

On the identity of *Isoperla curtata* (Plecoptera: Perlodidae): behavioural and molecular approaches show the existence of two separate species

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Abstract

The identity of *Isoperla curtata* Navás, 1924, an Iberian endemic, has been questioned since its description. Marked variability in pigmentation, wing length, penial armature and ecology of populations have been noted. To clarify the taxonomic status of *I. curtata* we examined variation in mating calls and cytochrome c oxidase subunit I sequences for two populations from north-central and southern Spain. Results of both approaches support the presence of two species. The north-central population corresponds to the nominal taxon, *I. curtata*, while southern populations represent a new species, *Isoperla morenica*, described herein.

Key words: stoneflies, drumming, DNA sequences, systematics, *Isoperla morenica* n. sp.

Introduction

Isoperla curtata Navás, 1924 is a species endemic to the Iberian Peninsula, collected from a wide altitudinal range (from 50 to 1800 m) (Tierno de Figueroa *et al.* 2003). It was described from external features, mainly colour, reduction of wings, wing venation, and body size (Navás 1924). The type locality was Cercedilla, near Madrid, Spain. In 1952, Aubert published a redescription of this species with many more details and better illustrations, including figures of the male penial armature and scales (Aubert 1952). He studied specimens from Cercedilla and Navarredonda (near Ávila, Spain) and considered this species as an endemic of the mountains of central Spain (Aubert 1952). According to him, this taxon could not be included in any of the previously described *Isoperla* species-groups. Four years later, Aubert (1956), studying material from a wider geographical area including Sierra de Guadarrama (central Spain) and Cantabric Mountains (northern Spain), observed for the first time that there is a marked variability in the size and arrangement of the penial armature and scales, in pigmentation, and in wing length (with macropterous, brachypterous and micropterous individuals) in relation to the altitude. In this paper, he wrote that the polymorphism of *I. curtata* could not be easily divided geographically to form subspecies. He remarked that it would be interesting to re-examine this species on the basis of more abundant material. Later, Aubert (1963a) commented on ecological niche partitioning between populations from the Douro basin (northwest Spain) which inhabited permanent mountain streams and populations from Sierra Morena (South Spain) inhabiting temporary streams. Specimens of the latter populations were always macropterous with general body colour of light yellow, while specimens from Douro exhibited the full range of wingedness and their coloration was dark.

Biological data for this species were scarce until mating, oviposition, vibrational communication, nymphal growth, feeding habits and secondary production studies were carried out on a population from the Sierra Morena (Tierno de Figueroa *et al.* 2000, López-Rodríguez *et al.* 2009).

The aim of this study is to clarify the taxonomic relationships of two polymorphic populations, one from north-central and the other from southern Spain. To determine this relationship we used a behavioural approach that involved describing and comparing species-specific male mating calls that may act as a reproductive isolation

mechanism (Stewart *et al.* 1983; Tierno de Figueroa & Sánchez-Ortega 1999). Additionally, we examined sequence variation in a fragment of the widely used mitochondrial gene cytochrome c oxidase subunit I (COI).

Material and methods

Behavioural approach

Adults of *I. curtata* were collected by the authors from the following Spanish localities:

North-central populations:

1) Garganta de Iruelas, Alberche basin (included in Tajo basin), Ávila, Sistema Central, 786 m, Coordinates: Lat 40.40425 Long -4.56775. May, 27th 2009. 1 male.

2) Garganta de la Olla, Tiétar basin, (included in Tajo basin), Cáceres, Sistema Central, 645 m, Coordinates: Lat 40.11917 Long -5.77685. May, 14th 2009. 3 males.

Southern populations:

3) Arroyo de la Nava del Rey, Jándula basin (included in Guadalquivir basin), Ciudad Real, Sierra Morena, 600 m, Coordinates: Lat 38.53031 Long -3.80594. May, 21st 2009. 5 males, 2 females.

4) Río Despeñaperros, Guadalén basin (included in Guadalquivir basin), Jaén, Sierra Morena, 560 m, Coordinates: Lat 38.37432 Long -3.50802. May, 1st 1998. 6 males (data published in Tierno de Figueroa *et al.* 2000).

For mating call recording and analysis we used the methods of Tierno de Figueroa *et al.* (2009): adults of *I. curtata* were placed in different crystal pots with a piece of paper at the opening. Drumming signals were recorded immediately or within 1–2 days of collection. Adults were recorded in a well lit room at a constant temperature of approximately 20 °C. The calls were recorded using a microphone (100–16000 Hz; 44 dBV/Pascal) placed in light contact with the paper surface and attached to a computer. Audacity® software version 1.2.6 was used for recording and analyzing the calls. In each call, the following parameters were analysed: number of beats per sequence and interval duration between beats (inter-beat duration), number of sequences per call and intervals between sequences (inter-sequence duration).

Molecular approach

Specimens were collected by the authors in the following sites and dates and stored in 99% ethanol (some of them after drumming recording):

North-central populations:

1) Garganta de Iruelas, Alberche basin (included in Tajo basin), Ávila, Sistema Central, 786 m, Coordinates: Lat 40.40425 Long -4.56775. May, 27th 2009. 1 male.

2) Garganta de la Olla, Tiétar basin, (included in Tajo basin), Cáceres, Sistema Central, 645 m, Coordinates: Lat 40.11917 Long -5.77685. May, 14th 2009. 1 male, 1 female.

Southern populations:

3) Arroyo de la Nava del Rey, Jándula basin (included in Guadalquivir basin), Ciudad Real, Sierra Morena, 600 m, Coordinates: Lat 38.53031 Long -3.80594. May, 21st 2009. 2 males, 4 females.

4) Río Despeñaperros, Guadalén basin (included in Guadalquivir basin), Jaén, Sierra Morena, 560 m, Coordinates: Lat 38.37432 Long -3.50802. May, 9th 2007. 3 males, 4 females.

Males from Garganta de Iruelas and Garganta de la Olla, two males and two females from Arroyo de la Nava del Rey used for the molecular analysis were the same employed for drumming recording.

DNA extraction was carried out on single specimens using the Easy-DNA™ Kit (Protocol #3). DNA target amplification was carried out using PCR. LCO1490 and HCO2198 primers were used (Folmer *et al.* 1994) to obtain a 709 bp COI fragment. Primer characteristics are listed in Table 1.

TABLE 1. Primer characteristics.

Primer	Sequence	bp	Tm	G/C
LCO1490	5'-GGTCAACAAATCATAAAGATATTGG-3'	25	50.5°C	32%
HCO2198	5'-TAAACTTCAGGGTGACCAAAAAATCA-3'	26	55.3°C	34.6%

G/C, Guanine/Cytosine content; Tm, Melting temperature

Primers (Macrogen Inc. - Seoul, Korea-) were used in PCR with 1 µg DNA, using GoTaq®Green Master Mix (Promega) in a Eppendorf MiniCycler™ PTC-150-16 model (MJ Research, USA).

PCR products were separated by gel agarose electrophoresis 1% (w/v), containing 5 ng/µl GelRed™ Biotium and then purified and sequenced by Macrogen Inc. (Seoul, Korea). Chromatograms were manually adjusted with Chromas Lite 2.01 (Copyright© 1998-2005, Technelysium Pty Ltd). Sequences were analysed in BLAST (Altschul *et al.* 1997). Sequence alignment was carried out with ClustalW2 (Thompson *et al.* 1994) and manually adjusted using MEGA 4.0 (Tamura *et al.* 2007).

A *Perla* sp. sequence from GenBank (HM880064) was used as outgroup. All positions containing gaps and missing data were eliminated from the dataset.

MEGA 4.0 (Tamura *et al.* 2007) was employed to build phylogenetic trees using Neighbour Joining (NJ, Saitou & Nei 1987) and Maximum Parsimony (MP, Eck & Dayhoff 1966) approaches.

Results

Behavioural approach

A total of 82 male drumming calls from north-central and southern populations were recorded and analysed. Twenty-one female answers from the southern populations were also studied.

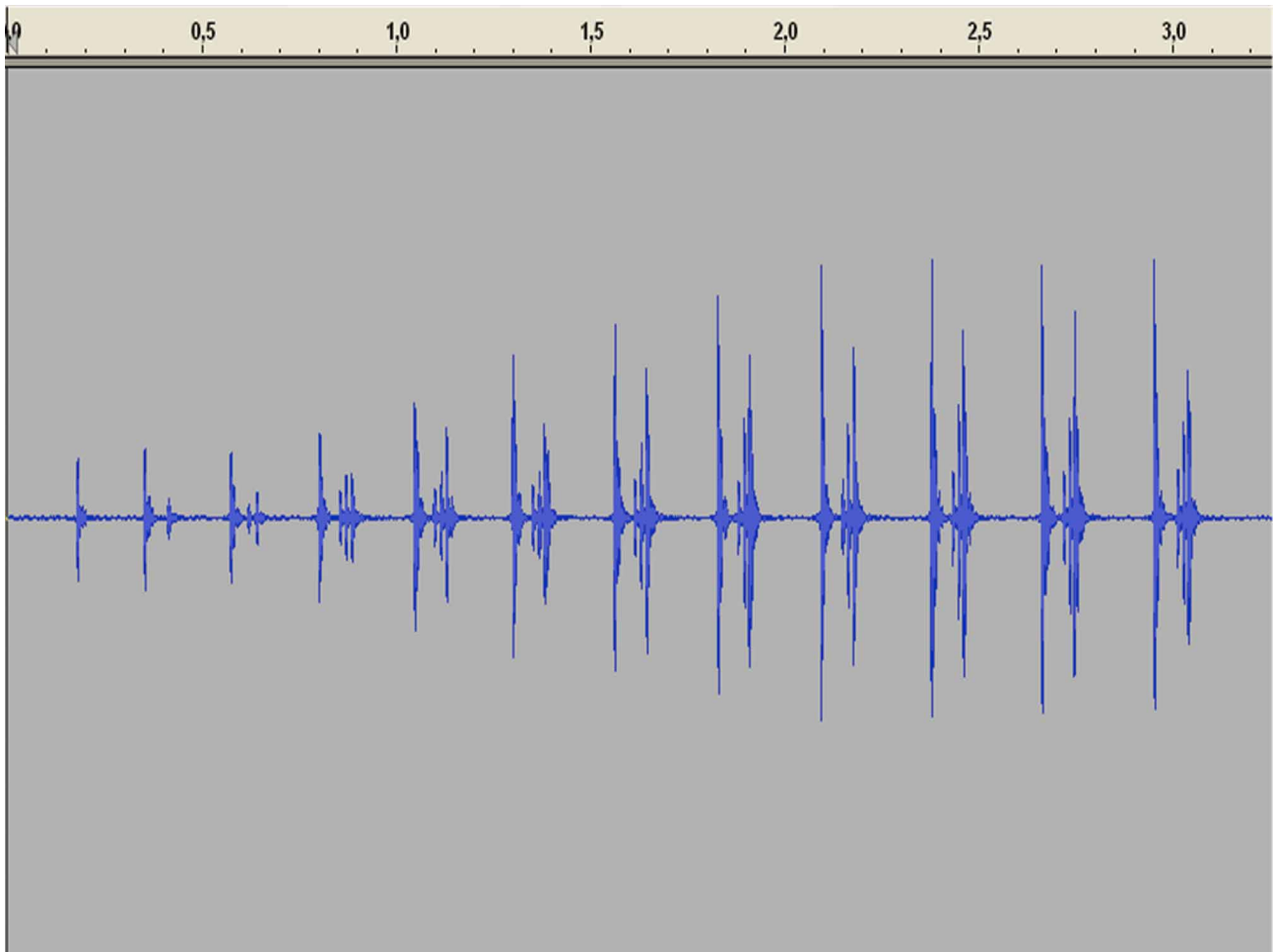


FIGURE 1. Drumming signal of a male from a north-central population (Garganta de Iruelas).

1) Males from north-central populations:

We obtained 34 male calls from the Garganta de la Hoya population (from 3 males) and 3 calls from the Garganta de Iruelas population (from 1 male). All the calls show a similar pattern. The drumming call consists on a

repetition of sequences (Fig. 1). The sequence can be repeated from 6 to 20 times (mean= 13.270; SD= 3.701; N= 37), with intervals between them from 0.019 to 0.235 s (mean= 0.152 s; SD= 0.038, N= 128). Each sequence consists of 1 initial beat that can be followed by a group of 1–4 beats (interval between the initial beat and the following ones range from 0.037 to 0.090 s, mean= 0.053 s; SD= 0.011; N= 108; intervals between the beats forming the group are more constant and range from 0.007 to 0.033 s; mean= 0.015 s; SD= 0.003; N= 171).

2) Males from southern populations:

A total of 45 calls of 5 males from Arroyo de la Nava del Rey were recorded. The male call consists of a repetition from 1 to 7 diphasic sequences (mean= 2.400; SD= 1.176; N= 45), the first phase with decreasing interbeat intervals and the second phase with short and approximately constant interbeat intervals (Fig. 2, Fig. 3). The interval between sequences is also variable (mean= 1.196 s, SD= 0.673; minimum= 0.073 s; maximum= 3.403 s; N= 63), such that almost all sequences may be considered as independent calls that are repeated a variable number of times.

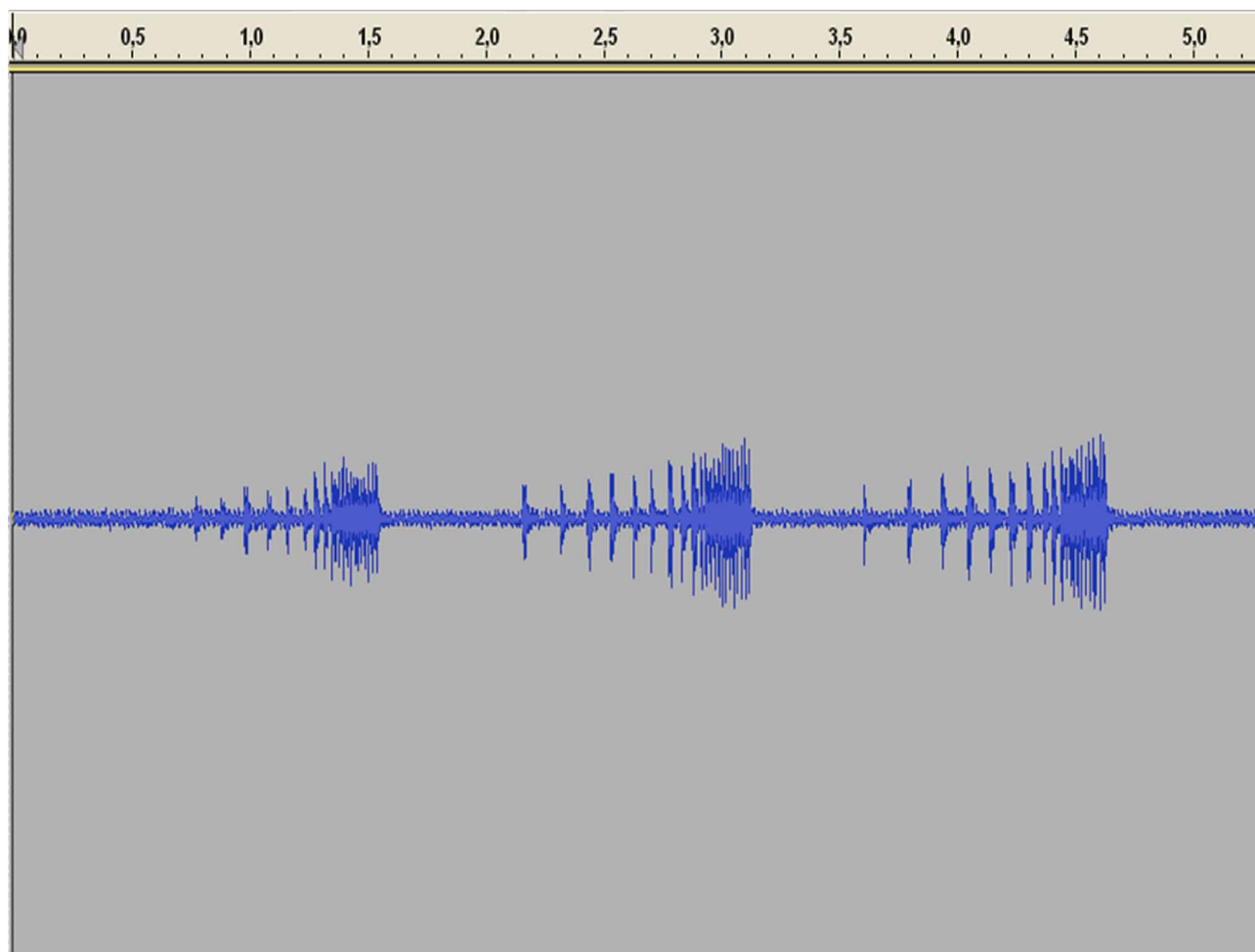


FIGURE 2. Drumming signal of a male from a southern population (Arroyo de la Nava del Rey).

Each diphasic sequence consists on a mean of 21.196 beats (minimum= 1, maximum= 39; SD= 4.113; N= 107). As said above, the intervals between beats decrease substantially from the first beat (phase I: mean number of beats= 8.630; minimum= 1; maximum= 14; SD= 2.936, N= 54; interbeat interval mean= 0.063 s; minimum= 0.009 s maximum= 0.485 s; SD= 0.045, N= 466), then are relatively even for the remaining beats (phase II: mean number of beats= 12.352; minimum= 5; maximum= 33, SD= 4.274, N= 54; interbeat interval mean= 0.016 s; minimum= 0.008 s; maximum= 0.142 s; SD= 0.009; N= 658).

Drumming calls of males from Despeñaperros had been previously published (Tierno de Figueroa *et al.* 2000) and are identical to those described above for the Arroyo de la Nava del Rey population.

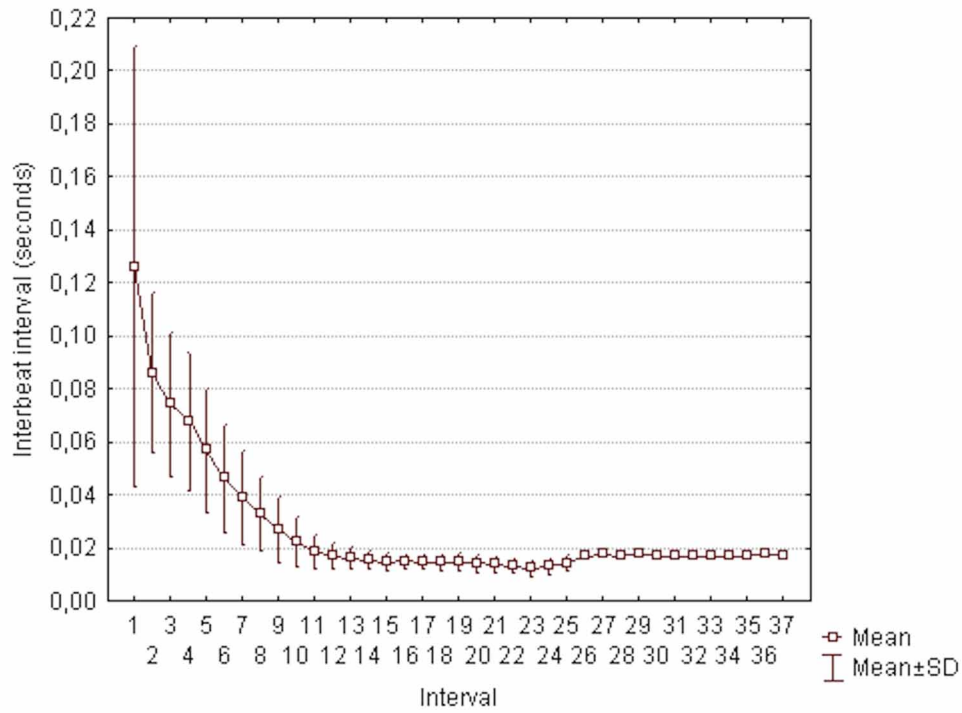


FIGURE 3. Time intervals between beats in a male drumming call from a southern population (Arroyo de la Nava del Rey).

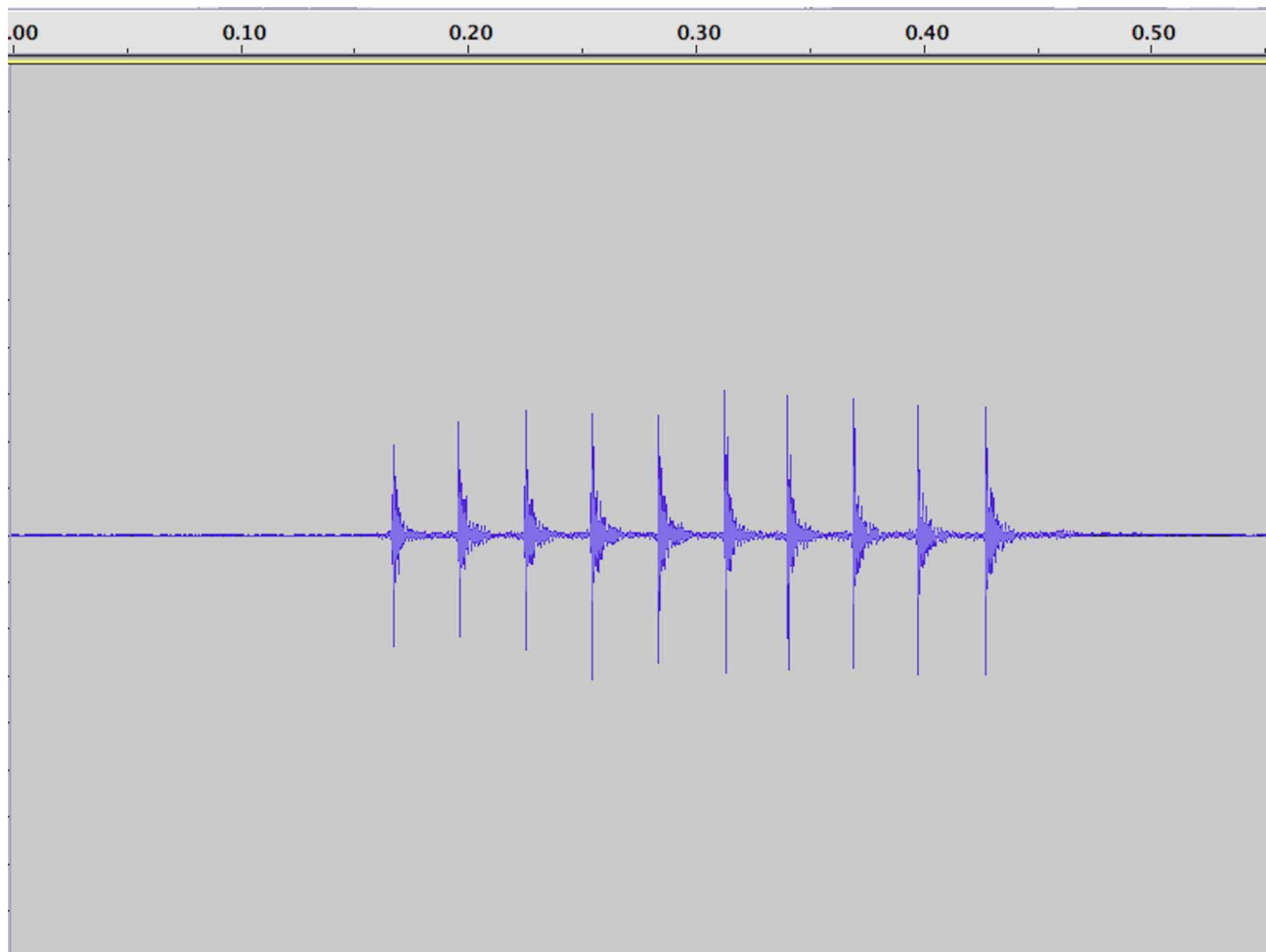


FIGURE 4. Drumming signal of a female from a southern population (Arroyo de la Nava del Rey).

3) Females from southern populations:

Twenty one answers of 2 females from Arroyo de la Nava del Rey were recorded and analysed. The drumming signals consist of a monophasic sequence composed of a variable number of beats (mean= 11.286, SD= 2.492; minimum= 6; maximum= 17; N= 21) with mean interbeat interval of 0.031 s (minimum= 0.028 s; maximum= 0.080 s; SD= 0.005; N= 216) (Fig. 4).

Molecular approach

A phylogenetic tree was obtained using the NJ approach (sum of branch length = 1.927) and a 505 bp fragment common to all specimens (Fig. 5). The tree obtained with the MP approach has an almost identical topology and because of this is not shown. Also, trees obtained using translated amino acids instead of base-pairs have an overlapping topology. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates; Felsenstein 1985) are shown next to the branches.

Southern populations clustered together and are clearly separated from north-central ones. This result is well supported by the bootstrap values.

In terms of nucleotide differences, the maximum distance is displayed between Olla and Despeñaperros populations (22.1%), while the minimum distance is that between Iruela and Olla ones (2.8%).

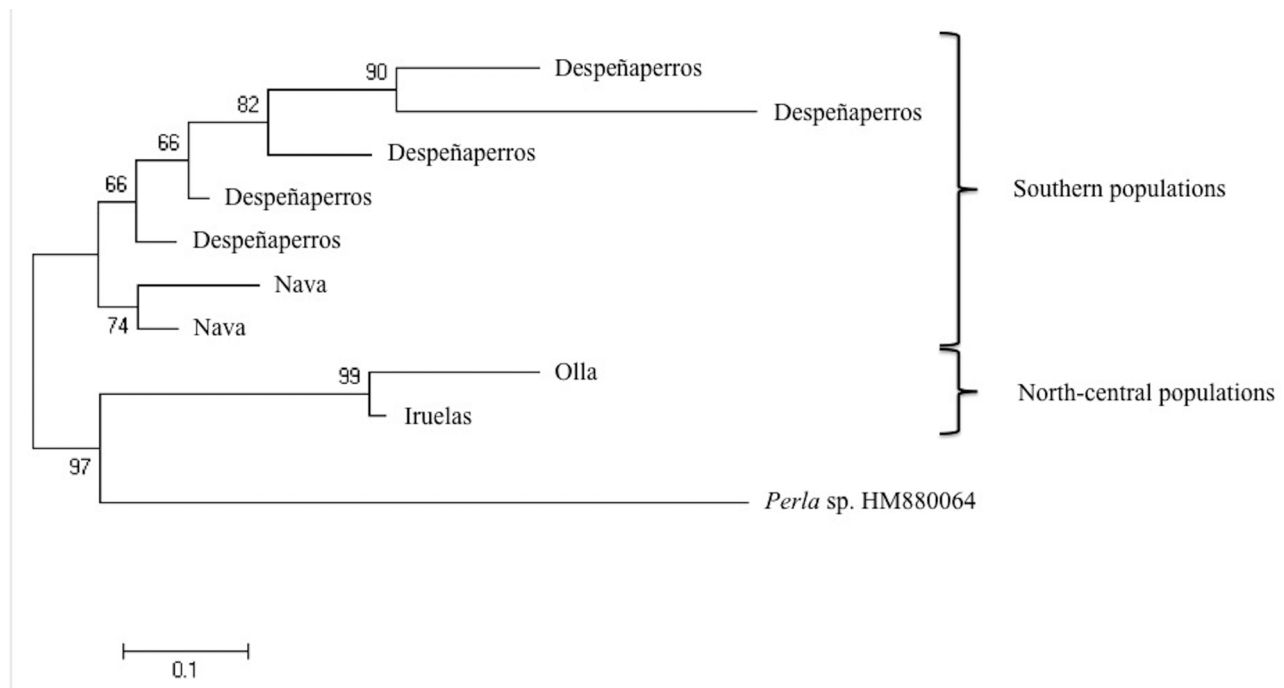


FIGURE 5. Neighbour Joining tree obtained from the 505 bp COI fragment for studied populations.

Discussion

The call patterns of males of the north-central and southern populations recorded in this study were qualitatively different. North-central populations displayed monophasic calls, while those from the south had diphasic calls. Additionally, the call of the north-central populations was notably different from those of all other *Isoperla* species studied in Spain (Tierno de Figueroa *et al.* 2002; Luzón-Ortega *et al.* 2010). This call is most similar to the European *Isoperla oxylepis* (Despax, 1936) (Rupprecht 1969), absent from Spain and clearly morphologically distinguishable. The southern population calls were completely different from that of other Palearctic *Isoperla* species. According to the basic types of Plecoptera calls (in relation to their complexity) listed by Stewart & Sandberg (2006), the north-central population's drumming signal could be included in type 2, while the southern population exhibited a type 3 call.

The role of drumming signals as reproductive isolation mechanisms in Plecoptera has been stressed many times and, therefore, they have been used for resolving systematic questions (Maketon & Stewart 1984; Stewart & Zeigler 1984). In this way Tierno de Figueroa & Sánchez-Ortega (1999) supported the distinction between *Isoperla nevada* Aubert, 1952 and *Isoperla grammatica* (Poda, 1761). Berthélemy (1979), using drumming signals, noted that a complex of species had been confounded under the name *I. grammatica* in the Pyrenees.

In the present study the two mating calls display differences well beyond the possibility that they are dialects within a single species. According to drumming behaviour, we can conclude there are two evolutionarily separate and isolated species.

Genetic data also suggest that north-central and southern Spain populations represent different species. Neighbor joining (Fig. 5) and ML trees clearly separate these populations with genetic distances exceeding 20%. Another study of genetic distances between two species of *Tyrrhenoleuctra* (Leuctridae) in Europe demonstrated almost 10% sequence divergence [*Tyrrhenoleuctra zavattarii* Consiglio, 1956 (Sardinia) vs. *T. tangerina* (Navás, 1922) (Spain)] (Fochetti *et al.* 2009).

Both approaches, drumming behaviour and molecular distances, are in agreement in discriminating a cryptic species that has been confused with *I. curtata*. Since *I. curtata* was described from material from central Spain, we argue that north-central populations correspond to the nominal taxon, *I. curtata*, while the southern populations belong to a new species described herein.

***Isoperla morenica* sp. n. Tierno de Figueroa & Luzón-Ortega**

Fig. 6

Type material. Holotype male. Spain: Jaén, Sierra Morena, Despeñaperros River, Coordinates: Lat 38.37432 Long -3.50802, altitude 560 m, 4-VI-2007, M.J. López-Rodríguez & J.M. Tierno de Figueroa leg.; Paratypes: Same locality, collection date and collectors, 4 males, 5 females.

The holotype, 2 males and 2 females paratypes are deposited in the Collection of the Museo Nacional de Ciencias Naturales (Madrid, Spain) with the identification codes: MNCN Ent N° Cat. 71379 for the Holotype and MNCN Ent N° Cat. 71380 for the Paratypes. The remaining paratypes (2 males and 3 females) are deposited in J. Manuel Tierno de Figueroa's collection in the Departamento de Biología Animal, Universidad de Granada (Granada, Spain).

Other material referred. Spain, Badajoz: Puerto de las Marismas, 750 m, 21-IV-1960, 5 nymphs (Aubert 1963a, b). Sevilla: Río Guadiamar, 300 m, 1979–80 (Puig & Gallardo 1985); Arroyo Aciago, 300 m, 17-IV-1979, 1 nymphs (Gallardo Mayenco 1990). Jaén: Sierra Morena, Arroyo del Rey, Órganos de Despeñaperros, 650 m, 26-V-1959, 1 male, 25 females, same location, 16-V-1960, 3 males, 3 females; Sierra Morena, Río Guarrizas, 450 m, 26-V-1959, 2 females; same location, 16-V-1960, 2 females; Sierra Morena, Confluence of Río Despeñaperros and Arroyo del Rey, 1-V-1998, 19 males, 30 females (Tierno de Figueroa *et al.* 2000); Despeñaperros River, 560 m, from I-2007 to VI-2007, many nymphs and adults (López-Rodríguez *et al.* 2008, 2009; Sanz *et al.* 2010). Andalucía: Sierra Morena, Río Panados, Venta del Aire, 700 m, 27-V-5-1959, male/s (Aubert 1963a, b). Ciudad Real: Sierra Morena, Arroyo de la Nava del Rey, Jándula basin, 600 m, Coordinates: Lat 38.53031 Long -3.80594, 21-V-2009, 13 males, 12 females, J.M. Luzón-Ortega leg.

The citations of *I. curtata* for the Baetic Cordillera (Cádiz and Jaén; southern Spain) referred to below are reported with doubt and must be checked because many of the identifications were made from nymphs before the nymph was described.

Spain, Cádiz: Río Palmones, Algeciras, 50 m; Río Grazalema, Grazalema, 700 m (Aubert 1963b). Spain, Jaén: Sierra de Cazorla, Río Guadalentín, Nava de San Pedro, 1300 m, 28-V-1959, 5 nymphs; id., 15-V-1962, 32 nymphs (Aubert 1963a, b) and "Nacimiento del Guadalquivir", 1350 m, 1975–78 (González del Tánago & García de Viedma 1983).

Diagnosis. This large, yellowish-brown species is macropterous in both sexes. Male penial armature is present as a long, flat patch, covered with large, flat, triangular scales with a blunt tip. No accessory armatures are present. The female subgenital plate is broadly semicircular, occupies the median 2/3rd of the sternum, and does not extend to past the posterior edge of the 8th sternum. Nymphs are distinguished by the characteristic light pentagonal-shape interocellar spot.

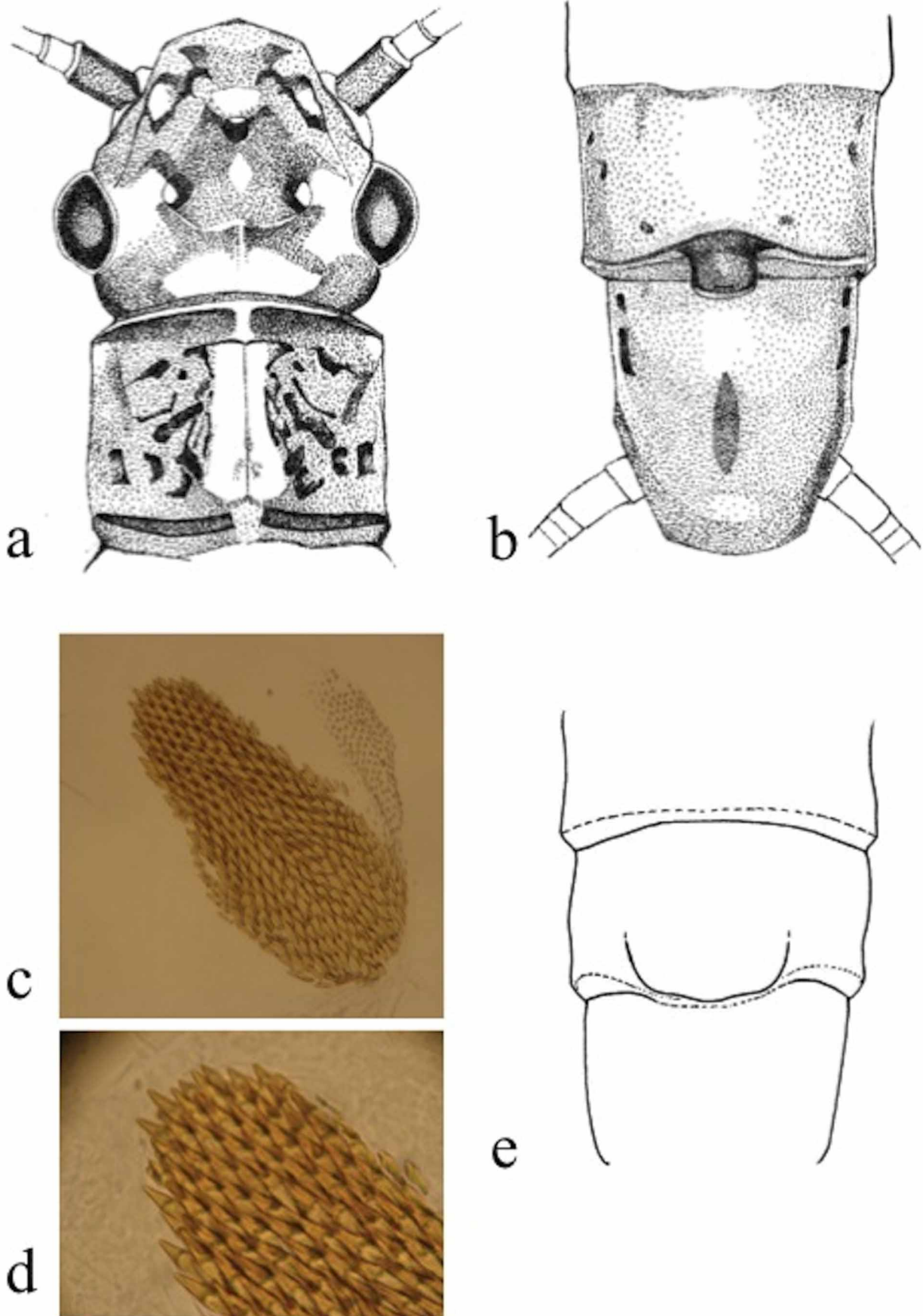


FIGURE 6. *Isoperla morenica* sp. n. Head and pronotum (a), ventral view of the male abdomen (b), penial armature (c), detail of the penial armature showing the scales (d), and ventral view of the tip of female abdomen (e).

Male (Figs. 6b, 6c & 6d): Forewing: 9.9–11.7 mm, n= 5 (Holotype: 11.2 mm). Total length: 10.9–13.7 mm, n= 5 (Holotype: 10.9 mm). Macropterous. Lobe of the tip of the 8th sternum rectangular (length/width ratio approximately 0.77) and considerably darker, much contrasted with the general yellow colour of the abdomen. General colour yellowish-brown. Head yellow with darker areas forming characteristic pattern (Fig. 6a), interocellar area dark except central diamond-shaped pale spot. Pale spot anterior to median ocellus, pale spots median to compound eyes, frontoclypeus light brown, dark bands lateral to pale occipital. Antennae brown, especially in the basal segments. Pronotum light brown, rectangular, vermiculated and darker at both sides, of light brown colour in general, with a centrally interrupted, pigmented line at the anterior and posterior edges. A yellow band in the middle of the pronotum. Mesonotum dark with a yellow area in the anterior third where a central vertical dark line and two lateral horizontal dark bands appear. Metanotum dark. Abdominal tergites brown, paler at the apex. Ventrally, both thorax and abdomen are yellow. Legs yellow with a dorsal brown band. Cerci yellow-brown, palest basally. Paraprocts dark. Penial armature flat, long (length: 0.40–0.50 mm, n= 4; width: 0.12–0.21 mm) slightly narrower at the apex. Scales (length: 0.38–0.43 mm n= 4, width: 0.10–0.13 mm) not mucronated. Accessory armatures absent.

Female (Fig. 6e): Forewing: 11.1–13.0 mm. Total length: 12.9–14.7 mm. Macropterous. General colour similar to the male. Subgenital plate curved occupying approximately 2/3 of the sternum width and not covering any part of sternum 9.

Nymph: Refer to that described in López-Rodríguez *et al.* (2008) under the name *I. curtata*.

Remarks. The penial armature and scales of *I. morenica* **n. sp.** are similar to that described by Aubert (1952, 1956) for *I. curtata*. Nevertheless, the armature size of *I. curtata* described by Aubert (1952) for individuals coming from the north-central Iberian Peninsula (0.28 mm length, 0.08 mm width) is smaller than that of *I. morenica* **n. sp.**

Habitat and ecology. This species occurs in temporary streams between 450 and 750 m. It emerges in late spring (May or June). For more biological data refer to Tierno de Figueroa *et al.* (2000) and López-Rodríguez *et al.* (2009).

Distribution. Spain: this species has been confirmed from the Sierra Morena in the southern Iberian Peninsula. We consider it endemic to southern Spain. The species may also be present in the Baetic Cordillera (southern Iberian Peninsula), but this should be confirmed by future collections of adults.

Etymology. The specific name, *morenica*, refers to the mountain range from which the species has been collected: Sierra Morena.

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Literature cited

- Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D.J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research*, 25, 3389–3402.
- Aubert, J. (1952) Plécoptères récoltés par Mr. F. Schmid en Espagne. *Eos*, 28, 249–270.
- Aubert, J. (1956) Contribution a l'étude des Plécoptères d'Espagne. *Mémoires de la Société vaudoise des Sciences naturelles*, 11, 209–276.
- Aubert, J. (1963a) Les Plécoptères de la Péninsule Iberique. *Eos*, 39, 23–107.
- Aubert, J. (1963b) Les Plécoptères des cours d'eau temporaires de la Péninsule Ibérique. *Mitteilungen der Schweizerischen Entomologischen Gesellschaft*, 35, 301–315.
- Berthélemy, C. (1979) Mating call and taxonomy in Pyrenean *Isoperla*. *Gewässer und Abwässer*, 64, 71–72.
- Eck, R.V. & Dayhoff, M.O. (1966) *Atlas of Protein Sequence and Structure*. National Biomedical Research Foundation, Silver Springs, Maryland, 215 pp.
- Felsenstein, J. (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, 39, 783–791.
- Fochetti, R., Sezzi, E., Tierno de Figueroa, J.M., Modica, M.V. & Oliverio, M. (2009) Molecular systematics and biogeography of the Western Mediterranean stonefly genus *Tyrrhenoleuctra* (Plecoptera, Insecta). *Journal of Zoological Systematics and Evolutionary Research*, 47, 328–336.
- Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cyto-

- chrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3, 294–299.
- Gallardo Mayenco, A. (1990) Distribución de los Plecópteros en el río Guadamar (Sevilla). *Ecología*, 4, 333–337.
- González del Tánago, M. & García de Viedma, M. (1983) Consideraciones acerca de los Ephemeroptera, Odonata y Plecoptera del Coto Nacional de las Sierras de Cazorla y Segura. *Miscellanea Zoologica*, 7, 53–66.
- Navás, L. (1924) Mis excursiones entomológicas del verano de 1924. *Broteria*, 21, 131–134.
- López-Rodríguez, M.J., Tierno de Figueroa, J.M. & Alba-Tercedor, J. (2008) Description of the nymph of *Isoperla curtata* Navás, 1924 (Insecta, Plecoptera). *Zoologica baetica*, 19, 85–88.
- López-Rodríguez, M.J., Tierno de Figueroa, J.M., Fenoglio, S., Bo, T. & Alba-Tercedor, J. (2009) Life strategies of 3 Perlodidae species (Plecoptera, Insecta) in a Mediterranean seasonal stream of Southern Europe. *Journal of the North American Benthological Society*, 28, 611–625.
- Luzón-Ortega, J.M., López-Rodríguez, M.J. & Tierno de Figueroa, J.M. (2010) Confirmation of the presence of *Isoperla rivulorum* (Pictet, 1841) (Plecoptera, Perlodidae) and first data of the male drumming call in the Iberian Peninsula. *Boletín de la Asociación española de Entomología*, 34, 441–443.
- Maketon, M. & Stewart, K.W. (1984) Further studies of the drumming behavior of North American Perlidae (Plecoptera). *Annals of the Entomological Society of America*, 77, 770–78.
- Puig-García, M.A. & Gallardo, A. (1985) Selección de hábitat de la población de Plecópteros del río Guadamar (S-España). In: *Resúmenes 3^{er} Congreso de la Asociación Española de Limnología*. Asociación Española de Entomología, León, pp. 121–122.
- Rupprecht, R. (1969) Zur artspezifität der trommelsignale der Plecopteren (Insecta). *Oikos*, 20, 26–33.
- Saitou, N. & Nei, M. (1987) The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology Evolution*, 4, 406–425.
- Sanz, A., Trenzado, C., López-Rodríguez, M.J., Furné, M. & Tierno de Figueroa, J.M. (2010) Study of the antioxidant defence in four species of Perloidea (Insecta, Plecoptera). *Zoological Science*, 27, 952–958.
- Stewart K.W. & Sandberg, J.B. (2006) Vibrational communication and mate searching behaviour in stoneflies. In: Drosopoulos, S. & Claridge, M.F. (Eds), *Insect sounds and communication. Physiology, behaviour, ecology and evolution*. Taylor & Francis, Boca Ratón, pp. 179–186.
- Stewart, K.W., Szczytko, S.W. & Stark, B.P. (1983) The language of stoneflies. *Bioscience*, 33, 117–118.
- Stewart, K.W. & Zeigler, D.D. (1984) The use of larval morphology and drumming in Plecoptera systematics, and further studies of drumming behaviour. *Annales de Limnologie*, 20, 105–114.
- Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007) MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular Biology Evolution*, 24, 1596–1599.
- Thompson, J.D., Higgins, D.G. & Gibson, T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research*, 22, 4673–80.
- Tierno de Figueroa, J.M., Luzón-Ortega, J.M. & López-Rodríguez, M.J. (2009) First record of male drumming call of the genus *Capnioneura* Ris, 1905 (Plecoptera, Capniidae). *Entomological Science*, 12, 359–362.
- Tierno de Figueroa, J.M., Luzón-Ortega, J.M. & Sánchez-Ortega, A. (2000) Male calling, mating and oviposition in *Isoperla curtata* (Plecoptera, Perlodidae). *European Journal of Entomology*, 97, 171–175.
- Tierno de Figueroa, J.M., Luzón-Ortega, J.M. & Stewart, K.W. (2002) The drumming of *Isoperla pallida* Aubert and *Guadalgenuis franzi* (Aubert) (Plecoptera, Perlodidae), and review and evolutionary considerations of Southern Iberian Peninsula Perlodidae. *Zoological Science*, 19, 871–875.
- Tierno de Figueroa, J.M. & Sánchez-Ortega, A. (1999) The male drumming call of *Isoperla nevada* Aubert, 1952 (Plecoptera, Perlodidae). *Aquatic Insects*, 21, 33–38.
- Tierno de Figueroa, J.M., Sánchez-Ortega, A., Membiela-Iglesia, P. & Luzón-Ortega, J.M. (2003) *Plecoptera*. In: Ramos, M.A. et al. (Eds.), *Fauna Ibérica*, vol. 22. Museo Nacional de Ciencias Naturales, CSIC, Madrid, 404 pp.