



MORPHOLOGY AND SYSTEMATIC POSITION OF TWO *LEUCTRA* SPECIES (PLECOPTERA: LEUCTRIDAE) BELIEVED TO HAVE NO SPECILLA

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ABSTRACT

Leuctra bidula Aubert and *L. ketamensis* Sánchez-Ortega & Azzouz were believed to lack specilla, other sperm transmitting organs were not known. We show that both have a normal penis attachment bulb displaced deep into segment IX. Unlike in most other *Leuctra* it is not visible from outside. From the bulb issue two narrow sperm ducts surrounded by a common tube. Details differ between the two species. In *L. bidula* the inner paraproct lobes remain visibly connected to the bulb. The rest of the paraprocts remains close to sternite IX, but in vertical position. *L. ketamensis* lacks such visible connection, the modified paraprocts lie caudo-dorsally. Transformation of the outer paraproct lobes into a supraanal hooklet in *L. nigra* (Olivier) provides a model for the origin of long prongs and associated dorsal structures issuing laterally from the paraprocts of *L. bidula* and *L. ketamensis* whose closest relatives within genus *Leuctra* are unknown.

Keywords: Penis attachment bulb, sperm transmitting organ, paraproct lobes, spermathecal sclerite, *Leuctra bidula*, *Leuctra ketamensis*

INTRODUCTION

The family Leuctridae Klapálek 1905 is composed of the subfamilies Leuctrinae Klapálek 1905 and Megaleuctrinae Zwick 1973. The latter comprises only one genus with six species in western North America and in Korea (DeWalt et al. 2014). The Leuctrinae include 11 genera and nearly 350 species. The west Palaearctic region is inhabited by three genera. *Pachyleuctra* Despax 1929 and *Tyrrhenoleuctra* Consiglio 1957 are endemic to the West-Mediterranean region, each with a few species. In

contrast, *Leuctra* Stephens 1836 is the most species-rich genus of the entire family, counting almost 200 species occurring on both sides of the Atlantic Ocean: in eastern North America, Europe, Middle East and North Africa. The European *L. fusca* (Linnaeus 1758), extends to east Siberia but the diversity of the genus ends in the Caucasus, with *L. kopetdaghi* Zhiltzova 1972 as an eastern outlier (DeWalt et al. 2014).

In Plecoptera, the male gonopore is situated between sternites 9 and 10, the structure of the copulatory organ itself is very different between families. Males

of Nemouridea (except Taeniopterygidae) have no eversible penis, sperm transfer is performed by other structures of the terminal segments. Capniidae and Leuctridae employ components of the paraprocts, the so-called fusion plate in the former, the so-called specilla in the Leuctridae (Zwick 1973, 1980). However, two west-Mediterranean species, *Leuctra bidula* Aubert 1962 and *L. ketamensis* Sánchez-Ortega & Azzouz 1997 were in the excellently illustrated original descriptions said to lack specilla. Other copulatory organs were not mentioned.

The purposes of the present paper are:

- 1 Identify the copulatory organs of *L. bidula* and *L. ketamensis*;
- 2 Supplement the species descriptions accordingly;
- 3 Assess placement of the two species among Leuctrinae.

METHODS

Specimens were studied with dissecting and compound microscopes (various models, WILD M8, Leica DMLS, Motic SMZ168, and USB Digital Microscopes (Veho Microcapture 40x-200x and Veho Microcapture 20x-400x V1.3), at magnifications up to 630x. A Pentax Optio VG-10 camera was used for the photographs. The abdominal tip of one male each was removed, segments IX & X were dehydrated and made semi-transparent in clove oil, for study with a compound microscope. Eventually, the terminal segments were permanently mounted on glass slides, in Euparal.

In addition to the two target species, representatives of all genera of Leuctridae (except *Calileuctra* Shepard et Baumann 1995) and several *Leuctra* species from the authors' collections were also compared.

DESCRIPTIONS

The general appearance of both species is typical of Leuctridae: small head with protruding eyes, narrow pronotum, wings rolled around the slender body, legs relatively delicate and short. Wing venation, thoracic sternal features, and the simple, unmodified one-segmented cerci (Figs. 1-4, 9, 10, 13) agree with genus *Leuctra*. The tergal ornamentation of males of both species includes two strong teeth on tergite VI and well-developed median pigmentation on

tergites VII, VIII and IX (Figs. 1, 2, 9). Males lack a ventral vesicle on sternite IX which is medially membranous between lateral sclerotizations (Figs. 3, 10).

Unlike other *Leuctra*, males of both species lack the typical slender specilla universally present in genus *Leuctra*. Also, the male paraprocts are of complex shape, including two thin anterior blade-shaped expansions, two lateral rounded plates and two median laminar expansions projecting upwards (Figs. 5, 6, 9, 11, 12).

Females possess two lateral lobes on the posterior edge of sternite VII (Figs. 4, 13), their spermatheca lacks an internal sclerite.

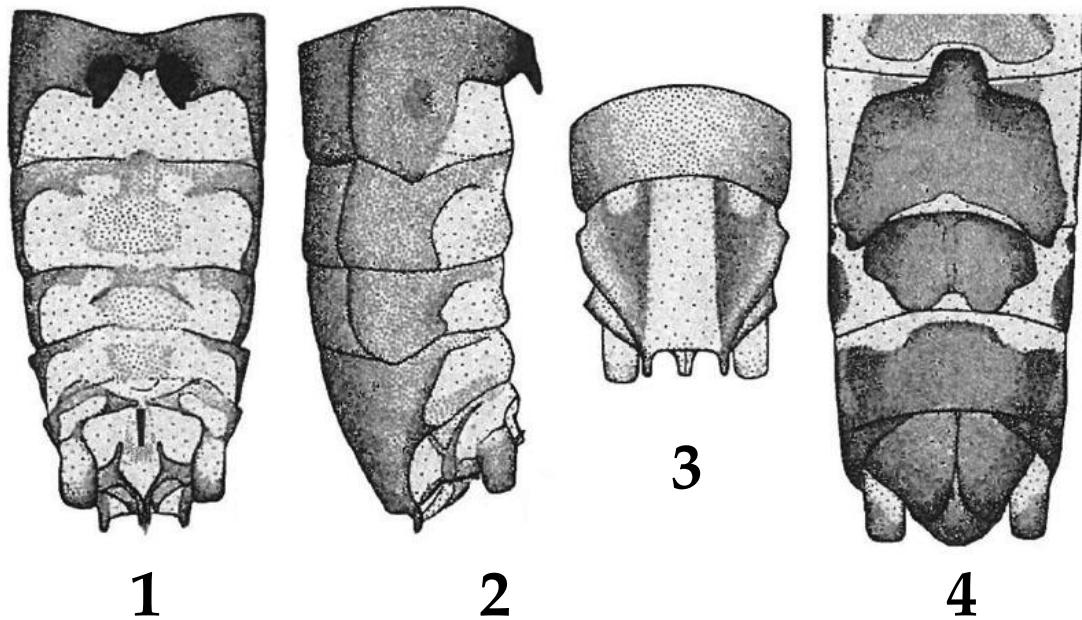
The very precise drawings in the original descriptions of both species permit easy identification of the species. Some are here reprinted (Figs. 1-4) and supplemented by drawings and photographs, to permit direct comparisons. However, morphology and homology of male parts were doubtful without consideration of internal structures visible only by transparency. Our descriptions and illustrations focus on these details.

Leuctra bidula Aubert 1962

(Figs. 1-8, 16)

Material examined. Paratypes: 1♂, 1♀, Serranía de Ronda, Sierra Bermeja, road 339 between Torre de Baños and Ronda, 700-800m, Málaga, Spain, 19.04.1960, Aubert collection (Lausanne Museum, Switzerland). Other material: 1♀, Arroyo Bolaje, Sierra Bermeja, 680 m, UF0854, Málaga, Spain, 10-12-1994, Tierno de Figueroa leg, Tierno de Figueroa's collection (University of Granada, Spain). 1♀, road from San Pedro de Alcántara to Ronda, near the Puerto de la Refriega, Rio Genal tributary, 1300m, Málaga, Spain, 21-02-1996, Vinçon coll.

Male terminalia (Figs. 1-3, 5-8). Tergite VI with strong anterior margin and a pair of hook-shaped sclerotized teeth (Fig. 1). Tergites VII and VIII with central pigmented area connected to the anterior margin. Tergite IX with median pigmented spot. The large sternite IX (SIX) without ventral vesicle, with median membranous area, ending in two postero-lateral nipples between which the soft middle is bent up a little (Figs. 1-3, 5). Tergite X (TX) is caudally truncate, the pilose soft epiproct projects a little over



Figs. 1–4. *Leuctra bidula*. Male abdomen: 1, Dorsal. 2, Lateral. 3, Ventral, Sternite IX. Female abdomen: 4, Ventral, showing the pregenital and subgenital plates (1-4 after Aubert 1962).

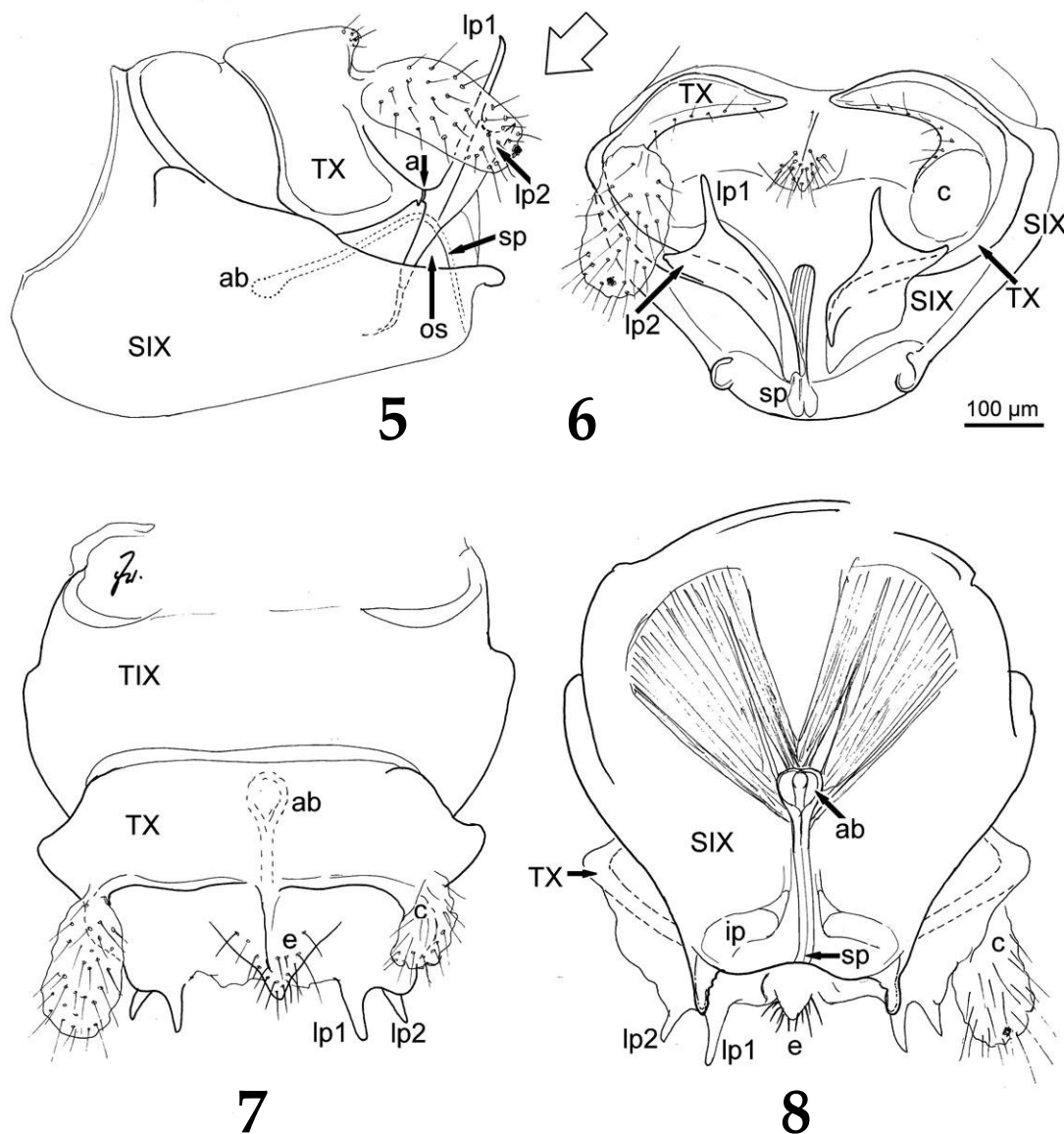
the transverse edge, its rod-like base is barely sclerotized (Figs. 1, 6, 7). Ventrolaterally, TX forms a short sclerite bracket which articulates (a) with the paraprocts (Fig. 5). The paraproct is in an unusual almost vertical position (Figs. 5, 6). Medially from the suture (a) the paraproct consists of a plate which carries dorsally a short outer process (lp2) and more medially a very long spine (lp1). Ventrally, the paraproct sclerite narrows, descends deeply into SIX and is in ventral view seen to form a conical pocket with anterior extensions extending towards the attachment bulb (ab) of the penis (see below); details not visible. Cerci short and truncated.

The specilla (sp) are hidden between the paraprocts and project as a narrow transparent structure which curves down. In side view, an open space (os) remains between it and the sclerotized paraprocts (Figs. 5, 6). At the front of this downcurving projection a delicate transparent tube enclosing two still narrower (diameter ~10 µm)

sperm ducts is seen by transparency, in side view (Fig. 5). The ventral end is concealed behind the caudal membrane of SIX but seems to be slightly bilobed. More basally the tube is bent down at a right angle and extends forward to a typical leuctrid attachment bulb to which the combined penis and paraproct retractors attach.

Female terminalia (Fig. 4). Sternite VII strongly sclerotized, with median anterior rounded lobe and two postero-lateral triangular lobes slightly overlapping sternite VIII on each side of the subgenital plate. Sternite VIII: subgenital plate with two median rounded lobes (Fig. 4). Cerci short and truncated. Paraprocts triangular with rounded posterior tip.

Ecology and distribution. Species occurring in mountain brooks and brooklets of the Sierra Bermeja (Málaga, Spain), in the southern part of the Baetic system (700-1300m) (Fig. 16). The adults emerge in the cold season (IX–IV).

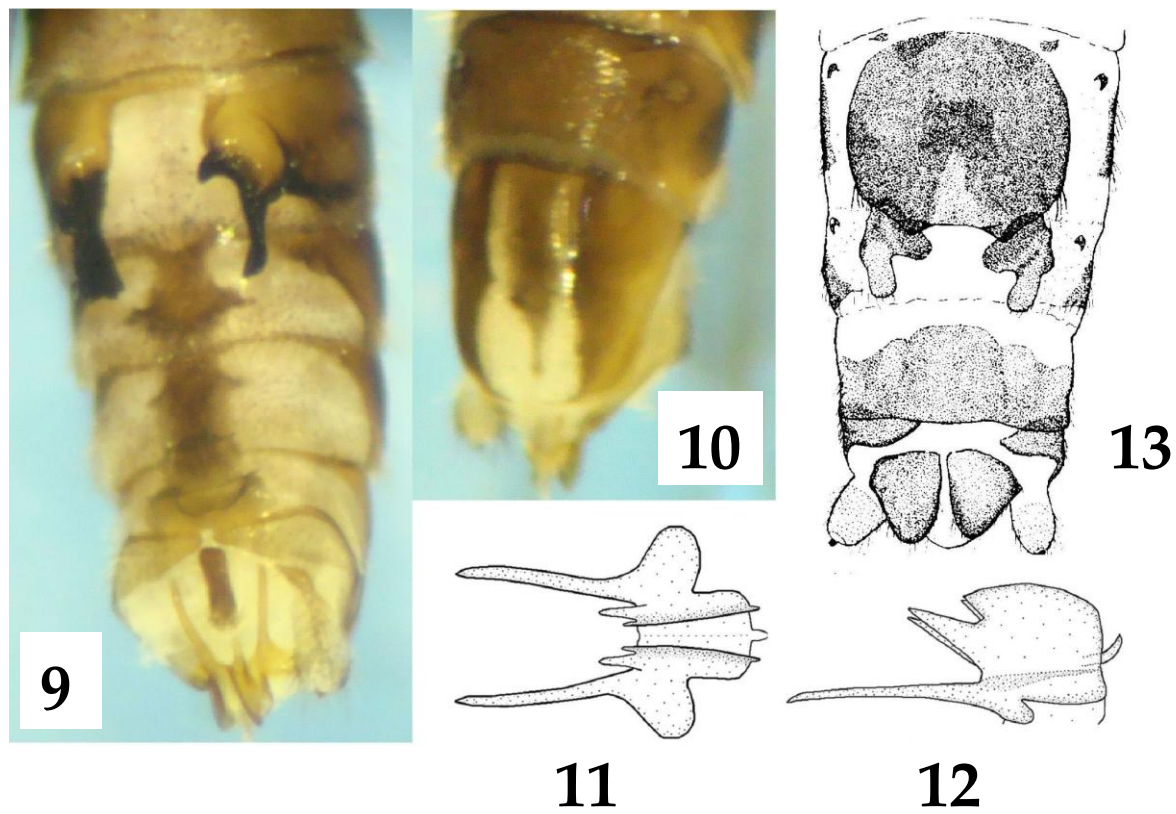


Figs. 5–8. *Leuctra bidula*, male. Segments IX and X, shown as transparent: 5, Lateral. 6, Oblique caudal view; open arrow in Fig. 5 indicates direction of view. 7, Dorsal. 8, Ventral. All to the same scale, semi-diagrammatic: a, articulation; ab, attachment bulb; c, cercus and cercus attachment site, respectively; e, epiproct; ip, invaginated ventral portion of paraproct; lp1, lp2, lateral processes 1 and 2, respectively; os, open space; SIX, TIX, TX, sternites and tergites IX and X, respectively; sp, specilla with sperm ducts.

***Leuctra ketamensis* Sánchez-Ortega & Azzouz 1997**
(Figs. 9–12, 14–16)

Material examined. Holotype: 1♂, Achraf Stream, 4 km of Tlata Ketama, 1400m, Al-Hoceïma, Rif, Morocco, 34°53'30"N, 4°35'50"W, 23-10-1993.

Paratypes: 1♀, Achraf Stream, 4 km of Tlata Ketama, 1400m, Al-Hoceïma, Rif, Morocco, 34°53'30"N, 4°35'50"W, 29-10-1993; idem, 3♀, 23-10-1994. 1♀, Oued Ketama, Tlata Ketama, 1210m, Al-Hoceïma, Rif, Morocco, 34°50'15"N, 4°34'10"W, 29-10-1993. The specimens remain in the Sánchez-Ortega collection



Figs. 9–13. *Leuctra ketamensis*. Male abdomen: 9, Dorsal. 10, Ventral, sternite IX; 11, Paraprocts, dorsal. 12, Paraprocts, lateral. Female abdomen: 13, Ventral, showing the pregenital and subgenital plates (13 from Sanchez-Ortega & Assouz, 1997).

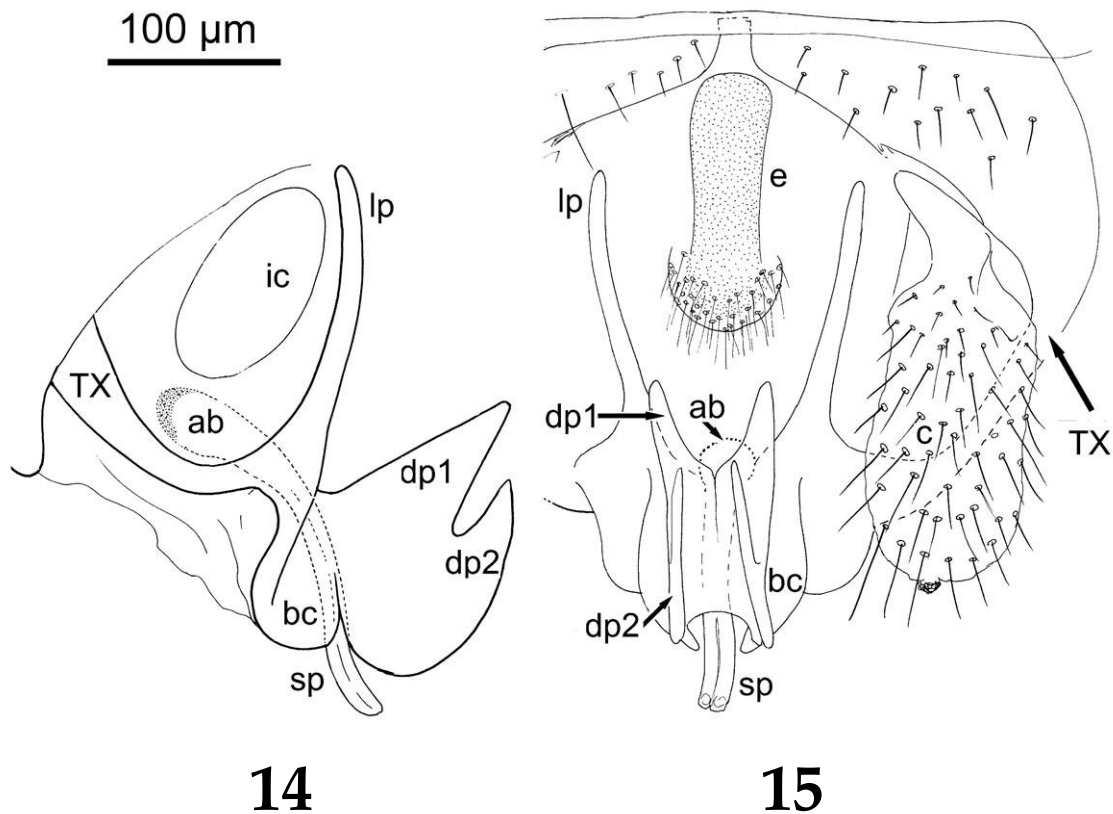
(University of Granada, Spain). Other material: 1♀, Ketama, above Azila, Tidirhine Mount, 1600m, Al-Hoceïma, Rif, Morocco, 13-11-2013. 1♂, SW Tazzeka Mount, below Bab Taka Pass, Oued el Abod tributary, 1400m, Taza, Middle-Atlas, Morocco, 13-11-2013, Vinçon leg.

Habitus. Typical of *Leuctra* except that in the male the maxillary palpus is a little longer than usual.

Male terminalia (Figs. 9–15). Tergite V with bell-shaped median membranous area. Tergite VI with entire anterior margin and a pair of strongly sclerotized processes; each process having two teeth, a small one, hook-shaped, on the internal side, and a long one extending posteriorly on half tergite VII, and ending in two sharp points (Fig. 9). Tergites VII and VIII without sclerotized process, but with central dark pigmented area connected to the anterior

margin. Sternite IX (SIX) with parabolic caudal margin, largely soft, only a mediobasal strip and caudally narrowing lateral stripes sclerotized and dark, without ventral vesicle (Fig. 10). No connection to the structures which (by comparison with *L. bidula*) are the paraprocts. They are located high up above SIX, pointing forward and upward (Figs. 9, 11, 12, 14, 15).

Tergite X dorsally similar to other *Leuctra* species, except that the basal strip of the epiproct is strongly sclerotized (Figs. 9, 15). The ventrolateral sclerite brackets formed by TX not separated from the paraprocts by sutures. The brackets curve up, each supports a very long spine (lp) whose widely separated and slightly diverging apices lie alongside the epiproct (Figs. 11, 12, 14, 15). Caudally, the brackets connecting TX and paraprocts curve inward,



Figs. 14, 15. *Leuctra ketamensis*, male. 14, Terminal section of segment X and paraprocts in lateral view. 15, dorsal view of part of TIX and TX. Both to same scale, abbreviations as in Figs. 5–8, plus: bc, basal cone; dp1, dp2, dorsal paraproct processes 1 and 2; ic, insertion of cercus.

almost meeting medially. They support a large sclerotized basal cone (bc) high above sternite IX. Caudo-medially the cone is divided, each side supports a vertical outgrowth. It is in side view divided into a straight long process (dp1) and above it a shorter process with curved upper contour (dp2) (Figs. 11, 14). In dorsal view, the longer processes (dp1) are contiguous, with a delicate anterior suture. The shorter processes (dp2) are widely separate (Figs. 12, 15).

Between the halves of the basal cone (bc) and beneath its dorsal processes projects a short median tube which represents the seemingly missing specilla (sp) (Figs. 11, 14, 15). At 630x, its paired caudal openings are visible. By transparency the tube can be

seen reaching far into the body (Figs. 14, 15). It arises from a typical attachment bulb into which the common gonoduct enters from below. In ventral view (not shown) the tube with the paired sperm ducts was obscured by some longitudinal sclerotization which in side view appeared like a ventromedian crest. This may be rudiments of the median components of the paraprocts, details not visible. Cerci short and truncated.

Female terminalia (Fig. 13). Sternite VII strongly sclerotized, with posterior edge terminating in two lobes overlapping anterior margin of sternite VIII. Sternite VIII: subgenital plate split into two parts by wide central membranous field; each lateral part is split into two lobes; the inner one oriented towards

the central part of the sternite, the outer one nearly parallel to the segment side and almost twice longer than the inner one (Fig. 13). Cerci short and truncated.

Ecology and distribution. Crenophilic species restricted to the highest points of the Rif and to the northern part of the Middle Atlas close to Taza (1250–1600m) (Fig. 16). The adults emerge in autumn (X–XI).

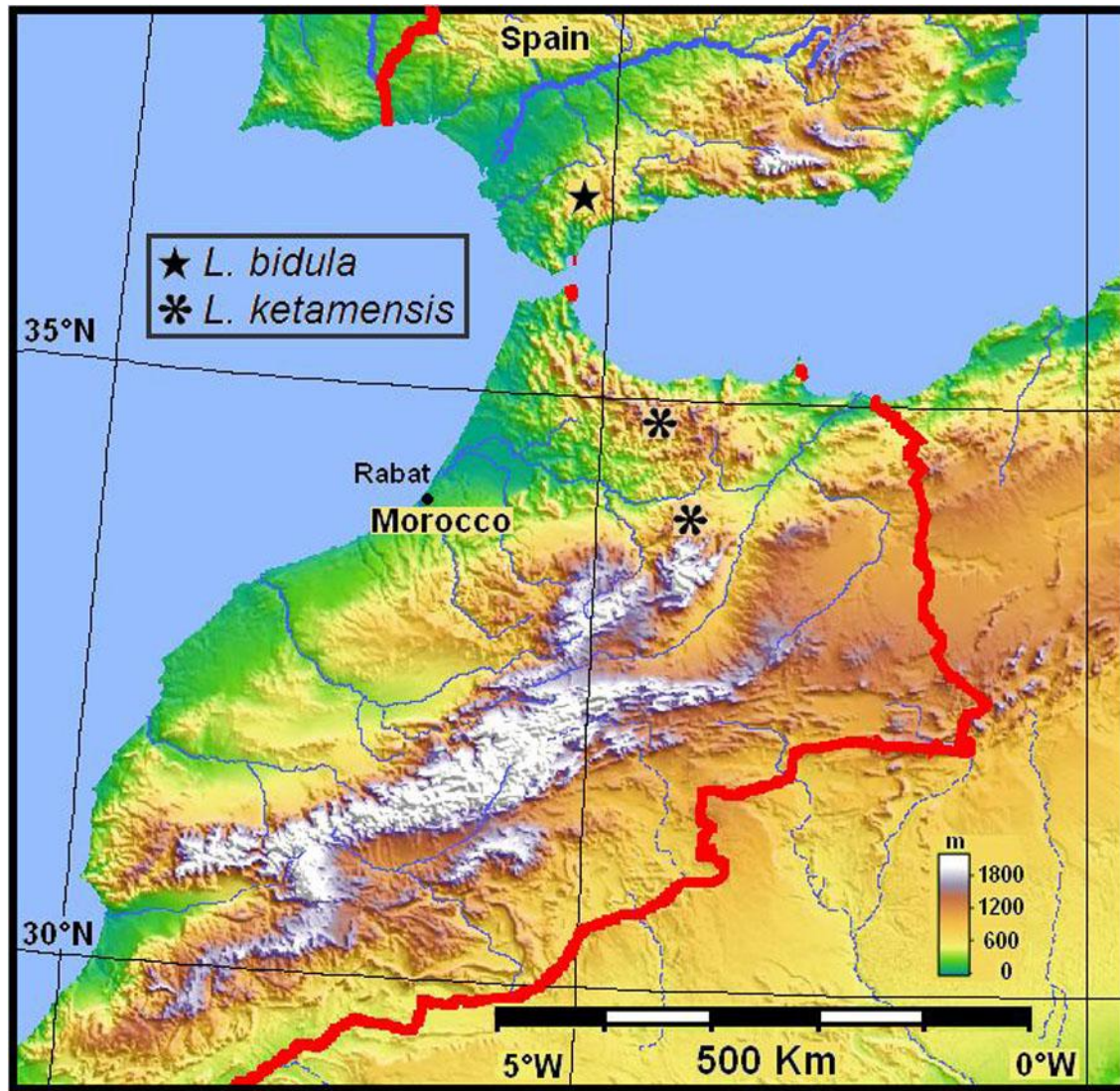


Fig. 16. Geographical distribution of *Leuctra bidula* and *L. ketamensis*.

CHARACTER ASSESSMENT AND TAXONOMIC PLACEMENT

Distinctive apomorphies in the inner anatomy of *Leuctra* were repeatedly described and illustrated (Klapálek 1896, Brinck 1956, Zwick 1973): *Leuctra* is unique in the family in that ovaries and testes are

anteriorly separate forming no loop or median string as it is generally the case in Plecoptera, also in the other Leuctridae (Zwick 1973). In *Leuctra*, the elongate follicles on each side form one dense terminal bundle opening into the wide sperm duct. The condition of the presently available material

precluded examination of these decisive details in *L. bidula* and *L. ketamensis*, their study is a task for the future.

The external male genitalia seem to exclude *Leuctra bidula* and *L. ketamensis* from *Leuctra*, and to call for a new genus. However, in all other characters the two species fit into genus *Leuctra* which differs from other Leuctridae by characters listed, for example, by Harper & Harper (1997, 2003) and Ravizza & Vinçon (1998). Some relevant observations concerning the placement of *L. bidula* and *L. ketamensis* are addressed below. We conclude that *Leuctra bidula* and *L. ketamensis* are no ancient relicts, are not the sister-group of any genus-group taxon among the Leuctrinae, but are secondarily modified species of genus *Leuctra*.

A sclerite ring inside the female receptacle is distinctive of genus *Leuctra* but is evidently secondarily missing in a number of undoubted *Leuctra* species. Absence of a spermathecal sclerite in the present two species does not speak against their membership in *Leuctra*.

An epiproct consisting of a band-like strip leading to a pilose terminal cap is typical of genus *Leuctra*. *Leuctra bidula* agrees, *L. ketamensis* differs gradually. Unsclerotized postero-central portions of tergites VI and following, and paired lateral tergal processes typical of male *Leuctra* are shared by *L. bidula* and *L. ketamensis*.

In *Leuctra* males the penial attachment bulb is normally visible from the outside, by transparency of the intersegmental membrane between sternites IX and X. In the present two species the bulb shifted deep into the body and is concealed. Strong retractor muscles on the attachment bulb inside SIX are visible in both *Leuctra bidula* and *L. ketamensis*, and paired sperm ducts surrounded by a soft common hull originate from the bulb. In *L. bidula*, the inner paraproct lobes visibly followed the inward move of the attachment bulb, anteriorly losing sclerotization and pigmentation. The delicate colorless unusually located specilla are therefore not easily visible and were believed to be missing. In *L. ketamensis*, there is no visible connection between penis tubes and paraprocts any longer. We do not know how during copula the bodies are positioned, if the females have modified internal structures, and how sperm transfer

with the unusual specilla is performed.

The two species differ in details of shape and position of the specilla and sperm ducts, and much in structure and position of the middle and outer portions of the paraprocts. In *L. bidula*, the slender ventrolateral bracket of TX is separated from the paraprocts by a suture, as is typical of Leuctrinae. In this respect, *L. ketamensis* is more derived, its caudal sclerotized arch is undivided. The arch is also in an exceptionally raised position high above S9, with the paraprocts in dorsal position. The long dorsolateral processes (lp, lp1 and lp2) arising from the arched sclerite bracket are homologous between the two species and seem in turn to be homologous to the lateral paraproct sclerite observed in many *Leuctra* species. Usually this is a simple rectangular plate, e.g., in *L. inermis* Kempny 1899. Only in *L. nigra* (Olivier 1811), the plate is dorsally extended and forms a hooklet above the anus (Aubert 1962, his fig. 18).

Tergal ornamentations are enormously variable in the genus. Shape and size of processes, their fusion into a single median structure, additional segments involved, or conversely the complete reduction of processes are mainly used to define species groups within *Leuctra* (e.g., Harper & Harper 1997, 2003, Ravizza & Vinçon 1998). No species group is shared between Europe and America which suggests separate evolution since the Atlantic Ocean opened (Harper & Harper 2003).

Aubert (1962) and Sánchez-Ortega & Azzouz (1997) included *L. bidula* and *L. ketamensis* and several other species in the *nigra*-group. Even though it provides an example of how the peculiar dorsal components of the paraprocts may have originated, *L. nigra* is certainly no close relative. In other structures, males of *L. nigra* are average representatives of genus *Leuctra*, females have a typical spermathecal sclerite, but the median sclerite visible between the lobes of the subgenital plate is exceptional. The closest relatives of *L. bidula* and *L. ketamensis* are probably among the other species at the south-western end of the genus' range. From the illustrations, *L. estrela* Aubert 1962 is rather similar in male tergal structures and the extravagant female subgenital plate. A multi-character-search is needed to identify the sister taxon of *L. bidula* and *L. ketamensis* – another task for the future.

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