

Systematics and ecology of *Linderiella baetica* n. sp. (Crustacea, Branchiopoda, Anostraca, Chirocephalidae), a new species from southern Spain

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ABSTRACT

Linderiella baetica n. sp. (Anostraca, Chirocephalidae), is described from a temperate Mediterranean climate, episodic temporary fresh water body in Puerto Real (Cádiz, South Spain). *Linderiella baetica* n. sp. is characterized by the display of the shape of the basomedial outgrowth on the male second antenna, margin structure of praepipods, structure of medial margin of endopod, penis basal projections, horn-like process on the inner side of the female antenna, female genital somites and of a combination of morphological features involving egg morphology. The study includes a morphological analysis using optical and scanning electron microscopes. Based on the differential diagnosis, a dichotomous key for males, females and resting eggs to *Linderiella* species is proposed. The diagnosis is complemented with phenology of the species and accompanying fauna and aquatic vegetation, and physico-chemical evolution of water along monitoring.

KEY WORDS

Crustacea,
Branchiopoda,
Anostraca,
Linderiella,
Spain,
new species.

RÉSUMÉ

Systématique et écologie de Linderiella baetica n. sp. (Crustacea, Branchiopoda, Anostraca, Chirocephalidae), une nouvelle espèce du sud de l'Espagne.

Linderiella baetica n. sp. est décrite d'une mare temporaire d'eau douce à Puerto Real (Cádiz, sud de l'Espagne), sous climat méditerranéen. *Linderiella baetica* n. sp. se caractérise par une nouvelle combinaison de caractères morphologiques mise en rapport avec la forme baso-médiale de la deuxième antenne du mâle, la marge des lames préépiprodiales, la structure de la marge de l'endopodite, les structures basales du pénis, l'excroissance corniforme de la marge interne de l'antenne de la femelle, les segments génitaux de la femelle, et la combinaison des traits morphologiques des œufs. L'étude comprend une analyse morphologique en utilisant la microscopie optique et électronique. Une clé dichotomique basée sur les diagnoses différentielles, des mâles, femelles et œufs de *Linderiella* est proposée. Les diagnoses sont complétées par l'écologie de l'espèce basée sur les caractéristiques physico-chimiques de l'eau, associée à la phénologie de la végétation et la faune d'accompagnement.

MOTS CLÉS

Crustacea,
Branchiopoda,
Anostraca,
Linderiella,
Espagne,
espèce nouvelle.

INTRODUCTION

The family Linderiellidae was erected by Brtek (1964) who differentiated it from the family Chirocephalidae Daday de Dees, 1910 in which *Linderiella occidentalis* (*Pristicephalus occidentalis* sensu Linder 1941; *Branchinecta occidentalis* sensu Dodds 1923) was included based on the lack of seminal vesicles and of spinous cirrus on the eversible portion of the penis (Brtek 1964; Belk 1982). Latter on Rogers (2002) when in praise of a morphological confirmation to findings by Weekers *et al.* (2002), who proposed the suppression of the family Linderiellidae based on molecular data, reallocated again the genus *Linderiella* in the family Chirocephalidae based on its male genital structure. Currently, four species of *Linderiella* Brtek, 1964 are known: *Linderiella africana* Thiéry, 1986 from the Middle Atlas Mountains (Morocco), *L. massaliensis* Thiéry & Champeau, 1988 from southeast France, and *L. occidentalis* (Dodds, 1923) and *L. santarosae* Thiéry & Fugate, 1994, both from California, USA. In 1978, various specimens belonging to the genus *Linderiella* were found at a temporary pond close to the Los Tollos lagoon, El Cuervo (36°50'N, 6°01'W, southern Spain). The specimens were first referred to *L. occidentalis* (Alonso 1985), but further descriptions of *L. africana* and *L. massaliensis* together with direct

comparison with *L. occidentalis* specimens from thetype locality of this species (California) revealed that the Spanish specimens could belong to a new species (Thiéry & Champeau 1988; Alonso 1996). This batch of specimens got lost and the "Spanish species of *Linderiella*" (sensu Thiéry & Champeau 1988; Thiéry & Fugate 1994) remained undescribed. After repeated surveys carried out by the authors between 1985-2007 in more than 40 temporary pools sited in the surroundings of the original location, this taxon was found again in Puerto Real, Cádiz (36°32'N, 6°11'W), on February 12, 2007, 40 km away (Fig. 1A). Based on specimens collected in this new locality, the description of *Linderiella baetica* n. sp. is completed herein. Environmental conditions supporting this species are also described. Based on monitoring of physico-chemical characteristics and the associated flora and aquatic fauna of the pond during the flooding period, we discuss which is the ecological niche of *Linderiella baetica* n. sp., as well as the main factors that may determine the growth and development of this species.

MATERIAL AND METHODS

Samples were taken with a 100 µm mesh plankton net. Several specimens were fixed with 4% formalin

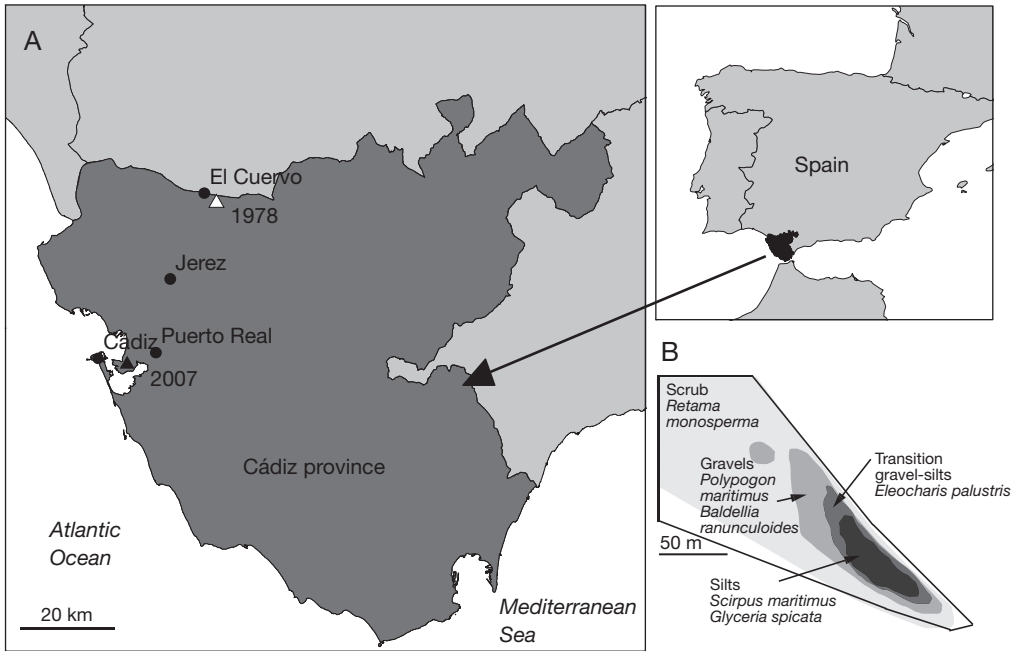


FIG. 1. — **A**, map of study area indicating location where *Linderiella* sp. was cited in 1978 by Alonso (1996) (Δ) and pond where *L. baetica* n. sp. was found in February 2007 (\blacktriangle); **B**, detail of pond shape, indicating associated vegetation and dominant sediment grain size. Grain size was calculated by using the Gradistat® v. 4.0 software, which uses a size scale modified from Udden (1914) and Wentworth (1922).

and camera lucida drawings were made with an Olympus® BH-2 compound microscope equipped with phase contrast optics. For scanning electron microscopy (SEM), adult *Linderiella baetica* n. sp. males and females were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, for 3 h. Then they were postfixed in 2% cacodylate-buffered osmium tetroxide for 1 h. Following several short washes in buffer, the samples were dehydrated in an ascending series of acetones, critical-point dried, sputter-coated with gold, and examined in a SEM microscope (FEI Company®, model Quanta® 200). To observe the eggs, the females with the most mature looking cysts were dissected and the cysts were removed. Air-dried cysts were mounted on SEM stubs using double stick tape. The cysts were then gold-coated and examined with a scanning electron microscope. Air drying was also used by Mura (1986, 1992a, b) for fairy shrimp cyst studies, and by Munuswamy & Subramoniam (1984) in their study of *Streptocephalus dichotomus* Baird, 1860

cysts. Comparison of cysts dried in alcohol with air-dried cysts showed no difference in morphology (Hill & Shepard 1997). Scaled images obtained by SEM were used to indicate measurements of the different body structures.

Once discovered the presence of *L. baetica* n. sp., the physico-chemical features of the temporary water body were monitored with a sampling frequency of 2-5 days. Conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$), pH, dissolved oxygen (% saturation) and temperature ($^{\circ}\text{C}$) were measured with Crison® portable meters. Filtered water samples (0.45 μm) were used for determination of additional water characteristics: sulphate, nitrite, inorganic phosphate and ammonium. The density of aquatic macro- and microinvertebrates were determined by selecting five sampling stations including the deepest area of the pond ($n = 1$), intermediate depth areas ($n = 2$) and shallow waters ($n = 2$). A bottomless cylinder (internal diameter = 0.34 m) was placed at each sampling station and 50 l were filtered with a 100 μm mesh sampling net

(total volume filtered = 200 l; sampling frequency = 2-5 days). Integrated samples were quantified under the stereomicroscope.

ABBREVIATIONS

MNCN Museo Nacional de Ciencias Naturales, Madrid, collection of non insect invertebrates;
 MNHN Muséum national d'Histoire naturelle, Paris;
 UCA Universidad de Cádiz.

SYSTEMATICS

Class BRANCHIOPODA Latreille, 1817

Order ANOSTRACA G. O. Sars, 1867

Family CHIROCEPHALIDAE Daday de Dèes, 1910

Subfamily CHIROCEPHALINAE

Daday de Dèes, 1910

Genus *Linderiella* Brtek, 1964

Linderiella baetica n. sp.

(Figs 2-9)

Linderiella occidentalis – Alonso 1985: 191, 192, fig. 5.

“Spanish species of *Linderiella*” – Thiéry & Champeau 1988: 70, 75, 77. — Thiéry & Fugate 1994: 654.

Linderiella sp. – Alonso 1996: 55, 56, figs 21, 22.

MATERIAL EXAMINED. — Puerto Real, Cádiz, Charco Carretones, II.2007, holotype mature ♂ 6 mm in length, preserved in 4% formalin with glycerol (MNCN 20.04/7963).

Allotype: same data as holotype, mature ♀ 7.5 mm in length, preserved in 4% formalin with glycerol (MNCN 20.04/7964).

Paratypes: same data as holotype, 1 mature ♀ paratype 7 mm, 1 mature ♂ paratype 5.5 mm (MNHN-Bp817); 5 mature ♂♂ and 5 mature ♀♀ preserved in 4% formalin with glycerol (MNCN 20.04/7965); 20 mature ♂♂ and 20 mature ♀♀ preserved in 4% formalin, and 10 mature males and females preserved in 100% ethanol (UCA-0023-00).

ETYMOLOGY. — The name of this species corresponds to “*Hispania Baetica*”, the name of the old Roman province comprising the south of the Iberian Peninsula (present Andalusia), where it was discovered.

HABITAT. — Charco Carretones in Puerto Real, Cádiz (36°32'N; 6°11'W; elevation = 9 m), is a shallow temporary water body with a surface of about 0.8 ha and a maximum depth of 0.4 m surrounded by bush (*Retama monosperma*

(L.) Boiss.). Flooding of this pond is directly dependent on the rainfall in autumn and winter. The water body lies within an abandoned quarry with bedrock composed of silty soils in the deepest area and with gravel around the margins (Fig. 1B). During the last decade, this pond was filled for 3-6 months in four winters between 1996 and 2007, coinciding with exceptional high autumn or winter rainfall. Water mineralization ranged from 300 to 600 $\mu\text{S}\cdot\text{cm}^{-1}$, water colour is slightly yellow and turbidity is low (subjective visual appreciation). Macrophytic vegetation and accompanying aquatic fauna is summarized in Table 1.

GEOGRAPHIC DISTRIBUTION. — Up to now the species has been found only in Cádiz, the southernmost Spanish province.

DESCRIPTION

Male

Total length of mature individuals (cercopod setae included) 5.2-7.8 mm (average = 6.6 mm, n = 30). Body unpigmented or slightly red. Head rounded with elliptic nuchal organ (Fig. 2A). Antennule longer than proximal segment of antenna with three long subdistal setae and 12 aesthetascs (Fig. 2A, B, F). Antenna (Figs 2A-D; 7A) two-segmented, proximal segment soft, corrugated, with patches of microtuberculate integument and sensillae distributed on dorsal and dorsolateral surface; basomedial sub-elliptical outgrowth with free face covered with smooth, slightly curved short spinules (Figs 2E; 7C, D). Distal segment longer than proximal counterpart, slender, slightly curved inward and tapering distally, with row of transverse ridges on inner surface (Figs 2A-D; 8B); distinctive acute small tooth terminally on segment. Labrum (Fig. 2G) subquadrangular lacking distal protuberances and with setulose lateral margins; short, fleshy setulose tongue-like process subdistally on posterior surface. Mandibles as figured in Figure 7E. Phyllopodia with gross structure typical for genus (Figs 3A; 5E). Praepipod (PE) subdivided into two leaf-like acuminate portions with smooth margins. Epipod (EP) with smooth margin and blunt end except on 11th thoracic limb where it is acute (Fig. 3C). Exopod (EX) broad, provided with short proximal spine-like setae on outer margin; pectinate scales present basally to feathered setae. Endopod (EN) expanded distally; distinct acute projections at base of proximal marginal setae (Fig. 3: I-VII),

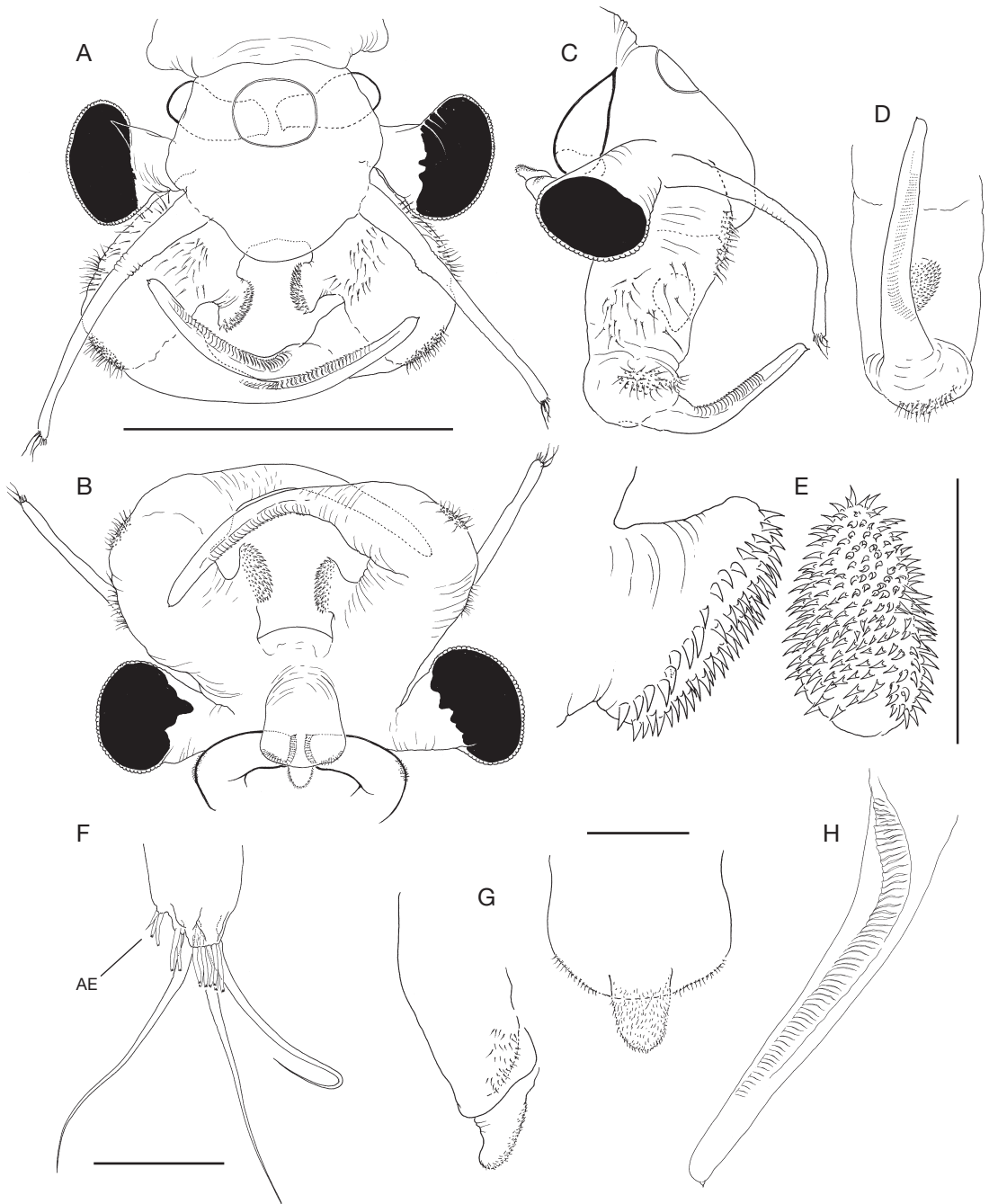


FIG. 2. — *Linderiella baetica* n. sp., male (holotype): **A**, head, dorsal view; **B**, head, ventral view; **C**, head, lateral view; **D**, antenna, ventrolateral view; **E**, basomedial outgrowth on second segment of antenna, lateral (left) and ventral (right) views; **F**, tip of antennule; **G**, labrum lateral view (left) and posterior side (right); **H**, first segment of antenna showing ridges, ventral view. Abbreviation: **AE**, aesthetascs. Scale bars: **A-D**, 1 mm; **E**, 0.2 mm; **F-H**, 0.1 mm.

TABLE 1. — Macrophytic vegetation and accompanying aquatic fauna of *Linderiella baetica* n. sp.

	Species
Macrophytic vegetation	<i>Eleocharis palustris</i> (L.) Roemer & Schultes; <i>Scirpus maritimus</i> L.; <i>Baldellia ranunculoides</i> (L.) Parl.; <i>Glyceria spicata</i> Guss.; <i>Polypogon maritimus</i> subsp. <i>maritimus</i> Willd.
Branchiopoda	Notostraca: <i>Triops cancriformis</i> Lamarck, 1801 Spinicaudata: <i>Cyzicus grubei</i> Simon, 1886 Anostraca: <i>Chirocephalus diaphanus</i> Desmarest, 1823; <i>Streptocephalus torvicornis</i> Waga, 1842; <i>Tanyastix stagnalis</i> Linnaeus, 1758 Anomopoda: <i>Ceriodaphnia</i> sp. (undescribed species); <i>Alona azorica</i> Frenzel & Alonso, 1988; <i>Dunhevedia crassa</i> King, 1853
Ostracoda	<i>Eucypris virens</i> Jurine, 1820; <i>Cypridopsis hartwigi</i> Müller, 1900; <i>C. parva</i> Müller, 1900.
Copepoda	Calanoida: <i>Hemidiaptomus maroccanus</i> Kiefer, 1954; <i>Diaptomus cyaneus</i> Gurney, 1909; <i>Mixodiaptomus incrassatus</i> Sars, 1903 Cyclopoida: <i>Diacyclops bicuspidatus</i> Claus, 1857; <i>Metacyclops minutus</i> Claus, 1863
Hemiptera	Corixidae: <i>Corixa affinis</i> Leach, 1818; <i>Corixa panzeri</i> Fieber, 1848; <i>Sigara semistrata</i> Fieber, 1848; <i>Sigara lateralis</i> Leach, 1818 Notonectidae: <i>Anisops sardeus</i> Herrich-Schäffer 1850 Gerridae: <i>Gerris</i> sp.
Vertebrata	Salamandridae: <i>Pleurodeles waltl</i> Michachelles, 1830 Anura: Pelobatidae: <i>Pelobates cultripes</i> Cuvier, 1829; Hylidae: <i>Hyla meridionalis</i> Boettger, 1874 Squamata: Colubridae: <i>Natrix maura</i> Linnaeus, 1758

reaching maximum size at fifth to seventh limbs; integument on medial portion of endopod of latter limbs wrinkle-patterned. First endite of first 10 thoracic limbs with three submarginal setae on anterior surface, two most distal reduced and spine-like, placed close to each other (Fig. 3B). Eleventh thoracic limb lacking of proximal seta on anterior surface (Fig. 3C). Second endite (Fig. 3B) with two unequal submarginal setae on anterior surface close to proximal angle. Third to fifth endites of second to 11th thoracic limbs (Fig. 3: II-XI) each with two short anterior setae and 3, 2, 2 long, plumose posterior setae respectively; endites of first thoracic limb with 3, 5 and 3 anterior setae. Posterior setae as in other limbs (Figs 3I; 5E). Genital somites (= abdominal somites 1-2; Fig. 4A, B) swollen and partially fused; posterior somite with posterodorsal pair of warty outgrowths each provided with sensilla; non-retractile portion of penis elongated and cylindrical, extending to fourth abdominal somite (Figs 4C; 7F), each bearing medio-distal process with medial surface covered with short curved denticles (Figs 4F; 8A). Eversible part of penis extending to fifth abdominal somite, conical with lateral integument wrinkle-patterned and with spine-like apex (Figs 4E; 8C). Post-genital somites

each with a pair of posteroventral warty outgrowths with sensillae (Fig. 4A-C); third, fifth, seventh and eighth abdominal somites each with an additional pair of posterodorsal outgrowths, one pair laterally on eighth somite. Cercopods (Fig. 4A, D) twice length of telson and five times longer than their base width. Surface of cercopods covered with tiny pectinate scales (Fig. 8D). Setae at tip of cercopods implanted as in Figure 4D, longest being longer than the cercopod itself.

Female (Fig. 8E)

Total length (cercopods setae included) 6.4-8.3 mm (average = 7.3 mm, n = 30); mature females (length = 7.3 ± 0.5 , n = 30) were significantly longer ($P < 0.0001$, t-test) than mature males (6.6 ± 0.5 , n = 30). Body unpigmented or with dorsal part slightly tinged in blue. Antenna (Figs 5A, B, D; 9A, B) slightly shorter than antennule; medial side of proximal segment bearing basal, distally directed horn-like blunt process; lateral margin of segment with several patches of microtuberculate integument, and with sparsely set sensillae distributed as figured. Distal segment of antenna reduced, tongue-like, tapering. Thoracic limbs with acute projections at base of proximal marginal setae of endopod absent or weakly developed. Epipodite

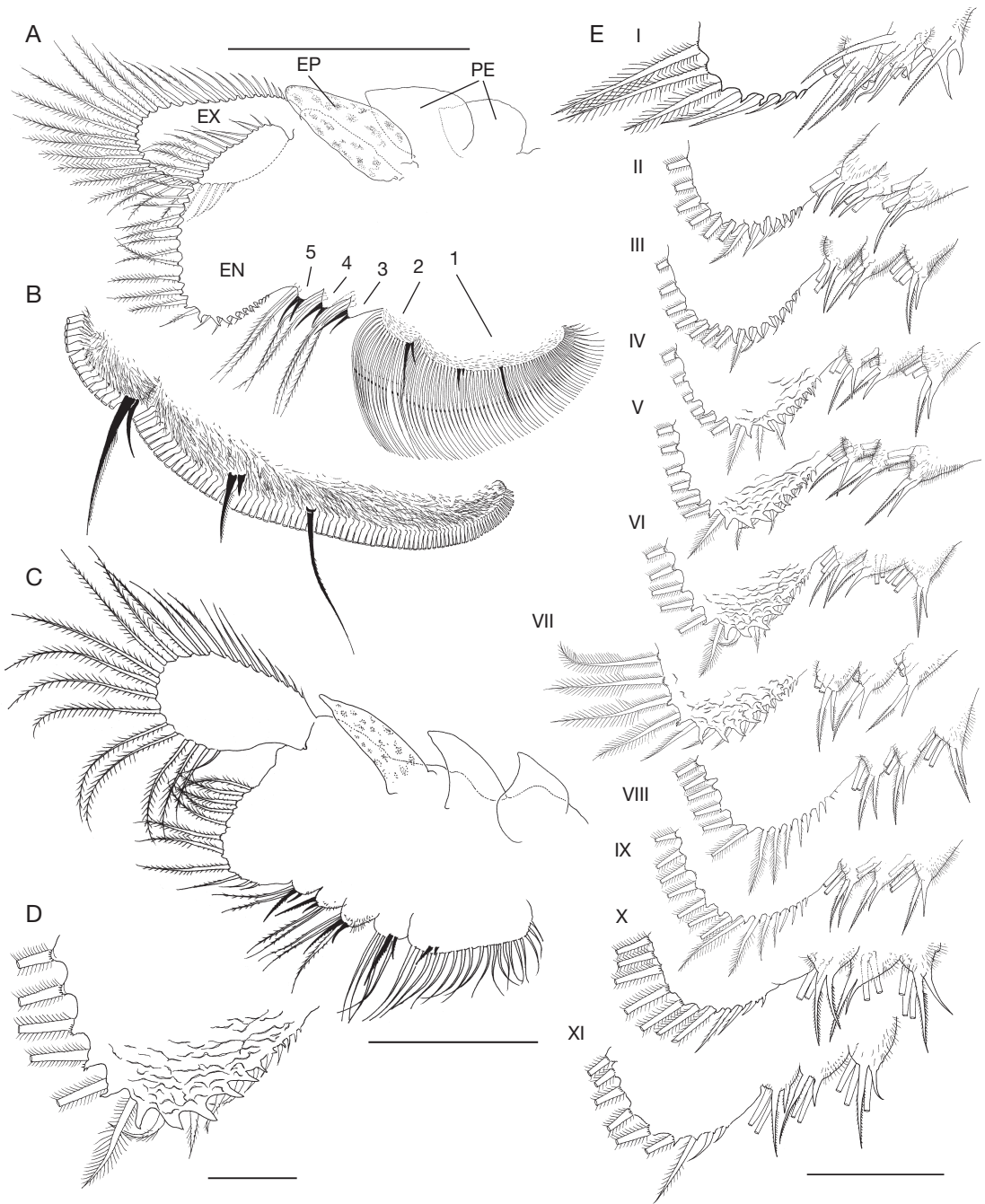


FIG. 3. — *Linderiella baetica* n. sp., male (holotype): **A**, right fifth thoracic limb, anterior view; **B**, detail of endite 1; **C**, right 11th thoracic limb, anterior view; **D**, detail of acute projections on base of proximal setae of medial margin of endopod; **E**, endites 3, 4 and 5 and proximal part of endopod of first to eleventh thoracic limbs. Abbreviations: 1-5, endites 1-5; I-XI, first to eleventh thoracic limbs; EN, endopod, EX, exopod, EP, epipod, PE, praepod. Scale bars: A, C, 0.5 mm; D, 0.1 mm; E, 0.25 mm.

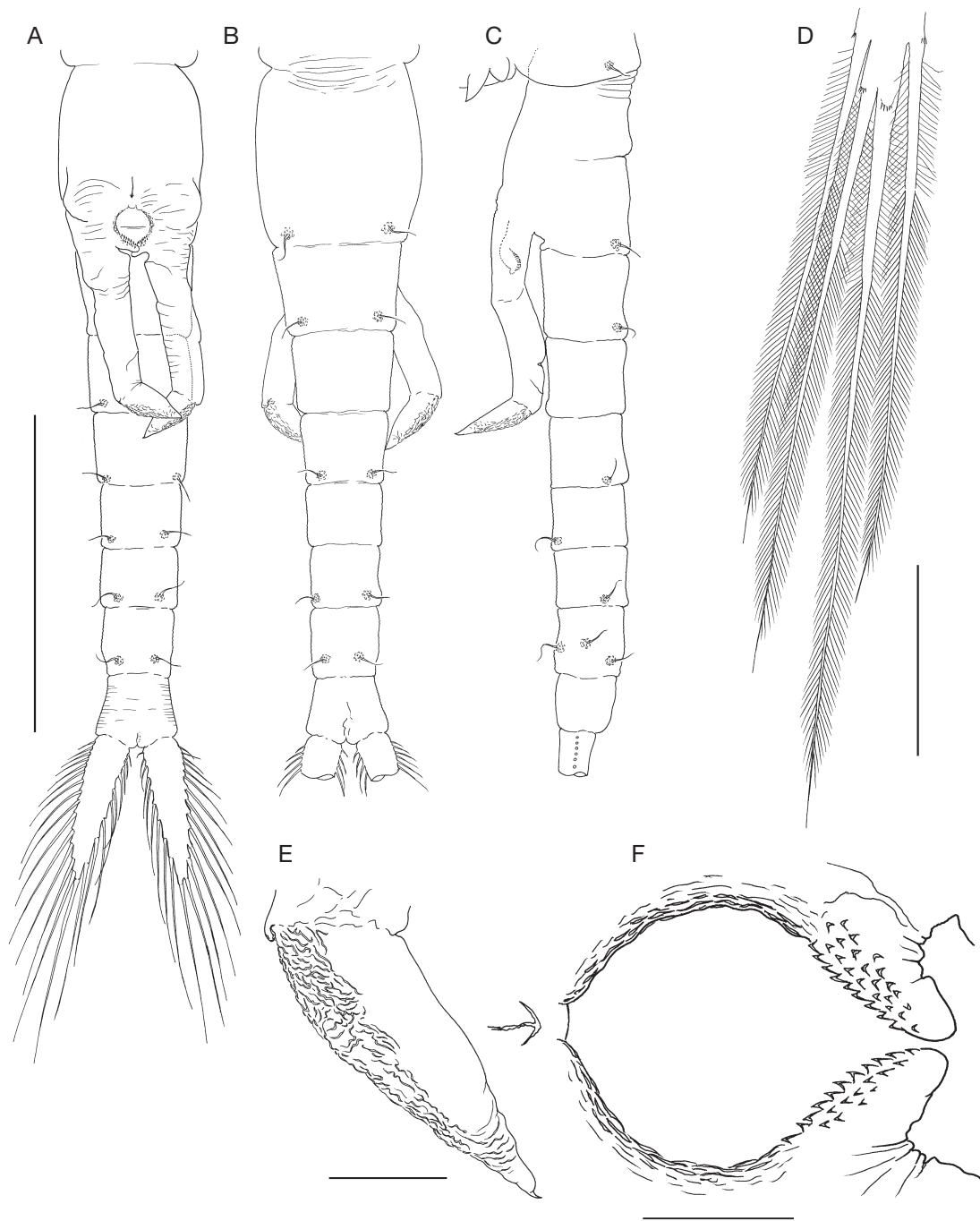


FIG. 4. — *Linderiella baetica* n. sp., male: **A**, genital and abdominal somites plus telson and cercopods; **B**, genital and abdominal somites and telson, dorsal view; **C**, genital and abdominal somites and telson, right lateral view (everted penes are shown in A-C); **D**, detail of tip of cercopods; **E**, eversible tip of right hemipenis; **F**, ventral surface of non-retractile part of penis, showing finely spined digitation, ventral view. Scale bars: A-C, 1 mm; D, 0.15 mm; E, F, 0.1 mm.

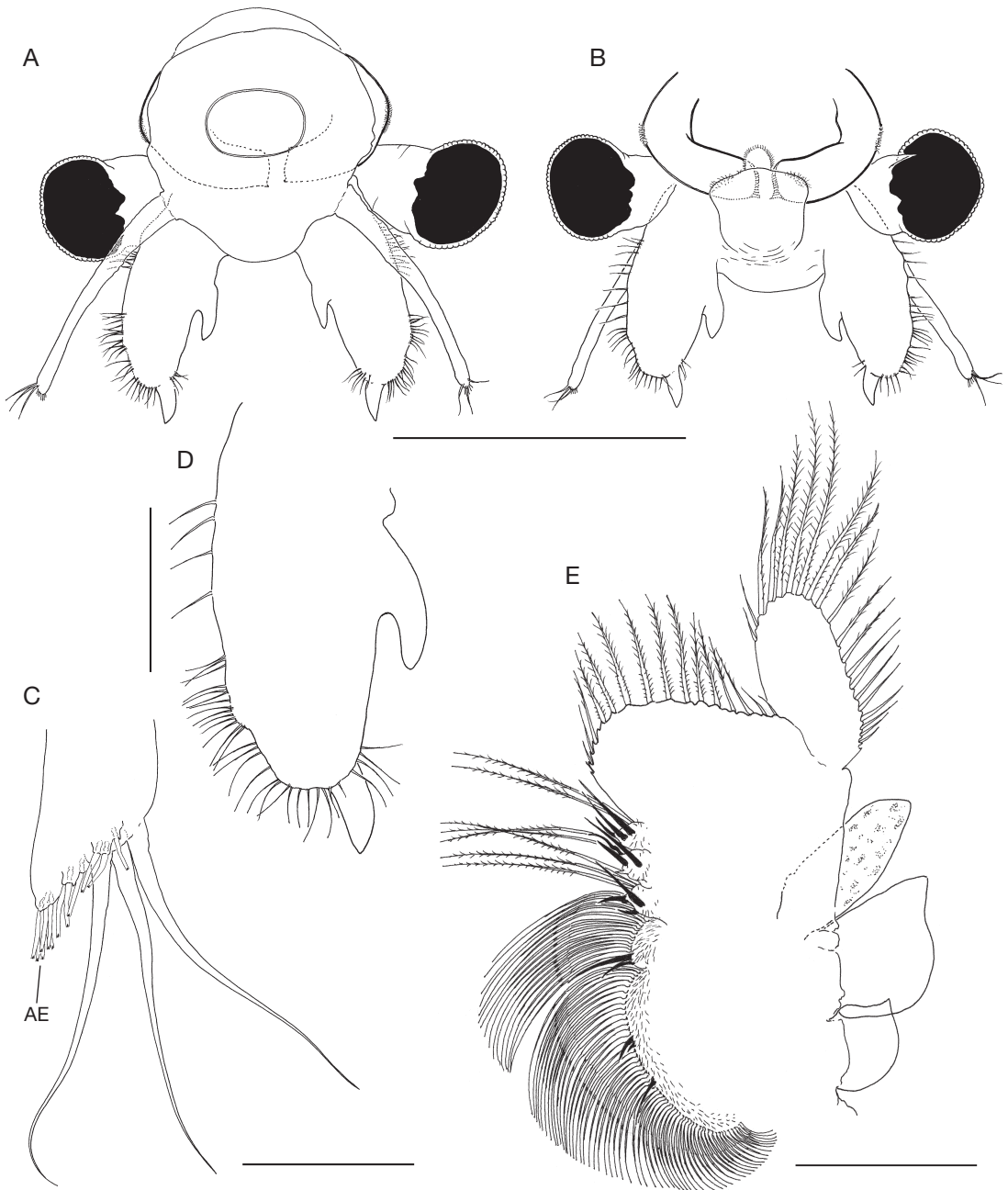


FIG. 5. — *Linderiella baetica* n. sp., female (allotype): **A**, head, dorsal view; **B**, same, ventral view; **C**, tip of antennule; **D**, right antenna, dorsal view; **E**, right first thoracic limb, outer view. Abbreviation: **AE**, aesthetascs. Scale bars: A, B, 1 mm; C, 0.05 mm; D, 0.25 mm; E, 0.5 mm.

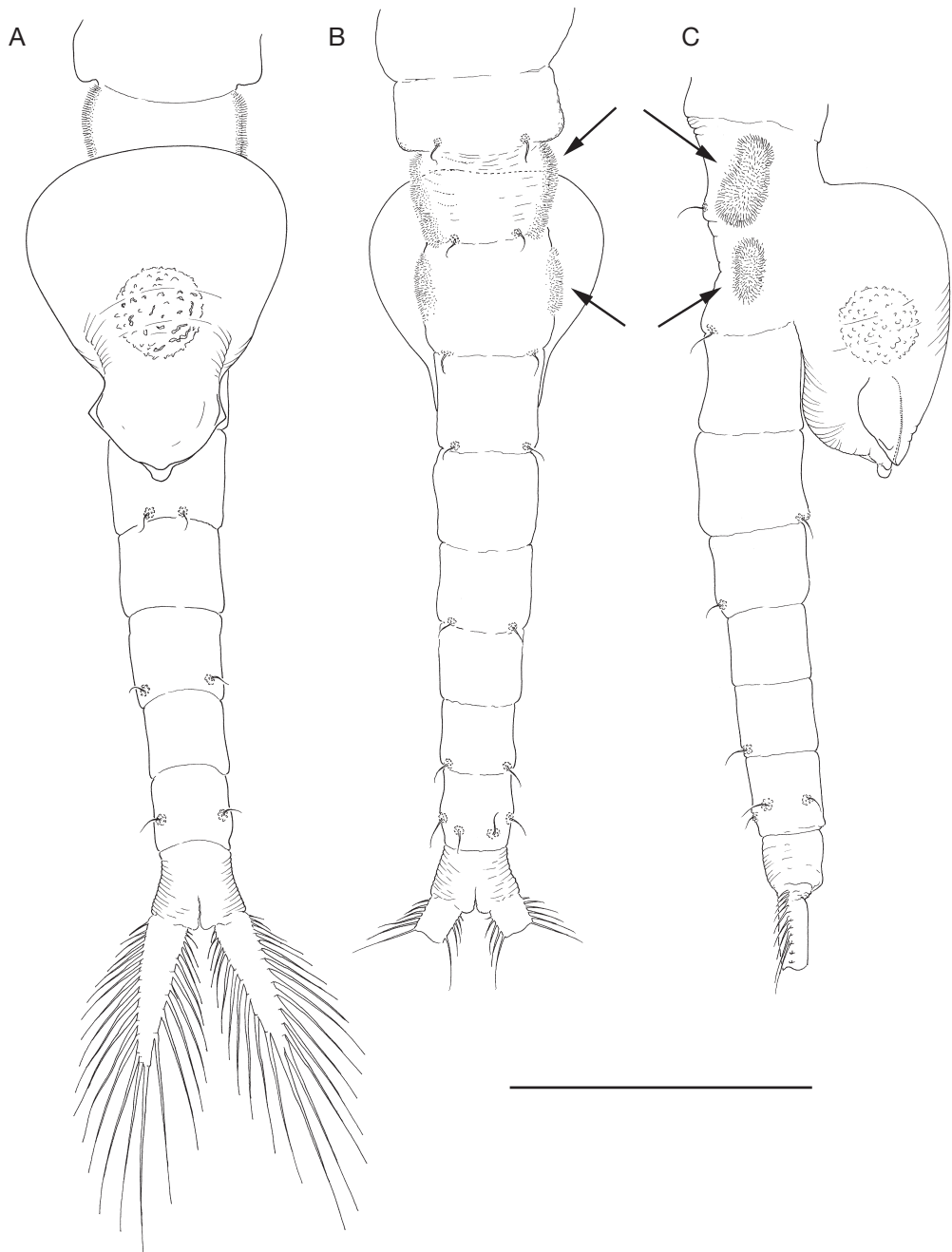


FIG. 6. — *Linderiella baetica* n. sp. female (allotype): **A**, ovisac, abdominal somites and telson with cercopods, ventral; **B**, ovisac, abdominal somites and telson, dorsal; **C**, ovisac and abdomen, right lateral. Arrows in B and C show small setae aggregates on somites 1 and 2. Scale bar: 1 mm.

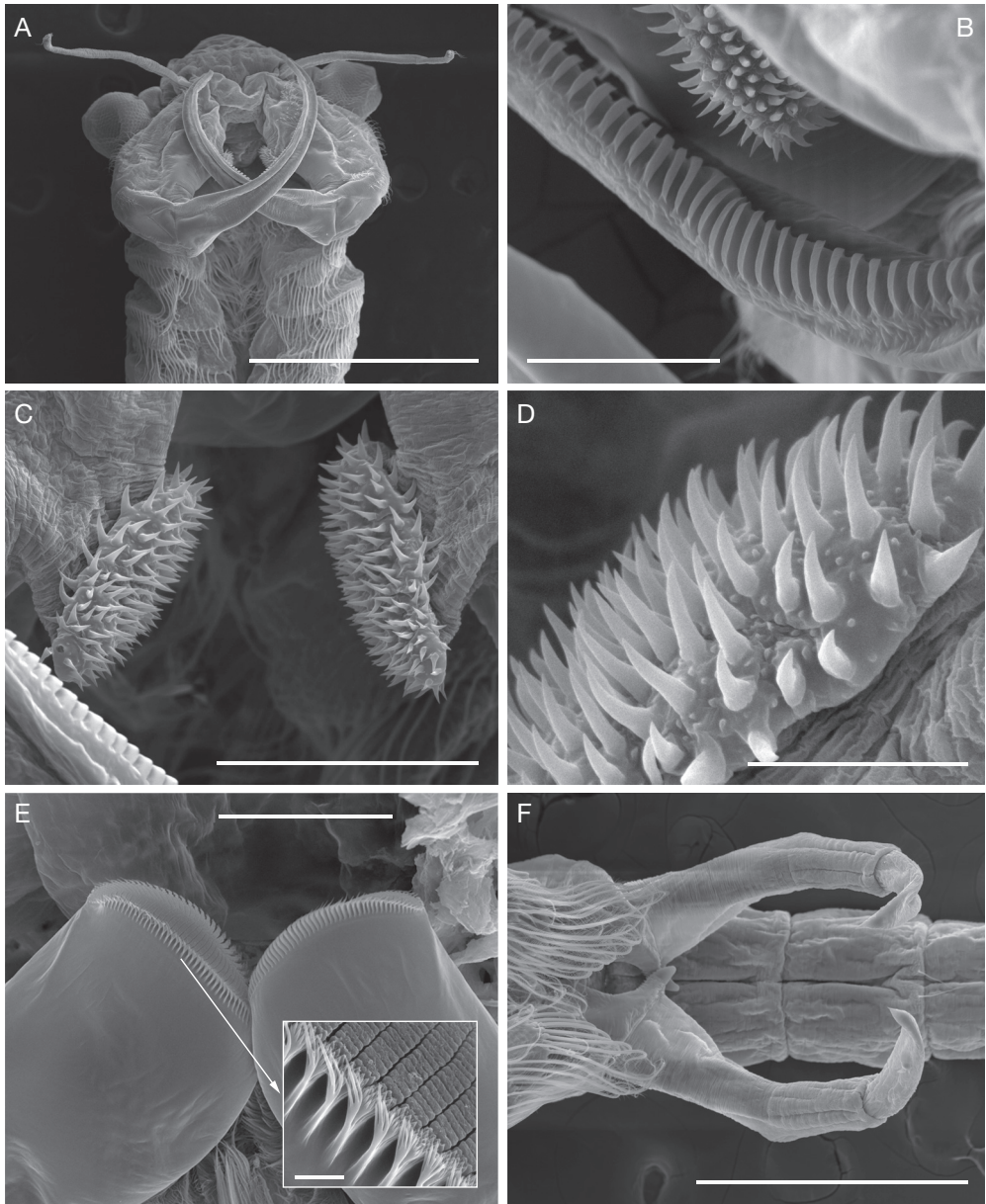


FIG. 7. — *Linderiella baetica* n. sp., male: **A**, head, ventral; **B**, detail of transverse ridges on second segment of antenna; **C**, **D**, basomedial outgrowth of first segment of antenna; **E**, mandibles with details of inner side; **F**, everted penis, ventral view. Scale bars: A, 1 mm; B, 0.1 mm; C, E, 0.2 mm; D, 50 μ m; E inset, 5 μ m; F, 0.5 mm.

wider than in male. Genital somites (= abdominal somites 1-2) completely fused, but retaining respective dorsolateral warty outgrowths (Fig. 6B). Lateral sides

of each somite displaying field of tiny hair-like setules (Figs 6B, C; 9C, D). Brood pouch globose (Fig. 6) extending to middle of fourth abdominal somite, bright

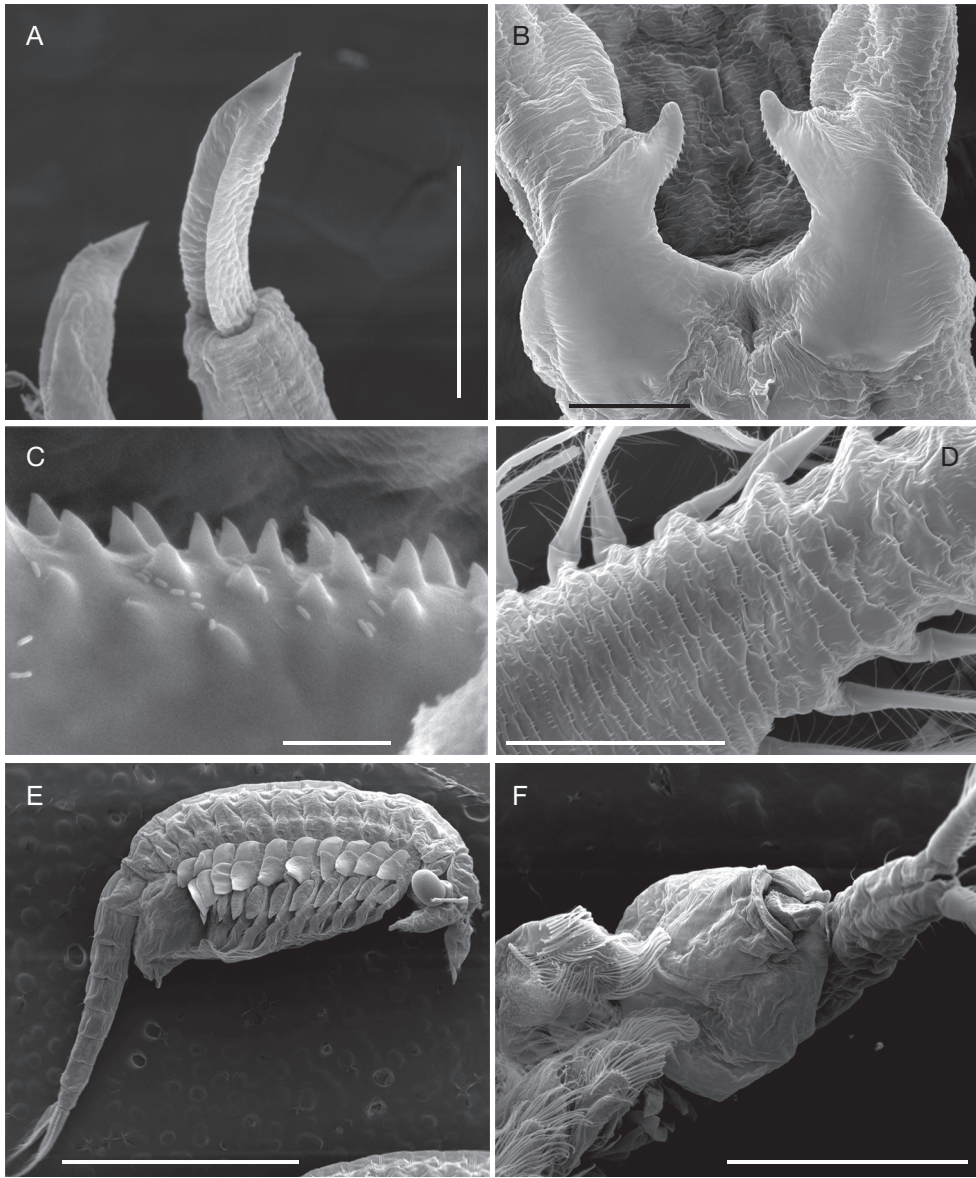


FIG. 8. — **A-D**, *Lindieriella baetica* n. sp. male; **A**, detail of tip of eversible part of penis; **B**, inner outgrowth on non-retractile part of penis; **C**, small spines on inner side of penis; **D**, detail of ventral surface of cercopods; **E, F**, *Lindieriella baetica* n. sp. female; **E**, right lateral view; **F**, brood pouch showing the pore opening. Scale bars: A, 0.2 mm; B, D, 0.1 mm; C, 20 μ m; E, 2 mm; F, 1 mm.

blue in living specimens. Anterior part of brood pouch sub-spherical; posterior third laterally constricted but not depressed. Pore opening subdistally with ventral lip provided with pointed lateral expansions (Fig. 6A). Resting eggs 259-318 μ m in diameter (average = 290

$\pm 16 \mu$ m, n = 20); egg shell covered with numerous trumpet-shaped spines 26-33 μ m long (average = $30 \pm 2 \mu$ m, n = 20) combined with few acute spines (Fig. 9E, F). A maximum number of 14-18 eggs per brood pouch was observed.

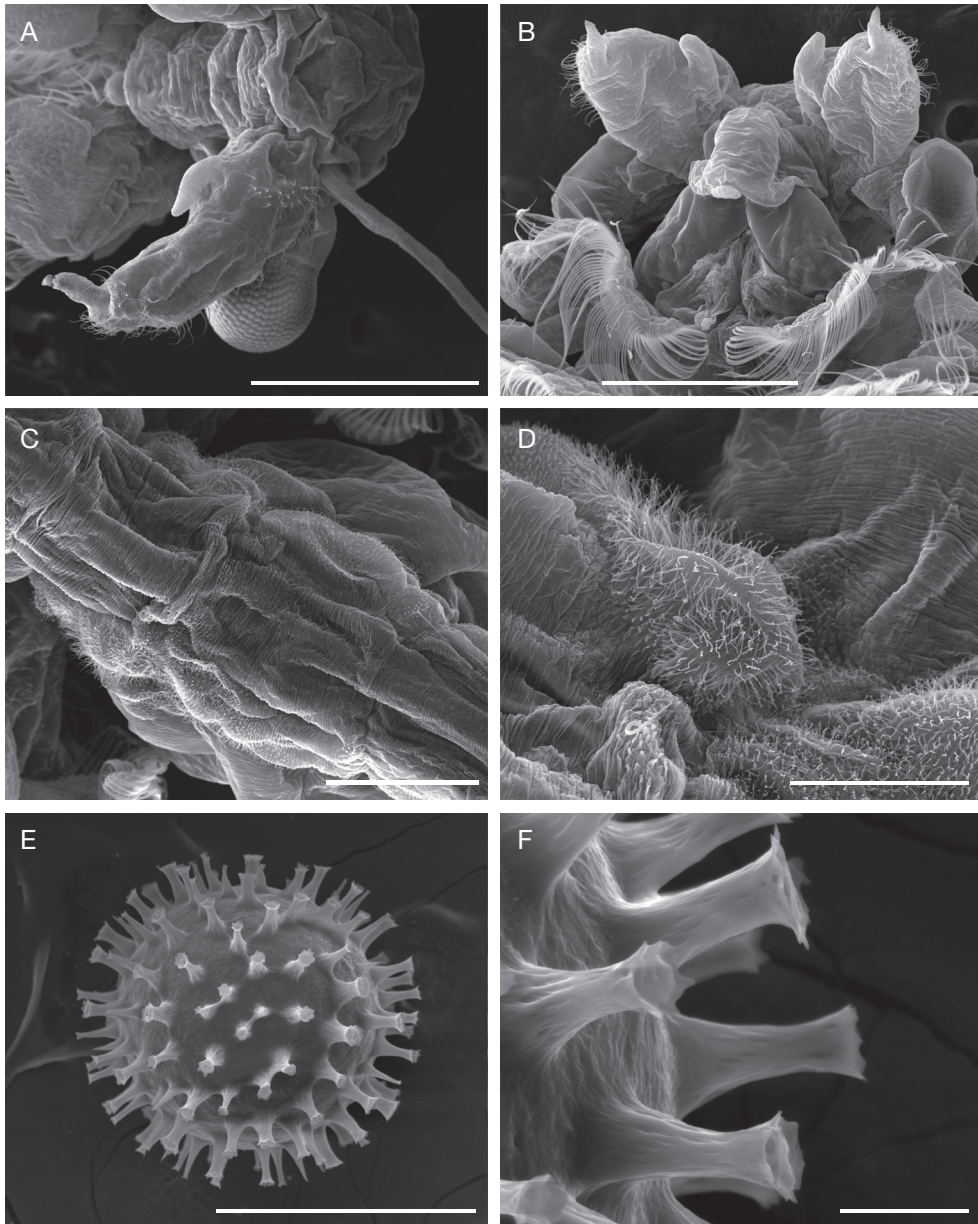


FIG. 9. — *Linderiella baetica* n. sp., female: **A**, horn-like process extending parallel to inner side of left antenna; **B**, antenna and labrum, ventral; **C**, **D**, abdominal somites 1 and 2 showing aggregates of hair like setules; **E**, whole egg showing coronate spines; **F**, detail of the "trumpets". Scale bars: A, 0.5 mm; B, 0.4 mm; C, E, 0.2 mm; D, 0.1 mm; F, 20 μ m.

SPECIES ECOLOGY

Linderiella baetica n. sp. colonizes a shallow (maximum depth = 0.5 m, Fig. 10A), episodic pond. During the last decade, this pond was filled for

c. 3-6 months in the winters 1996-1997, 2002-2003, 2003-2004, and 2006-2007, coinciding with exceptionally high autumn or winter rainfall (Fig. 10B). Maximum densities of *Linderiella baetica*

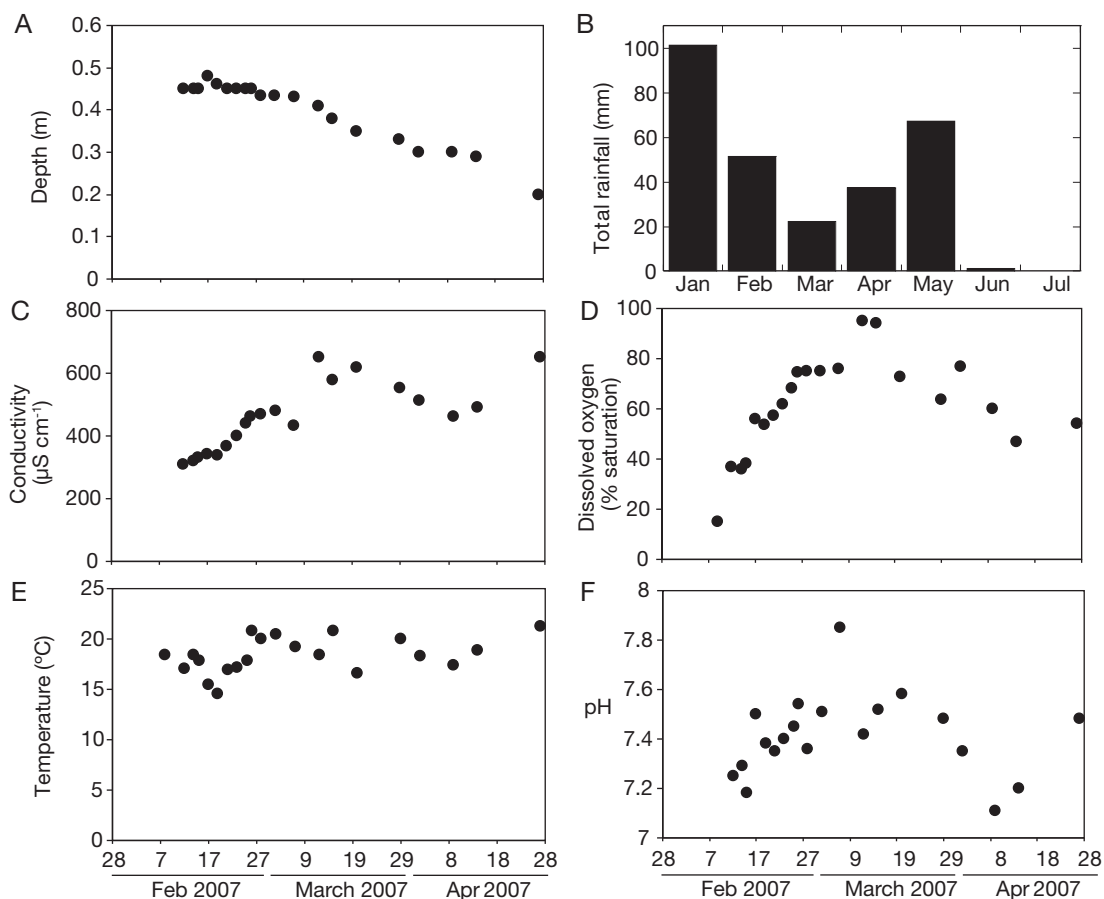


Fig. 10. — Physico-chemical characteristics of Charco Carretones along the course of flooding: **A**, maximum depth; **B**, monthly rainfall along the course of the study; **C**, conductivity; **D**, dissolved oxygen; **E**, water temperature; **F**, pH.

n. sp. occurred within the first stage of flooding (12 February–12 March 2007) (Fig. 11A), coinciding with minimum conductivities (mean \pm SD = $391 \pm 63 \mu\text{S}\cdot\text{cm}^{-1}$, $n=12$), and the lowest dissolved oxygen concentrations ($59 \pm 15\%$, $n=12$) (Fig. 10C, D). Temperature was quite constant during the whole sampling period ($18 \pm 2^\circ\text{C}$) (Fig. 10E), whereas pH varied between 7.1–7.8 (Fig. 10F). *Lindleriella baetica* n. sp. coincided with other Anostracan species such as *Tanymastix stagnalis* Linnaeus, 1758 (Fig. 11B) and *Chirocephalus diaphanus* Desmarest, 1823 (Fig. 11C), *Streptocephalus torvicornis* Waga, 1842 (Fig. 11D), two calanoid copepods (*Diaptomus cyaneus* Gurney, 1909 and *Hemidiaptomus maroc-*

canus Kiefer, 1954, Fig. 11G, H), one ostracod (*Eucypris virens* Jurine, 1820, Fig. 11J) and large (length = 2.9 ± 0.9 mm, $n=35$) (undetermined) turbellaria species (Fig. 11K). The decline of *L. baetica* n. sp. coincided with a clear change in the invertebrate community composition. Small chydorid species (*Alona azorica* Frenzel & Alonso, 1988 and *Dunhevedia crassa* King, 1853) and other calanoid copepod (*Mixodiaptomus incrassatus* Sars, 1903) became dominant (Fig. 11F, H). Small ostracoda species like *Cypridopsis hartwigi* Müller, 1900 and *C. parva* Müller, 1900 replaced *Eucypris virens* (Fig. 11J) and even small turbellaria (length = 0.64 ± 0.09 mm, $n=18$) substituted the larger

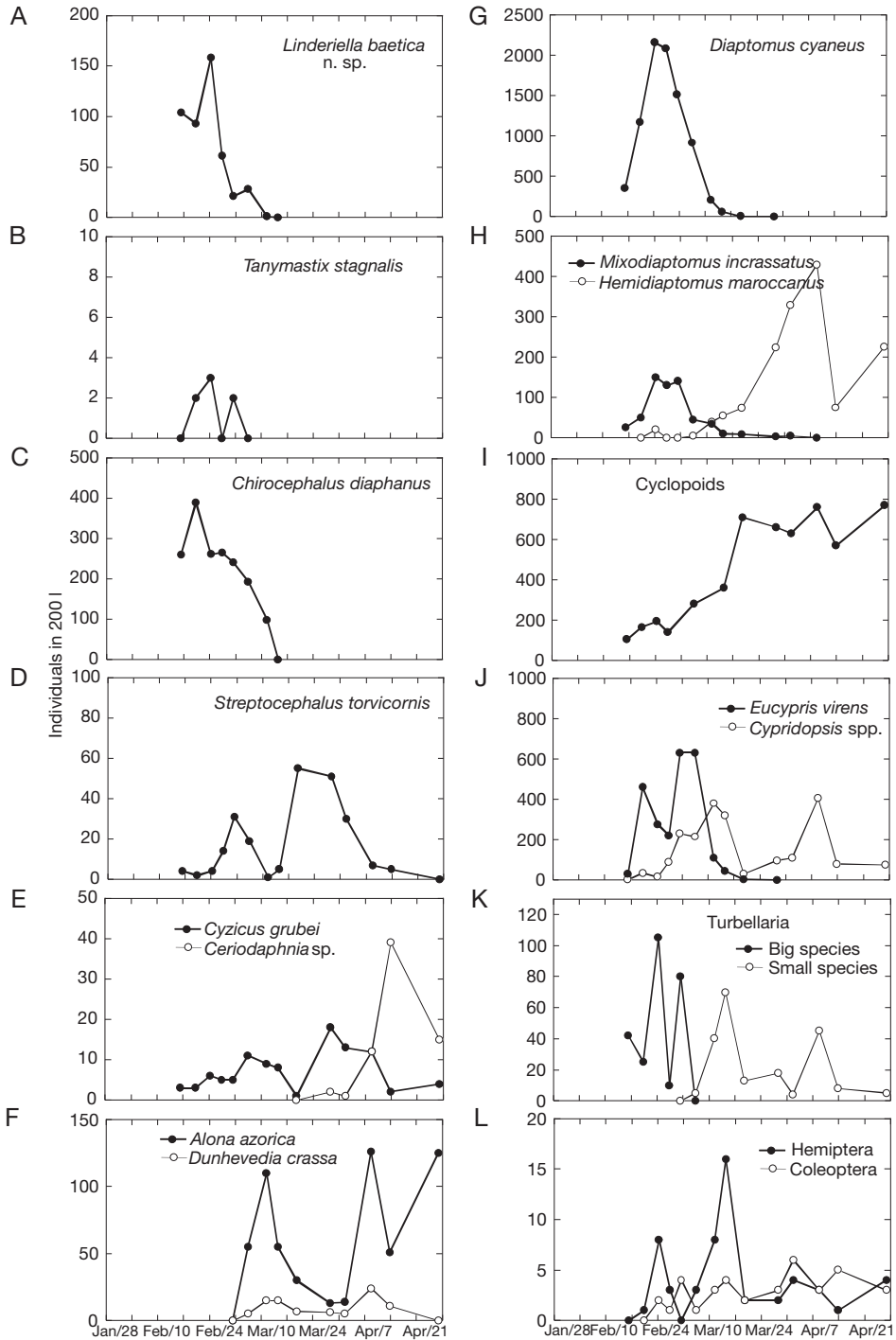


FIG. 11. — Phenology of the main invertebrate accompanying species, including *Linderiella baetica* n. sp.

TABLE 2. — Comparative features between the known *Linderiella* Brtek, 1964 species.

	<i>L. occidentalis</i>	<i>L. santarosae</i>	<i>L. africana</i>	<i>L. massaliensis</i>	<i>L. baetica</i> n. sp.
Spinose plateau of basomedial outgrowth on inner side of male antenna	triangular	triangular with smooth, elongated slender tip	sub-circular	sub-ellipsoidal with finger-like tip	sub-ellipsoidal
Margin of praepipods	serrate	serrate	smooth	serrate	smooth
Acute projections on base of proximal setae of medial margin of endopod	absent	weakly developed	strongly developed	strongly developed	strongly developed
Penis basal projections	blunt, with few denticles	sharp, with few denticles	sharp, with small tubercles	blunt, with many denticles	blunt, with many denticles
Inner horn-like process on basal segment of female antenna	Long, sharp	short, blunt	short, sharp	long, sharp	short, blunt
Lateral fields of setulae on female genital somites	absent	absent	present	present	present
Pointed lateral expansions in ventral lip of brood pouch pore opening	absent	absent	absent	absent	present
Shape of spines of resting eggs	acute	tulip-shaped	acute	acute	trumpet-shaped

species found during the initial stages (Fig. 11K). At the end of the flooding period, *Ceriodaphnia* sp. (undescribed species of *Ceriodaphnia*) became dominant (Fig. 11E).

Assuming that the resting cysts hatched soon after the flooding of the studied pool (at the end of January), the life span of *L. baetica* n. sp. was estimated in *c.* 1.5-2 months. The ratio male/female in *L. baetica* n. sp. varied between 2.0-7.7 (within the period 17 February-3 March), but decreased up to 0.27 on 7 March, coinciding with the population decline. Sexual dimorphism in size for *Linderiella baetica* n. sp. was found: mature females (length = 7.3 ± 0.5 , $n = 30$) were significantly longer ($P < 0.0001$, *t*-test) than mature males (6.6 ± 0.5 , $n = 30$).

REMARKS

Linderiella was already considered by Thiéry & Champeau (1988) as a genus very uniform morphologically. All species share the basic structure of male and female antennae, male and female genitalia, phyllopodia and spiny-patterned resting eggs shell surface. The characteristics used to separate *Linderiella baetica* n. sp. from its congeners are not exclusive but displayed in a unique combination

in this species. Only its resting eggs morphology is unique within the Anostraca. The here so-called trumpet-shaped spines were already considered as a diagnostic character (Thiéry & Champeau 1988; Thiéry & Fugate 1994; Alonso 1996) for this species before the present description, since resting egg morphology is a valuable taxonomical character for anostracans (Thiéry & Gasc 1991; Mura 1991, 1992a, b). Resting eggs of *L. santarosae* resemble those of *L. baetica* n. sp. in having distally expanded spines, but they end in several acute cusps (tulip-shaped) whereas have coronate flattened rim (trumpet-shaped) in *L. baetica* n. sp. Also, spine density in *L. santarosae* (15-44 per 0,1 mm²) is higher than in *L. baetica* n. sp. (10 per 0,1 mm²). Combination of flat top and acute spines also has been described for *L. occidentalis* (Thiéry & Fugate 1994) although in *L. baetica* n. sp. the proportion of trumpet-shaped spines is significantly higher. The lateral fields of hair-like setulae in female genital somites are an exclusive characteristic of *L. africana*, *L. massaliensis* and *L. baetica* n. sp. among anostracans. These hairy surfaces likely facilitate the clutching of the female by the male antennae, as occurs with similar structures in other anostracans, namely the pre-genital dorsal

outgrowths in some *Galaziella* Naganawa & Orgiljanova, 2000 species (Naganawa & Zagas 2003) or the expanded and warty pre-genital somites in *Parartemia* Sayce, 1903. The proximal medial margin of the endopod of the phyllopodia is frequently reinforced by short robust spine-like marginal setae (i.e. *Branchinecta* Verrill, 1869, *Branchipus* Schaeffer, 1766, *Tanymastix* Simon, 1886, *Branchinella* Sayce, 1903) or integument acute projections in the base of marginal setae (*Chirocephalus* Prévost, 1820, *Linderiella* Brtek, 1964). Such projections

are well developed in the Mediterranean species *L. massaliensis*, *L. baetica* n. sp. and *L. africana*. In North American species these acute projections are lacking or are weakly developed. The aforementioned and other differential characteristics are summarized in Table 2.

Following this differential diagnosis, a dichotomous key to *Linderiella* species is proposed. Considering that only one sex may be caught during samplings, dichotomous keys for males, females and resting eggs are proposed.

KEY TO THE MALES OF *LINDERIELLA* BRTEK, 1964

1. Outgrowth on inner side of basal segment of antenna with smooth elongated slender tip *L. santarosae*
— Outgrowth on inner side of basal segment of antenna without such distal extension ... 2
2. Outgrowth on inner side of basal segment of antenna acute triangular-shaped *L. occidentalis*.
— Outgrowth on inner side of basal segment of antenna sub-ellipsoidal or subcircular ... 3
3. Margin of praecipods serrate *L. massaliensis*
— Margin of praecipods smooth 4
4. Penis basal projections sharp, with small tubercles *L. africana*
— Penis basal projections blunt, with many denticles *L. baetica* n. sp.

KEY TO THE FEMALES OF *LINDERIELLA* BRTEK, 1964

1. Female genital somites displaying lateral fields of tiny hair-like setules 2
— Female genital somites smooth 4
2. Pointed lateral expansions in ventral lip of brood pouch pore opening ... *L. baetica* n. sp.
— Ventral lip of brood pouch without such expansions 3
3. Inner horn-like process on basal segment of antenna very short and sharp ... *L. africana*
— Inner horn-like process on basal segment of antenna long sharp *L. massaliensis*
4. Inner horn-like process on basal segment of antenna short blunt *L. santarosae*
— Inner horn-like process on basal segment of antenna long sharp *L. occidentalis*

KEY TO THE RESTING EGGS OF *LINDERIELLA* BRTEK, 1964

1. Acute resting egg spines 2
— Coronate resting egg spines 4
2. Short (14-23 μm long) twisted resting egg spines *L. massaliensis*
— Longer straight conical resting egg spines 3
3. Long spines (average longitude: 38 μm) *L. africana*
— Shorter spines (27-33 μm) *L. occidentalis*
4. Tulip-shaped spines *L. santarosae*
— Trumpet-shaped spines *L. baetica* n. sp.

DISCUSSION

Ecological processes can be important in structuring the species assemblages in temporary waterbodies (King *et al.* 1996; Holland & Jenkins 1998). Both biotic and abiotic factors acting together regulate succession of crustacean assemblages in temporary ponds, directly controlling growth and longevity of particular species (Wolfenbarger 1999; Williams 2001). Therefore, characterization of both environmental variables and zooplankton phenology throughout the flooding cycle is the first step to give some insights on the main factors controlling the ecology and population dynamics of *L. baetica* n. sp. Anostracans are especially adapted to temporary waters, surviving the dry period as resting eggs. Among anostracans, different life-cycles have been documented. *R*-species whose dormant stages hatch shortly from sediment and commonly appear during the first days of inundation, while *K*-oriented are better suited to compete under density-dependent circumstances and dominate later stages (Lahr *et al.* 1999). *Lindieriella baetica* n. sp. showed a rapid colonization of the pond just after flooding, rapidly disappearing in 1.5 months time, thus suggesting a *R* strategy. The population observed was composed of one cohort corresponding to a high synchronic hatching. This is coincident with *Eubranchipus vernalis* (Weaver, 1943), *Eubranchipus oregonus* (Coopey, 1950), *Branchinecta mackini* (Brown & Carpelan, 1971) *Polyartemia forcifata* (Hellstrom & Nauwerck, 1971), *Tanyrastigites jbleitica* Thiéry & Brtek, 1985 and *Branchipus schaefferi* Fischer, 1834 (Thiéry 1987), *Tanyrastix stagnalis* (Alonso 1996; Mura & Zarattini 2000; Garcia-de-Lomas & Garcia 2004), *Branchinellites chudeaui* Daday, 1910 (Lahr *et al.* 1999).

Considering the environmental conditions in the water phase, *L. baetica* n. sp. occurred during the lowest dissolved oxygen concentrations in the pond, suggesting specific adaptations to hypoxia (e.g., synthesis of hemoglobin). Hemoglobin induction under hypoxia has been also widely reported in cladocera (*Daphnia* spp.) and anostracans (*Artemia* spp.) (Kobayashi & Hoshi 1982; Heip *et al.* 1978), but also in other branchiopod taxa that coincided with *L. baetica* n. sp. during the first stage of flooding (*Triops*, *Cyzicus*) (Ar & Schejter 1970; Horne &

Beyenbach 1971; Weber & Vinogradov 2001). It is therefore feasible that this increase in dissolved oxygen exposed *L. baetica* n. sp. to greater levels of interspecific competition with *K*-species dominating later stages and likely with a poorer adaptation to extreme conditions. In this sense, the synchronic appearance of *Lindieriella baetica* n. sp. with the anostracan *Tanyrastix stagnalis* and *Chirocephalus diaphanus* and the calanoids *Diaptomus cyaneus*, *Hemidiaptomus maroccanus* suggest an ecological segregation in their spatial distribution and food supplies (Mura 1991; Thiéry 1991). Coexistence of *Tanyrastix stagnalis* and *Chirocephalus diaphanus* was reported by Mura (1991), who found significant differences as to size, time in attaining sexual maturity, fecundity and life span, thus favouring a niche separation by size and by time. Given the lower longevity and body length of *L. baetica* n. sp. with respect to the *Lindieriella* sp. specimens found by Alonso (1996) (female body length = 15 mm), and other *Lindieriella* species (see Table 1), we hypothesize that the exponential increase of *Streptocephalus torvicornis* and *Mixodiaptomus incrassatus* were likely responsible of a premature decline of *L. baetica* n. sp. population. This hypothesis can be also applicable to *Chirocephalus diaphanus* whose specimens reached lower body lengths (15-20 mm) than those frequently found in nature (up to 35 mm) (Lake 1969; Mura 1991; Alonso 1996). However, interspecific competition might not be the only factor explaining the population dynamics of *L. baetica* n. sp., and the premature decline observed could be also the result of predation, as revealed by the abundance of macroinvertebrates (Hemiptera and Coleoptera) (Fig. 11L) and newt (*Pleurodeles waltl* Michachelles, 1830) larvae (Garcia-de-Lomas pers. obs.). Hemiptera (e.g., Notonectidae) and Dytiscidae larvae are known to predate on large-bodied zooplankton including anostracans (Arnér *et al.* 1998; Steiner & Roy 2003), whereas newt larvae are known to predate on a variety of preys, including cladocera and other active swimmers such as large branchiopods (Díaz-Paniagua 1983; Santos *et al.* 1986; Rodríguez-Jiménez 1985; Thiéry 1987; Joly *et al.* 1999).

Our data support the sexual dimorphism in size advanced for *Lindieriella* (Doods 1923; Heath

1924; Thiéry & Champeau 1988; Thiéry & Fugate 1994), as females of the new species were found to be significantly greater than males. The male/female ratio for *L. baetica* n. sp. was 2.0-7.7 but cannot be compared with other species in the genus because the lack of data. The steady increase of female numbers in March might be explained by the higher death rates in males, because of their greater activity and continuous seeking and courting mates (Lahr *et al.* 1999). The rest of accompanying anostracans showed lower ratios of male/females than *L. baetica* n. sp., with values of 0.4 ± 0.1 ($n = 7$) in *Chirocephalus diaphanus*, and 1.3 ± 0.9 ($n = 8$) in *Streptocephalus torvicornis*.

The accompanying large phyllopods in Charco Carretones appeared also in the site where the *Linderiella* sp. was originally cited (in 1978) (Alonso 1985, 1996). This suggests that the species was originally distributed in vernal pools throughout the rangelands of southern Spain. Unfortunately, the original location described by Alonso (1996) was significantly damaged because of mining, agriculture and urbanization, and the repeated surveys in search for the "Spanish *Linderiella*" in this area have been fruitless. The taxa accompanying *L. baetica* n. sp. also showed some similarities with those found accompanying other *Linderiella* species. For example, *Chirocephalus diaphanus* and calanoids of the genera *Diaptomus*, *Hemidiaptomus* and *Mixodiaptomus* accompanied *L. massaliensis* (Thiéry & Champeau 1988), whereas *C. diaphanus* and *Triops cancriformis* Lamarck, 1801 were present in temporary ponds containing *L. africana* (Thiéry 1986). Amphibians such as *Bufo* sp. and *Hyla* sp. were also present in vernal pools containing *L. santarosae* (Thiéry & Fugate 1994). These data, together with the ephemeral condition and low conductivity of water reveal common features of ponds containing *Linderiella* species. Given the low degree of morphological differentiation, the thesis of an ancestral taxon proposed by Thiéry & Champeau (1988) and Eng *et al.* (1990) gains additional support from the description of *L. baetica* n. sp. The presence of biogeographical barriers such as the Pyrenees (between *L. massaliensis* and *L. baetica* n. sp.) and the Straits of Gibraltar (between *L. baetica* n. sp. and *L. africana*) provides further

evidence of the allopatric speciation. However, additional studies on the phylogeny of this genus are necessary to ensure the vicariant hypothesis (Thiéry & Fugate 1994).

Finally, considering that *Linderiella baetica* n. sp. has been found only in a single locality and that urbanization plans threaten the conservation of his habitat, we suggest that this species should be considered as critically endangered according to IUCN criteria (IUCN 2001).

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