expressed = 1
18. Aesthetasc on antennular segment III: normal = 0 / hypertrophied = 1
- Mandible
19. Number of setae on basis of palp: 3/2/1/0 = 0/1/2/3
- Maxillule
20. Seta representing exite on maxillary basis: present = 0 / absent = 1
21. Number of setae on exopod 1: 1-10 / 9-8 / 3-2 = 0 / 1 / 2
- Maxilla
22. Allobasis : hoop-like, or slightly enlarged = 0 / enlarged, forming notched triangular allobasis = 1
- Maxilliped
23. Seta representing praecoxal endite: present = 0 / absent = 1
24. Number of setae derived from 1st endopodal segment: 2/1/0 = 0/1/2
- Swimming legs
25. Articulation between endopodal segments 2 and 3 of leg 1: expressed = 0 / not expressed = 1
26. Inner coxal seta of leg 1: present = 0 / absent = 1
27. Inner seta on 1st exopodal segment of leg 1: present = 0 / absent = 1
28. Proximal inner seta derived from 2nd endopodal segment of leg 1: present = 0 / absent = 1
29. Proximal seta on inner margin of 3rd exopodal segment of leg 1: present = 0 / absent = 1
30. Proximal seta on inner margin of 3rd exopodal segment of leg 2: present = 0 / absent = 1
31. Proximal seta on inner margin of 3rd exopodal segment of leg 3: present = 0 / absent = 1
32. Proximal seta on inner margin of 3rd exopodal segment of leg 4: present = 0 / absent = 1
33. Middle seta on inner margin of 3rd exopodal segment of leg 4: present = 0 / absent = 1
- Female fifth swimming leg
34. Coxa-basis articulation: expressed = 0 / not expressed = 1
35. Free endopod with articulation on basis: expressed = 0 / not expressed = 1
36. Spine a (outer spine derived from 1st exopodal segment): present = 0 / absent = 1
37. Spine b (outer spine derived from 2nd exopodal segment): present = 0 / absent = 1
38. Spine d (proximal outer spine from 3rd exopodal segment): present = 0 / absent = 1
39. Spine e (distal outer spine derived from 3rd exopodal segment): present = 0 / absent = 1
40. Position of setal element g (inner distal element on exopod): margin = 0 / offset on segment surface = 1
41. Form of setal element g: articulated basally = 0 / spiniform, fused to segment = 1
42. Seta k (inner seta derived from second exopodal segment): present = 0 / absent = 1
43. Setal element C (subapical seta on apex of endopod): present = 0 / absent = 1
44. Setal element D (apical seta on apex of endopod): present = 0 / absent = 1
- Male fifth swimming leg
45. Articulation between coxa and basis: expressed = 0 / not expressed = 1
46. Free endopod with articulation on basis: expressed = 0 / not expressed = 1
47. Articulation between exopodal segments 2 and 3: expressed = 0 / not expressed = 1
48. Spine a (outer spine derived from 1st exopodal segment): present = 0 / absent = 1

49. Spine b (outer spine derived from 2nd exopodal segment): present = 0 / absent = 1
 50. Spine d (proximal outer spine from 3rd exopodal segment): present = 0 / absent = 1
 51. Spine e (distal outer spine from 3rd exopodal segment): present = 0 / absent = 1
 52. Position of setal element g (inner distal element on exopod): margin = 0 / offset on segment surface = 1
 53. Seta k (inner seta derived from 2nd exopodal segment): present = 0 / absent = 1
 54. Setal element C (subapical seta on apex of endopod): present = 0 / absent = 1
 55. Setal element D (apical seta on apex of endopod): present = 0 / absent = 1
- Body
56. Carapace-like extension from rear margin of cephalosome: absent = 0 / completely enclosing first pedigerous somite = 1

Taxonomy

Mexicophria gen. nov.

Diagnosis. Cephalosome not produced posteriorly into carapace-like extension. First pedigerous somite free. Female urosome 5-segmented, with genital and first abdominal somites fused to form genital double-somite; single copulatory pore opening mid-ventrally. Free abdominal somites separated by extensive arthrodistal membrane allowing telescoping of adjacent somites. Caudal rami armed with 6 setae. Antennules 27-segmented in female, with compound apical segment derived from ancestral segments XXVII–XXVIII. Proximal segment swollen dorsolaterally, forming bulbous lobe: segments II–X and XI–XII not completely separated along posterior margin; elongate aesthetascs on segments XI and XVI. Antennule 23-segmented in male; geniculate between segments 19 (XIX–XX) and 20 (XXI–XXIII); segment 21 (XXIV) free, segment 22 (XXV–XXVI) and apical segment (XXVII–XXVIII) double. Antenna with 1 seta on basis; exopod 7-segmented bearing 1, 1, 1, 1, 1, 1, 4 setae; endopod indistinctly 3-segmented bearing 2, 5, 7 setae. Mandible with 2 setae on basis, endopod with 4, 7 setae; exopod with 1, 1, 1, 3 setae. Maxillulary basal exite absent. Allobasis of maxilla with armature formula 4, 2. Maxilliped syncoxa lacking praecoxal seta; endopod 6-segmented, setal formula 2, 2, 2, 2, 2+1, 4. Swimming legs 1–4 biramous with 3-segmented rami except for 2-segmented endopod of leg 1; spine and seta formula as below for type species. Fifth legs uniramous in both sexes, located immediately adjacent to ventral midline, lacking intercoxal sclerite; segmentation sexually dimorphic, 2-segmented, with unsegmented protopodal segment and single exopodal segment in female, 3-segmented, with separate coxa and basis plus single exopodal segment in male.

Type species. *Mexicophria cenotocola* gen. et sp. nov., by original designation.

Etymology. The generic name is based on the name of the country where it is found, Mexico, combined with the ending – *ophria*, which is commonly used within the family.

Remarks. The fifth legs of the new genus are located immediately adjacent to the ventral midline of the somite and lack an intercoxal sclerite, as in several other speleophriid genera, but they are more strongly reduced than in most other genera. *Speleophriopsis*, *Speleophria*, *Boxshallia*, *Archimisophria*, *Huysia* and *Protospeleophria* all have a fifth leg in the female consisting of at least three segments. The enigmatic genus *Dimisophria* has a 2-segmented fifth leg (Boxshall & Iliffe 1987), but this genus can be distinguished from *Mexicophria* n. gen. by the lack of a bulbous lobe on the proximal segment of the antennule, and by mouthpart characters, such as the reduced setation on the mandibular endopod and the maxillulary exopod. *Expansophria* species vary in the form of the female fifth leg. In female *E. dimorpha* Boxshall & Iliffe, 1987 the fifth leg is 3-segmented, comprising an unarmed coxa, the basis with a single outer seta and a 1-segmented exopod with 2 apical setae but in *E. apoda* Boxshall & Iliffe, 1987, the female fifth leg is lacking. Within the family, this 3-segmented state is the most similar to that found in the new genus. However, the male fifth legs differ markedly between the new genus and *Expansophria*: in the former they are 3-segmented with 1-segmented exopod whereas known males of the latter genus have a 5-segmented fifth leg with a 3-segmented exopod (Boxshall & Iliffe 1987, Jaume & Boxshall 1996b).

The new genus exhibits reduced leg setation. The second endopodal segment of legs 2 to 4 bears only 1 inner seta and this is a unique character state for the family. The third exopodal segment of legs 1 to 4 carries 3, 4, 4, 3 inner margin setae, respectively, as in *Boxshallia*, *Huysia*, and *Protospeleophria* (Huys 1988, Jaume *et al.* 1998)

and 3, 4, 4, 3 outer and distal spines. These other genera do not share the latter formula: in *Boxshallia* and *Protospeleophria* it is 4, 4, 4, 4, while in *Huysia* it is 3, 4, 4, 4. The presence of flagellated tips on the outer spines of exopodal segments 2 and 3 of leg 1 is a fine scale similarity shared only with *Boxshallia* out of these three genera. *Boxshallia* and the new genus also share the possession of the conspicuous bulbous lobe on the proximal segment of the antennule.

The new genus can be distinguished from *Boxshallia* by its derived 2-segmented endopod of leg 1 since this ramus retains a 3-segmented state in *Boxshallia*. Another major difference between these two genera is the fifth leg. In *Boxshallia* the intercoxal sclerite is retained, the coxa and basis are separate in both sexes and the exopod is 2-segmented, plus the endopod is represented by an inner seta on the basis, so the leg is biramous (Huys 1988). In contrast, in the new genus the leg is uniramous, the exopod is 1-segmented in both sexes and the intercoxal sclerite is lacking.

***Mexicophria cenotocola* gen. et sp. nov.**

Type locality. Cenote Carwash (also known as Cenote Aktun Ha) near Tulum, Quintana Roo, Mexico; (location: 20°16' 26" N; 87° 29' 11"W).

Type material. Undissected holotype female (ECO-CHZ-07538) and 2 undissected paratype females (ECO-CHZ-07539) stored in the collections of ECOSUR, Chetumal, partly dissected male and 2 undissected female paratypes stored in collections of the Natural History Museum, London, Reg. Nos. NHMUK 2014.10-12.

Adult female. Body (Fig. 1A) cycloform, compressed laterally, colourless. Body length about 0.480 to 0.534 mm [varying with degree of telescoping of urosomites and degree of dorsal flexure of body]. Nauplius eye absent. Prosome 5-segmented, with first pedigerous somite not incorporated into cephalothorax. Dorsal shield of cephalosome lacking carapace-like extension on posterior margin. Rostrum sickle-shaped, powerfully developed, not fused to dorsal shield; ornamented with paired sensilla near middle of lateral margin (Figs 1A, 3A). Prosomal pedigerous somites with evenly rounded posterolateral corners. Urosome 5-segmented (Fig. 1B); genital and first abdominal somites completely fused to form elongate double-somite. Fifth pedigerous somite carrying fifth legs ventrally, adjacent to midline; posterior margin ornamented with smooth hyaline frill ventrally. Genital double-somite (Fig. 1B) with genital field located in anterior third; gonopores separate, in form of narrow sigmoid slits, each closed off by unarmed vestigial leg 6, located latero-ventrally on each side of single copulatory pore located in ventral midline; genital double-somite with conspicuous swelling midventrally in posterior third (Fig. 1A–B); posterior margin with continuous hyaline frill varying in width. Second, third and fourth (= anal) abdominal somites, with extensive arthrodiol membrane anteriorly, allowing telescoping inside posterior end of preceding somite. Anal somite with 2 dorsolateral sensilla, on either side of smooth anal operculum. Caudal rami short, just longer than wide; armed with 6 setae; anterolateral accessory seta (seta I of Huys & Boxshall 1991) absent, as in male (Fig. 1D).

Antennule (Fig. 2A) 27-segmented: segment 1 with dorsolateral margin swollen, forming ovoid protuberance distally; segments 2 to 10 densely packed, perhaps not completely separate posteriorly; segments 11 and 12 not completely separate posteriorly; apical segment double. Segmental armature as follows: segment 1, 3 setae; segments 2 to 10, 2 setae each; segment 11, 2 setae + long aesthetasc; segment 12, 1 seta, segments 13 to 15, 2 setae each; segment 16, 2 setae (1 very long) + long aesthetasc; segments 17 to 20, 2 setae each; segment 21, 2 setae + aesthetasc; segments 22 and 23, 1 seta each; segments 24 to 26, 2 setae (1 posterior) each; apical segment 27 (fused ancestral segments XXVII and XXVIII), 5 setae + aesthetasc.

Antenna (Fig. 2B) biramous: coxa unarmed, ornamented with distal spinule row; basis separate, armed with 1 inner distal seta. Endopod indistinctly 3-segmented; first segment with 2 unequal setae near mid distance along medial margin; second and third segments incompletely separated; second segment with 5 inner and distal setae; third segment with 7 setae on tip and ornamented with longitudinal row of setules along outer margin. Exopod distinctly 7-segmented; second segment longer than first and third, possibly compound; distal segment double; setal formula 1, 1, 1, 1, 1, 4.

Labrum an undivided muscular lobe.

Mandible comprising stout coxal gnathobase (Fig. 2C) and biramous palp (Fig. 2D); gnathobase with large ventral tooth, separated by small gap from slender curved tooth, and row of 5 simple teeth; dorsal seta present;

spinule row present near base of teeth. Mandibular palp with basis markedly longer than wide, bearing 2 unequal setae on inner margin, curved proximal seta longer than endopod; exopod indistinctly 4-segmented, apical segment double; setal formula 1, 1, 1, 3: endopod 2-segmented, proximal segment elongate with convex medial margin; setal formula 4, 7.

Maxillule (Fig. 3B) with large praecoxal arthrite bearing 12 elements, including pair of setae on posterior surface (Fig. 3C); coxa with single endite bearing 5 setae, and vestigial epipodite incorporated into segment margin, represented by 7 setae; basis with unarmed outer margin; 2 widely separated basal endites present, proximal endite discrete, armed with 3 setae; distal endite incorporated into segment, represented by 4 setae; exopod 1-segmented, lamella-like, with 8 setae, medial and lateral margins with setule rows; endopod with 2 free segments, proximal segment representing double segment subdivided by partial suture line; setal formula (4, 2), 5.

Maxilla (Fig. 3D) powerfully developed, 6-segmented. Praecoxa and coxa discrete; each with 2 endites, setal formula 5, 3, 3, 3. Allobasis with proximal (= basal) endite powerfully developed, drawn out distally into stout, non-articulating curved medial claw with 3 setae implanted around base; distal (= endopodal) endite weakly developed, represented by 2 unequal setae. Free endopod 3-segmented, with setal formula 2, 2, 4.

Maxilliped 8-segmented (Fig. 3E), comprising syncoxa with 3 defined endites, basis and slender 6-segmented endopod, with proximal endopodal segment free: praecoxal seta lacking, syncoxal endites with setal formula 0, 2, 4, 3; basis with 2 setae, distal setae bilaterally spinulate; endopodal setal formula 2, 2, 2, 2, 2+1, 4; distal segment longest.

Swimming legs 1 to 4 biramous (Fig. 4A–D), with 3-segmented rami except 2-segmented endopod of leg 1, due to failure of second and third segments to separate; members of each leg pair joined by intercoxal sclerite; incomplete hoop-like praecoxa remnant retained laterally on each leg; distolateral corners of endopodal segments 1 and 2 of legs 2 to 4 typically produced into conspicuous spinous processes, bifid on second segment of leg 2. Inner margin of first exopodal segment of legs 1 to 4 and outer margins of all endopodal segments of legs 2 to 4 ornamented with setules. Outer margins of exopod segments of leg 1 with scale-like ornamentation. Spine and seta formula for legs 1 to 4 as follows:

	coxa	basis	exopodal segments	endopodal segments
leg 1	0-0	I-1	I-1; I-1; II,I,3	0-1; 1,2,3
leg 2	0-1	1-0	I-1; I-1; III,I,4	0-1; 0-1; 1,2,3
leg 3	0-1	1-0	I-1; I-1; III,I,4	0-1; 0-1; 1,2,2
leg 4	0-1	1-0	I-1; I-1; II,I,3	0-1; 0-1; 1,2,2

Outer margin spines and terminal spine on exopod of leg 1 each with flagellate tip (Fig. 4A).

Members of fifth leg pair (Fig. 1C) located immediately adjacent to ventral midline, intercoxal sclerite lacking. Fifth leg slender, uniramous, 2-segmented; proximal segment representing undivided protopod, slightly wider than and shorter than distal segment, bearing outer basal seta; distal segment 3.5 times longer than wide, representing exopod, bearing 2 spiniform elements on distal margin; outer spine slightly longer than inner.

Adult male. Body (Fig. 1D) cycloform as in female, sexually dimorphic in antennules, legs 5 and 6, and in genital segmentation. Body length 0.476 mm. Urosome 6-segmented, with genital somite just longer than wide, and markedly larger than all other urosomites; free posterior margins of genital and first to third free abdominal somites with narrow strips of hyaline frill; segments not showing extreme telescoping as in females. Genital somite with paired ventral gonopores located close to posterior margin, closed off by opercular plates formed by sixth legs (Fig. 1E). Sixth legs each with 2 distal setae; posterior margin of somite with tooth-like protuberance just lateral to gonopore.

Antennules (Fig. 3F) bilaterally symmetrical, similar to those of female in general aspect, but indistinctly 23-segmented with articulations between densely packed segments 2 to 11 less complete than in female; segment 1 with dorsolateral margin swollen, forming ovoid protuberance distally, as in female; segments 2 to 11 partly fused, separated by incomplete articulations; segments 12 to 18 separate, with segment 15 (XV) forming sheath around proximal part of segment 16 (XVI); geniculate between segments 19 (XIX–XX) and 20 (XXI–XXIII); segment 21 (XXIV) free, segment 22 (XXV–XXVI) and apical segment (XXVII–XXVIII) double. Segmental armature as in female except as follows: segments 4, 7 and 8 each with 1 seta; double segment 19 (XIX–XX) bearing 1 seta, 1 modified plate-like element; triple segment 20 (XXI–XXIII) with 2 modified, plate-like elements plus 1 distal seta.

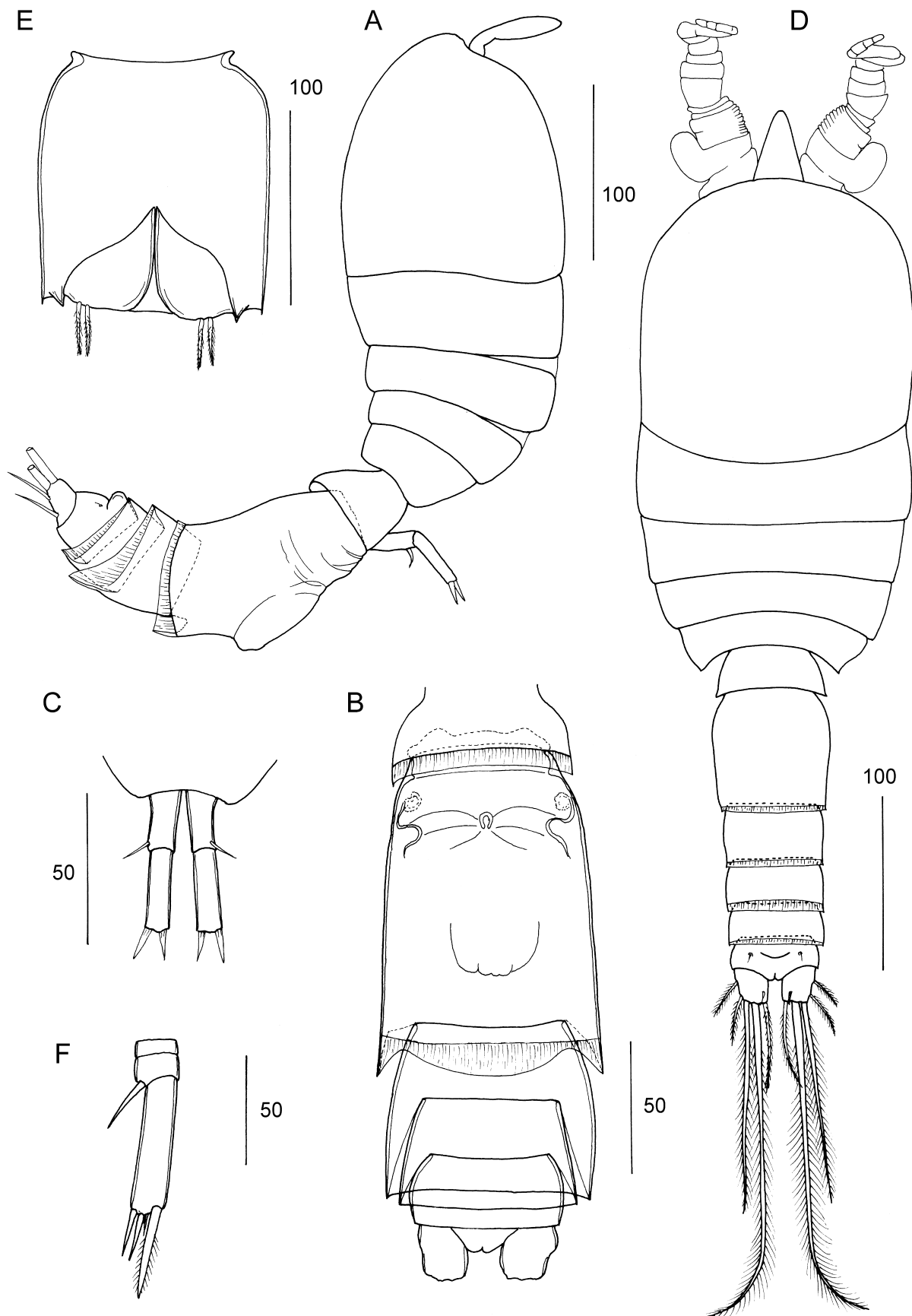


FIGURE 1. *Mexicophria cenoticola* gen. et sp. nov. Female: A, habitus, lateral; B, urosome ventral with fifth legs removed and caudal setae not figured; C, fifth legs, anterior. Male: D, habitus, dorsal; E, genital somite, ventral; F, fifth leg, anterior. Scale bars: A, D, E = 100 μ m; B, C, F = 50 μ m.

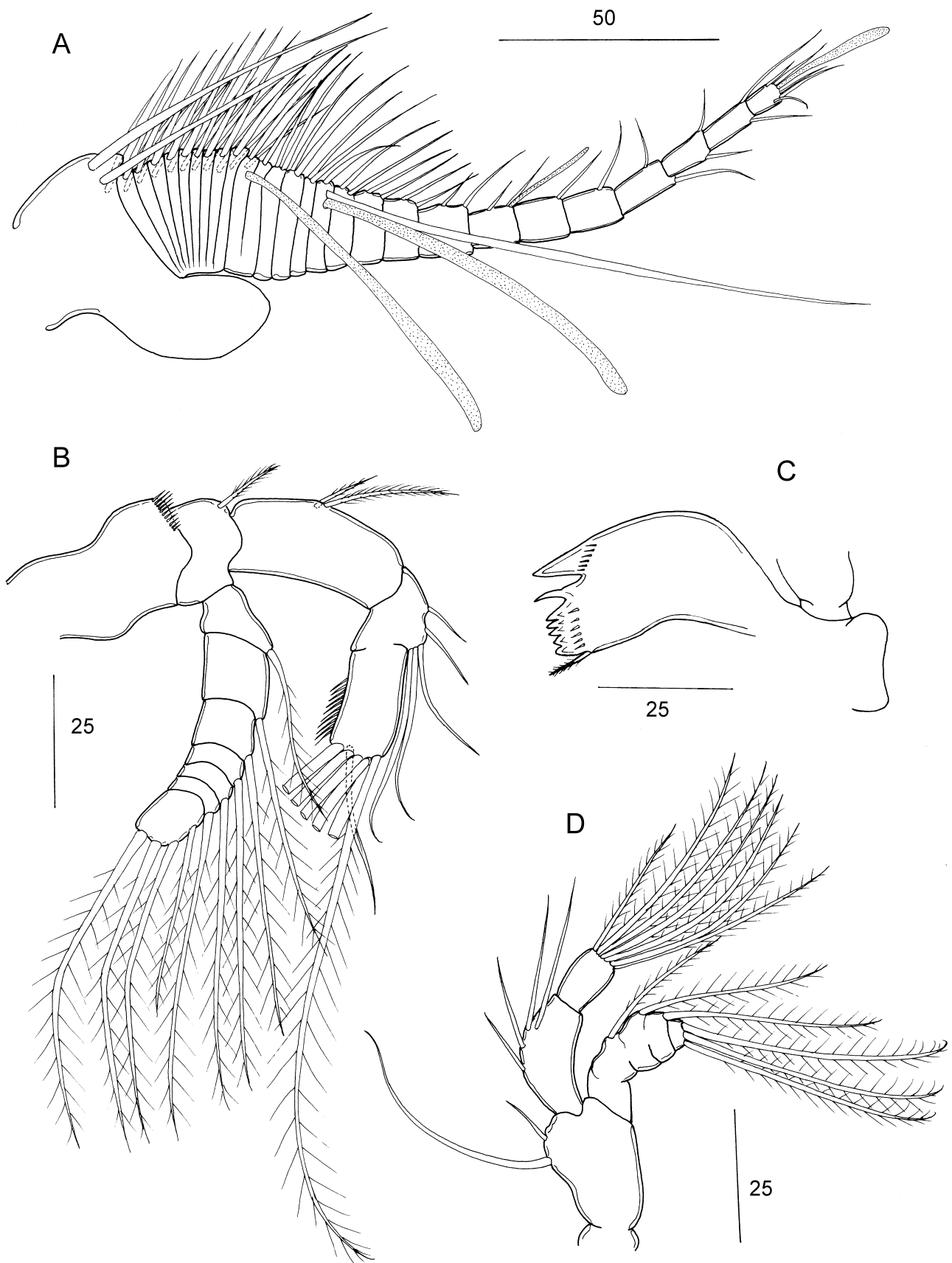


FIGURE 2. *Mexicophria cenoticola* gen. et sp. nov. Female: A, antennule; B, antenna; C, coxal gnathobase of mandible; D, mandibular palp. Scale bars: A = 50 µm; B–D = 25 µm.

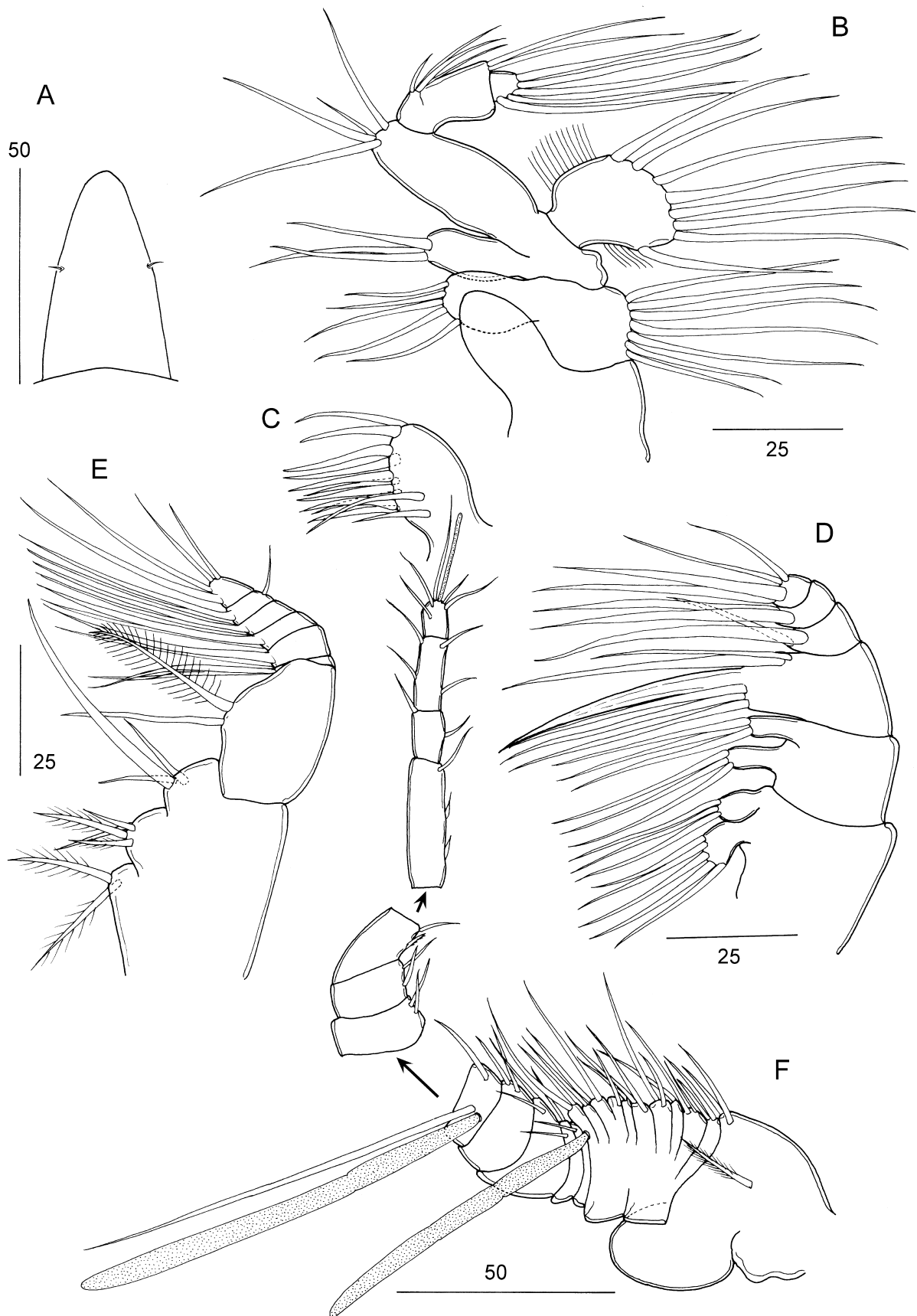


FIGURE 3. *Mexicophria cenoticola* gen. et sp. nov. Female: A, rostrum, frontal view; B, maxillule, with arthrite drawn separately; C, arthrite of maxillule; D, maxilla; E, maxilliped. Male: F, antennule (in 3 parts). Scale bars: A, F = 50 µm; B–E = 25 µm.

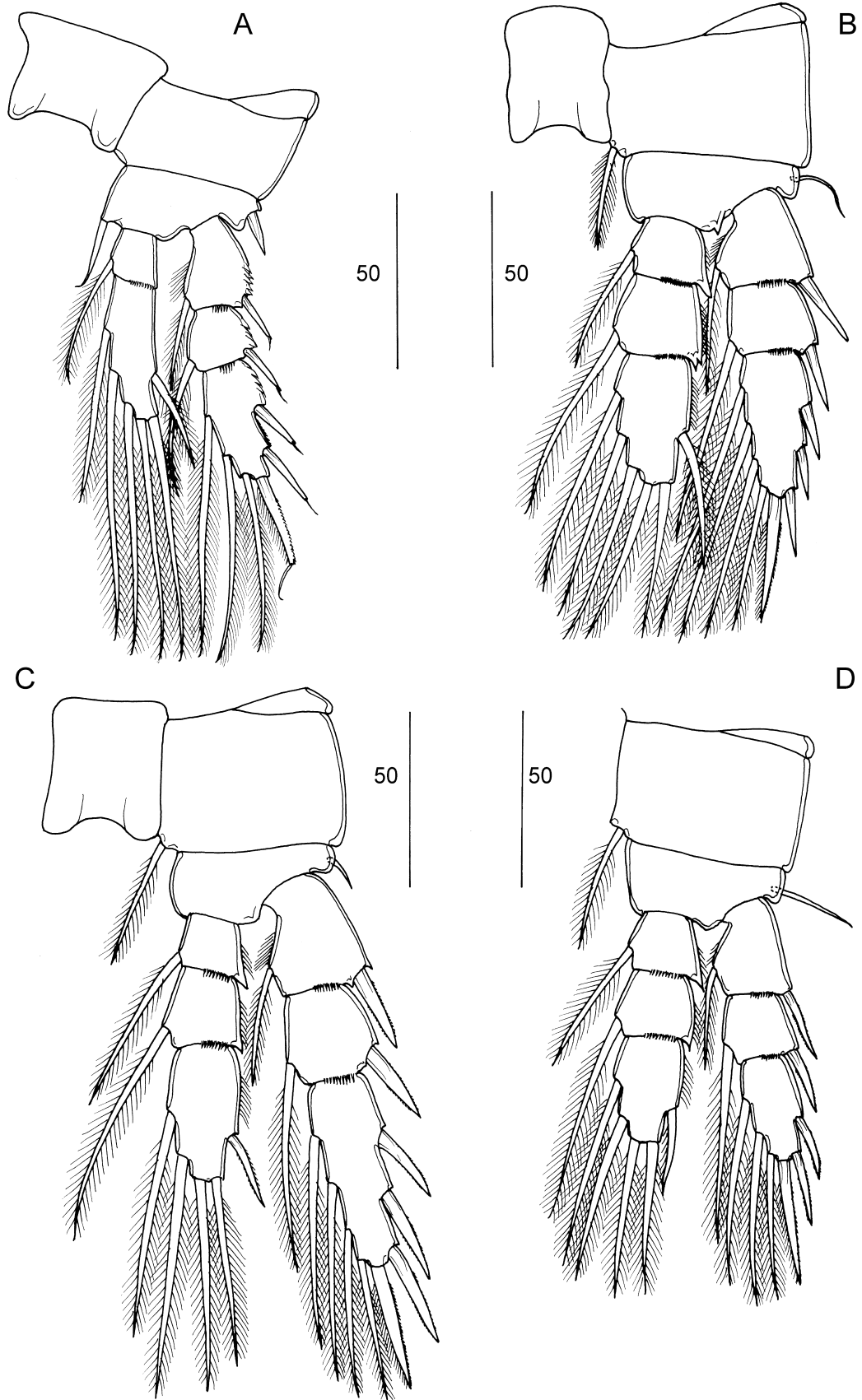


FIGURE 4. *Mexicophria cenoticola* gen. et sp. nov. Female: A, leg 1; B, leg 2; C, leg 3; D, leg 4. All scale bars = 50 μ m.

Members of fifth leg pair located immediately adjacent to ventral midline, intercoxal sclerite lacking. Fifth leg slender, uniramous, 3-segmented (Fig. 1F), comprising coxa, basis and 1-segmented exopod: coxa short, unarmed; basis slightly longer than coxa, bearing outer basal seta; distal (exopodal) segment 4.2 times longer than wide; narrower than coxa and basis, bearing 4 setal elements on distal margin, inner spine slightly more than half as long as segment, ornamented with minute pinnules bilaterally, adjacent spine minute, 2 outer spines about equal in length.

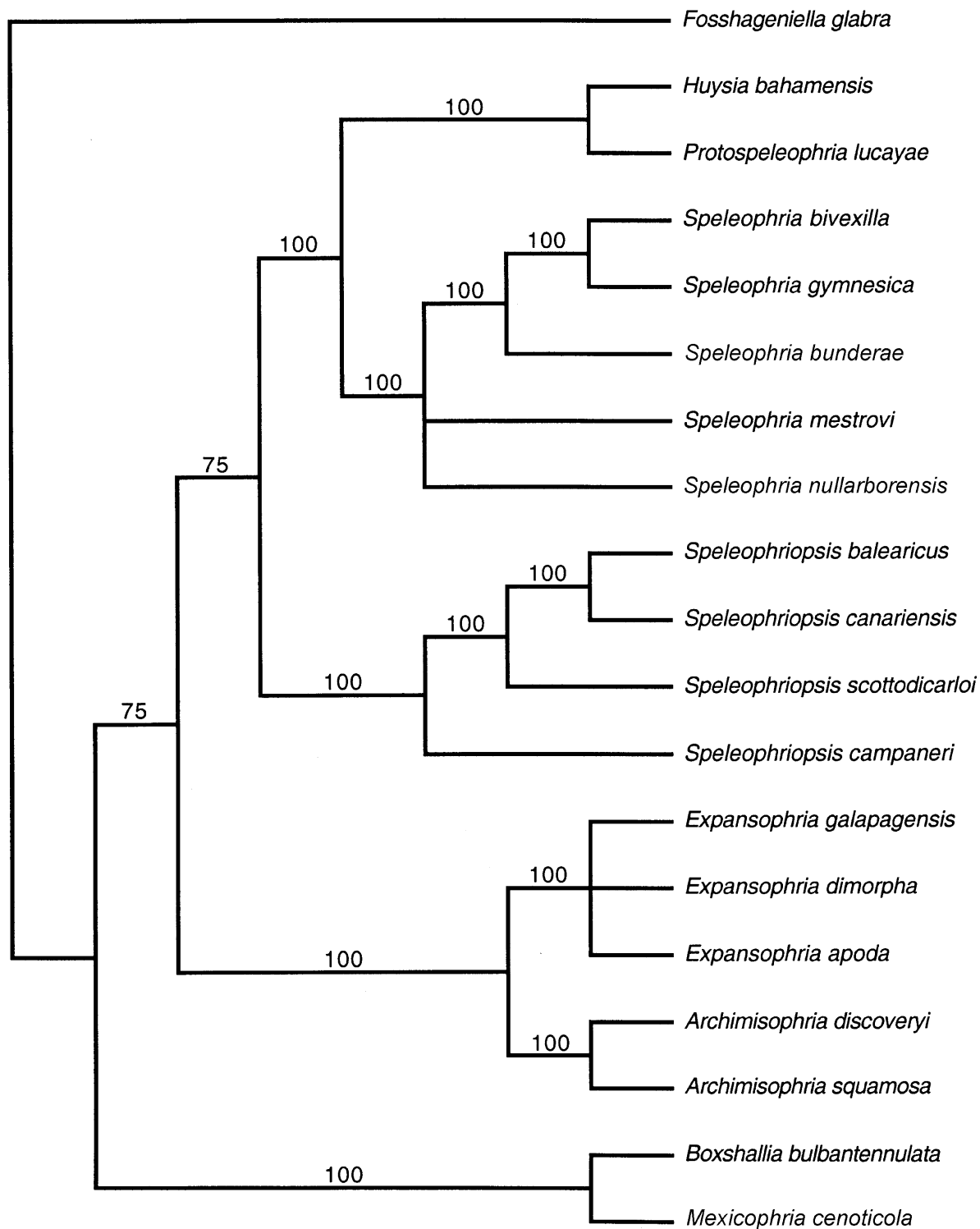


FIGURE 5. Majority rule (50%) consensus tree generated by PAUP, showing estimated phylogenetic relationships between genera of the family Speleophriidae, excluding the incompletely known *Dimisophria*.

Results of phylogenetic analysis

A Branch and Bound analysis recovered 12 equally parsimonious trees of branch length 112. The majority rule tree recovered four main clades (Fig. 5), each with 100 percent bootstrap support. The most basal offshoot was a clade comprising two monotypic genera, *Boxshallia* and *Mexicophria* n. gen., which was sister to the remaining speleophriids. The next offshoot was a clade again comprising two genera, *Expansophria* and *Archimisophria*, each of which was monophyletic. The remaining taxa were recovered in two clades, one consisting of a monophyletic *Speleophriopsis*, and the other made up of two subclades, one containing *Speleophria* and the other, *Huysia* and *Protospeleophria*. All of the existing genera are recovered as monophyletic in both the strict consensus and majority rule trees. The tree is fully resolved except for uncertainty within the genus *Speleophria*. Three species, *S. bivexilla*, *S. gymnesica* and *S. bunderae* cluster together but the relationships with the basal species *S. mestrovi* and *S. nullarborensis* are unresolved. Relationships within *Expansophria* are also unresolved, and because the incompletely known species *E. sarda* was excluded from the analysis, the uncertainty extends to all four described species.

Discussion

Mapping geographical distributions onto the tree (Fig. 5) presents some interesting distribution patterns. On the smallest geographical scale, sister species can occur in the same cave on a single island (*Huysia* and *Protospeleophria* on Norman's Pond Cay in the Exumas) (Jaume *et al.* 1998). Currently no evidence is available that indicates whether reproductive isolation between these two taxa evolved within the cave (i.e. "sympatrically"). However, given the amplitude of sea-level oscillations during the last half-million years, with a maximum highstand of around +13 to 20 m above present sea level 400,000 years ago (interglacial marine isotope stage (MIS) 11; Kindler & Hearty 2000, Raymo & Mitrovica 2012), and a minimum of ca. -140 m, 450,000 years ago (MIS 12; Rohling *et al.* 1998), it seems likely that previously separated populations could have secondarily come back into contact as the interconnections between cave systems changed with sea-level changes.

Sister taxa are also found on islands in the western Mediterranean and in the Canaries (*Speleophriopsis balearicus* and *S. canariensis*). There is a relatively short geographical distance separating these localities and this is reflected by other close relationships between these island cave faunas. For example, the cyclopinid *Muceddina multispinosa* Jaume & Boxshall, 1996 is found in caves on both Majorca and Sardinia in the western Mediterranean and on Lanzarote on the Canaries (Jaume & Boxshall 1996c). This species has also been found outside caves (Holmes & Gotto 2000) but still represents a faunal link between these islands as part of a regional fauna. The calanoid copepod *Stygocyclopia balearica* Jaume & Boxshall, 1995 occurs in anchialine habitats on both the Balearics and the Canaries (Jaume *et al.* 1999). Similarly, as highlighted by Bréhier & Jaume (2009), in their discussion of the relationships of the anchialine cave amphipod *Pseudoniphargus leucatus* Bréhier & Jaume, 2009, there are close similarities between cave crustaceans from the shores and islands of the western Mediterranean and those from the neighbouring Atlantic coast and islands.

There are several examples of amphiatlantic distributions within the Speleophriidae. *Speleophriopsis scottodicalloi* from Bermuda is the sister species of the eastern pairing of *S. balearicus* and *S. canariensis*, similarly *Boxshallia* from Lanzarote in the Canaries and *Mexicophria* n. gen. from Mexico are sister taxa, as are *Speleophria gymnesica* from Majorca and *S. bivexilla* from Bermuda. Similar amphiatlantic distribution patterns have been found in a range of other cave crustacean taxa including ostracods, amphipods and remipedes (Kornicker *et al.* 2007, Bauzà-Ribot *et al.* 2012, Koenemann *et al.* 2009). A vicariance-based explanation has been proposed to explain such distributions: namely that such amphiatlantic sister taxa were separated by the opening of the proto-Atlantic ocean in the later Mesozoic (e.g. Stock 1993, Boxshall & Jaume 2000). The availability of molecular sequence data has recently allowed this hypothesis to be tested. Bauzà-Ribot *et al.* (2012) analysed relationships between, and divergence times of, species of subterranean amphipods. They examined metacrangonyctids from islands on both sides of the Atlantic and their results supported an ancient origin of the family, prior to the Late Cretaceous. Molecular dating of the divergence suggested a major diversification between 96 and 85 million years ago that may have coincided with the opening of the Atlantic.

On the largest zoogeographical scale there are resolved sister group relationships between the terminal clade

comprising the three Atlantic species of *Speleophriopsis* and its sister taxon, *S. campaneri*, from the Indo-Pacific, and between the amphiatlantic *Speleophria gymnesica* / *S. bivexilla* pair and its sister taxon, *S. bunderae* from Western Australia. There must be similar distributional features within the unresolved *Expansophria* because it comprises species from the Mediterranean, the Canary Islands, Palau in the Indo-West Pacific and the Galapagos in the eastern Pacific. Explanations of such distribution patterns have been generated by numerous authors (e.g. Stock 1986, 1993, Boxshall & Jaume 2000, Danielopol *et al.* 2000, Kornicker *et al.* 2007). The current working hypothesis explaining these extreme disjunct distributions is based on the concept that the shallow benthic ancestors of these anchialine taxa lived around the margins of the Tethys Sea in Mesozoic times, and that this range was subsequently fragmented by plate tectonics – essentially a vicariance process (see Stock 1986, 1993, Danielopol & Rouch 2012). Colonization of subterranean habitats could have occurred prior to or subsequent to the fragmentation. This is essentially the same conclusion reached by Boxshall & Jaume (2000) to explain the relationships and habitat usage of misophrioid copepods. One apparent weakness of the Tethyan vicariance hypothesis has been the requirement for the anchialine taxa, such as the speleophriids, remipedes and thaumatoocypridid ostracods belonging to the genus *Humphreysella*, to persist in anchialine systems in geologically young caves on isolated oceanic islands, over timescales of tens of millions of years, through major tectonic movements and through significant changes in sea level (see Boxshall & Jaume 2000).

The predictive quality of the Tethyan hypothesis was articulated by Wagner (1994) who, after the discovery of a species of the thermosbaenacean *Halosbaena* Stock, 1976 in northern Western Australia (Poore & Humphreys 1992), hypothesized that the ostracod *Danielopolina* would be found there. This was confirmed by Danielopol *et al.* (2000) who reported on the discovery of *Danielopolina kornickeri* Danielopol, Baltanás & Humphreys, 2000 (now treated as the type species of a new monotypic genus, *Welesina kornickeri* by Iglíkowska & Boxshall (2013)). Yeates *et al.* (1987) and Poore & Humphreys (1992) outlined the geological and faunistic evidence supporting the inference that northwestern Australia could be considered as a remnant of the eastern Tethyan belt.

Recently, however, Karanovic & Eberhard (2009) described a second Australian speleophriid, *Speleophria nullarborensis* from the Roe Plains in the Nullarbor region of southern Western Australia. It was collected from Nurina Cave located about 30 km inland from the present coastline using a handnet at subsurface depths where the water had a salinity of 30.4 to 32.1 ppt. Karanovic & Eberhard (2009) stated that *S. nullarborensis* shared the greatest number of morphological characters with *S. gymnesica* but a sister-group relationship is not supported by the analysis presented here (Fig. 5), which was unable to resolve the relationships between *S. nullarborensis* and *S. mestrovi*, and a well-supported clade comprising *S. bivexilla*, *S. gymnesica* and *S. bunderae*. After analysis of detailed geological evidence, Karanovic & Eberhard (2009) concluded that the presence of *Speleophria* in the Roe Plains can be explained either by dispersal, or by a significant extension of the Tethyan track down to the southern coastal margin of Australia. We agree with Karanovic & Eberhard (2009) that more sampling is needed before such distribution patterns can be robustly analysed, and we urge that new samples be preserved for molecular analysis.

Molecular evidence in support of the Tethyan track hypothesis is beginning to be made available. Initial studies by Hunter *et al.* (2008) and Page *et al.* (2008) were focused on a selection of atyid shrimp species from the genus *Typhlatya* and near relatives. About a third of *Typhlatya* species occur in marine cave habitats but Botello *et al.* (2013) found that the genus, as constituted, was paraphyletic and that *Typhlatya* s. str. originated only at an estimated 30.7 million years ago. This is too recent to enable it to function as a good model taxon. In addition, atyids in general are unsuitable as a model to test the Tethyan vicariance hypothesis, because they can occur over a wide range of salinity regimes and are capable of surviving in open marine waters (e.g. Page *et al.* 2005). A better model taxon is provided by the subterranean metacrangonyctid amphipods. The divergence times found by Bauzà-Ribot *et al.* (2012) support an ancient origin of the family, and suggested a major diversification between 96 and 85 million years ago. This may have coincided with the opening of the Atlantic and provides the first robust evidence in support of a divergence time for amphiatlantic cave faunas that is in accord with the Tethyan vicariance hypothesis.

Acknowledgements

We would like to thank cave divers Joerg Hess and Lara Hinderstein who assisted with collections in Cenote Carwash. Field research in Mexico was supported by a collaborative science grant with Fernando Alvarez

(UNAM) from Texas A&M University-CONACyT. Specimens were collected under a permit issued to Fernando Alvarez.

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