

Confirmation of the presence of *Ischnura senegalensis* (Rambur, 1842) on the Canary Islands

R. A. Sánchez–Guillén & A. Cordero–Rivera

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Abstract

Confirmation of the presence of Ischnura senegalensis (Rambur, 1842) on the Canary Islands.— The presence of one or two species of damselflies of the genus *Ischnura* in the Canary Islands has been a matter of debate in the recent years. The first published records listed *I. senegalensis* as the only zygopteran inhabiting the archipelago, but this proved to be wrong, and until recently, all specimens of *Ischnura* captured in the islands were unanimously regarded as belonging to *I. saharensis*. Recent photographic evidence, however, is compatible with the presence of *I. senegalensis*. In this study, we give morphological and genetic evidence of the presence of *I. senegalensis* in the Canary Islands, and we discuss the importance of voucher specimens to correctly identify very similar species.

Key words: Odonata, *Ischnura*, Genetic identification, Island, Voucher specimens, Macaronesia

Resumen

Confirmación de la presencia de Ischnura senegalensis (Rambur, 1842) en las islas Canarias.— La presencia de una o dos especies de zigópteros del género *Ischnura* en las islas Canarias ha sido objeto de debate en los últimos años. Los primeros registros publicados señalaban que *I. senegalensis* era el único zigóptero presente en el archipiélago; pero resultó no ser correcto y hasta hace poco, todos los especímenes del género *Ischnura* capturados en las islas se clasificaban sin excepción como *I. saharensis*. No obstante, las recientes pruebas fotográficas son compatibles con la presencia de *I. senegalensis*. En el presente estudio aportamos pruebas morfológicas y genéticas de la presencia de *I. senegalensis* en las islas Canarias, y analizamos la importancia de los especímenes de referencia para identificar correctamente especies muy parecidas.

Palabras clave: Odonatos, *Ischnura*, Identificación genética, Isla, Especímenes de referencia, Macaronesia

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R. A. Sánchez–Guillén, Genome Integrity and Instability Group, Inst. de Biotecnología i Biomedicina (IBB), Univ. Autònoma de Barcelona, Campus UAB, 08193 Barcelona, Espanya (Spain).— A. Cordero–Rivera, Depto. de Ecología y Biología Animal, Univ. de Vigo, España (Spain).

Corresponding author: R. A. Sánchez–Guillén. E–mail: rguillenuvigo@hotmail.com

Introduction

The genus *Ischnura* is one of the most speciose genera of the family Coenagrionidae, with around 70 species with a worldwide distribution (Dumont, 2013). The Mediterranean is home to nine species: *I. elegans* (Vander Linden, 1820); *I. evansi* (Morton, 1919); *I. fontaineae* (Morton, 1905); *I. genei* (Rambur, 1842); *I. graellsii* (Rambur, 1842); *I. intermedia* (Dumont, 1974); *I. pumilio* (Charpentier, 1825); *I. saharensis* (Aguesse, 1958); and *I. senegalensis* (Rambur, 1842) (Boudot et al., 2009). Given the inherent dynamic nature of animal distributions, the species found at particular localities are expected to change over time, especially in a scenario of global warming and range expansions (Hickling et al., 2005; Sánchez-Guillén et al., 2013, 2014b).

To date, 14 species of Odonata (three Zygoptera and 11 Anisoptera) have been recorded in the Canary Islands, including one Macaronesian endemic genus (*Sympetrum nigrifemur*, also found in Madeira) (Weihrauch, 2013). However, only one zygopteran has been reported regularly on the islands. It was first identified as *Ischnura senegalensis* by Valle (1955), but this identification was questioned by Hämäläinen (1986), who examined Valle's specimens preserved in the Zoological Museum of the University of Turku, and confirmed that they belong to *Ischnura saharensis*. For many years, the Canarian *Ischnura* was considered to be represented by only one species, namely *I. saharensis*, and consequently the monograph by Báez (1985) lists it as the only species of Zygoptera in the Archipelago.

In most of the Mediterranean area, *I. elegans* and *I. pumilio* occur in sympatry. Other species overlap locally: *I. elegans* and *I. graellsii* in Spain, *I. elegans* and *I. genei* in the islands of Elba and Giglio (Italy), and *I. graellsii* and *I. saharensis* in Morocco (Boudot et al., 2009). Additionally, *I. fontaineae*, *I. saharensis*

and *I. graellsii* overlap in Morocco, while *I. elegans*, *I. evansi*, *I. fontaineae*, *I. pumilio* and *I. senegalensis* overlap in the Middle East (Boudot et al., 2009). Given that *I. senegalensis* has been recorded from Mauritania (Boudot et al., 2009) and Cape Verde Islands (Aistleitner et al., 2008), its presence in the Canary Islands is possible due to the frequent winds coming from the desert. These winds have been invoked as the explanation for the only record of *Platycnemis subdilatata* for the Canaries (Kalkman & Smit, 2002).

In recent years, there has been an on-going discussion about the presence of *I. senegalensis* in the Canaries (Weihrauch, 2013), particularly because several pictures uploaded by amateurs to web servers suggested its presence. Nevertheless, scientific evidence needs voucher specimens (Corbet, 2000), because this is the only way to re-examine data in the light of future taxonomic knowledge. Our initial goal, therefore, was to obtain a sample of Canarian *I. saharensis* for molecular analyses, but we only found specimens which morphologically resembled *I. senegalensis*. The main purpose of this work was therefore to determine whether mtDNA sequencing would confirm that our *Ischnura* specimens were *I. senegalensis*. Additionally, we investigated the phylogenetic relationships of seven Mediterranean *Ischnura* species (*I. elegans*, *I. fontaineae*, *I. genei*, *I. graellsii*, *I. pumilio*, *I. saharensis* and *I. senegalensis*) and discuss our findings in the light of the recent review by Dumont (2013).

Material and methods

We collected five *Ischnura* samples on 20 V 2007 near San Andrés (Santa Cruz de Tenerife, Canary Islands) (28° 30' 51.26" N and 16° 11' 31.53" W). Three males and two gynochrome females, which we identified as

Table 1. Taxon sampling of *Ischnura* genus: * Data not available.

Tabla 1. Muestreo de taxones del género *Ischnura*: * Información no disponible.

Species	N samples	Locality	Country	Date	Collector
<i>I. asiatica</i>	3	*	Japan	2006	Yuma Takahasi
<i>I. elegans</i>	3	Kaiserslautern	Germany	2006	Jürgen Ott
<i>I. fontaineae</i>	1	Za	Morocco	2009	Sánchez-Guillén
<i>I. genei</i>	3	Coginas	Sardinia, Italy	2008	Sánchez-Guillén
<i>I. graellsii</i>	2	Ribeira de Cobres	Portugal	2005	Sánchez-Guillén
<i>I. graellsii</i>	2	Saïdia	Morocco	2009	Sánchez-Guillén
<i>I. saharensis</i>	3	Ouarzazate	Morocco	2007	Sánchez-Guillén
<i>I. senegalensis</i>	3	San Andrés, Tenerife	Canary Islands	2007	Sánchez-Guillén
<i>I. senegalensis</i>	3	Reservoir Van Bach	Namibia	2007	Sánchez-Guillén
<i>I. senegalensis</i>	2	*	Japan	2006	Yuma Takahasi
<i>I. pumilio</i>	1	Kaiserslautern	Germany	2006	Jürgen Ott
<i>I. pumilio</i>	1	São Miguel	Azores	2003	Cordero-Rivera

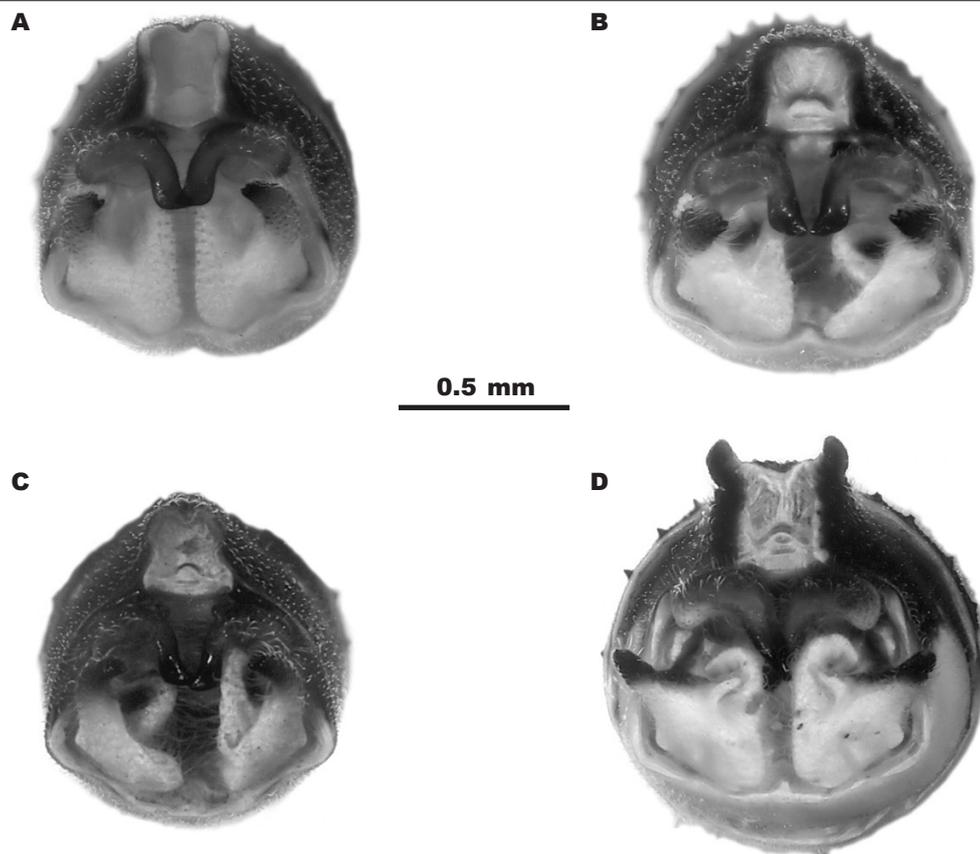


Fig. 1. Posterior view of the abdomen, showing the anal appendages, all at the same magnification. The specimens of *I. senegalensis* from Tenerife (young male, A; mature male, B) are indistinguishable from *I. senegalensis* from Namibia (C). All show the upper appendages in close contact, and the lower appendages convergent, findings that contrast with *I. saharensis* from Morocco (D), whose upper appendages cross, and whose lower appendages are divergent. Pictures taken with LAS software (Leica Microsystems).

Fig. 1. Vista posterior del abdomen donde se observan los apéndices anales. Todas las imágenes tienen el mismo aumento. Los especímenes de I. senegalensis de Tenerife (macho joven, A; macho maduro, B) son indistinguibles de los de I. senegalensis de Namibia (C). Todos presentan los apéndices superiores en estrecho contacto y los inferiores, convergentes, lo cual difiere de I. saharensis de Marruecos (D), cuyos apéndices superiores se cruzan y los inferiores, divergen. Imágenes tomadas con el programa informático LAS (Leica Microsystems).

Ischnura senegalensis, were captured and preserved in absolute ethanol for further DNA studies.

For the molecular identification, we combined two mitochondrial markers, cytochrome oxidase II and cytochrome b, because the use of several genetic markers improves species identification (Bergmann et al., 2013). DNA of three samples of putative *Ischnura senegalensis* from the Canary Islands, and one to three samples of each of the seven Mediterranean *Ischnura* species: *I. elegans*, *I. fountaineae*, *I. genei*, *I. graellsii*, *I. pumilio*, *I. saharensis*, and *I. senegalensis* was extracted from the thorax using a standard phenol/chloroform extraction protocol (Sambrook et al., 1989). Additionally, we included three samples of *I. asiatica* and one of *Enallagma basidens*, *E. cyathigerum*

and *Telebasis salva* as outgroups (Gene bank accession numbers: AF067669.1, AF067670.1, AF067681.1, AF067689.1, AF067690.1 and AF067701.1). Table 1 gives the localities of capture of these samples and the accession numbers. Extracted DNA was amplified by PCR for part of cytochrome oxidase II (673 bp), with the primers TL2–J–3037 and C2–N–3494 and C2–J–3400 and TK–N–3785 (Simon et al. 1994) (Gene bank accession numbers KC430114–KC430232), and cytochrome b (457 bp) with the primers CB–J–10933 and TS1–N–11683 (Simon et al., 1994) (Gene bank accession numbers KC430114–KC430232). The PCR program had an initial cycle of 95°C for 3 min, followed by 34 cycles at 95°C for 30 s, with annealing for 45s, an elongation phase at 72°C for 45s, and

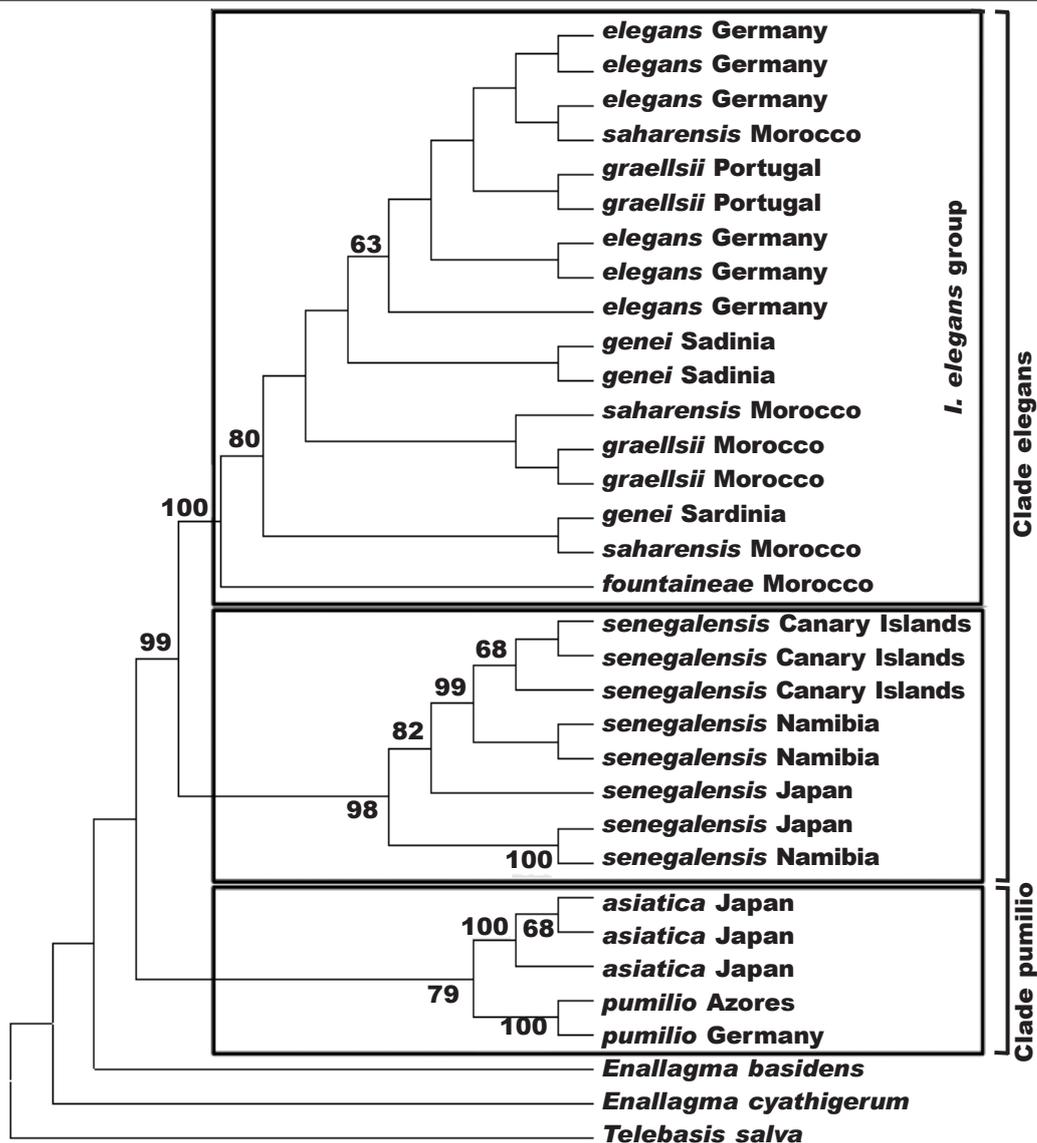


Fig. 2. Consensus neighbour-joining tree based on Kimura 2-parameters (K2P) with the rate of variation between sites being modelled with a gamma distribution (shape parameter = 1). Branches corresponding to partitions reproducing more than 50% of bootstrap replicates are shown.

Fig. 2. Árbol de consenso producido mediante el método del "vecino más cercano" (neighbour-joining) y basado en el modelo de Kimura con dos parámetros (K2P) donde la tasa de variación entre los sitios se define con una distribución gamma (parámetro de forma = 1). Se muestran las ramas correspondientes a las particiones que reproducen más del 50 % de las réplicas por remuestreo con reemplazamiento (bootstrap).

a final extension phase at 72°C for 10 min. It was performed in 10 µl and amplification conditions were as follows: 1–2 ng of DNA (2 µL), 5.0 µL of 2X Ready Mix™ PCR Master Mix (1.5 mM MgCl₂), 1µL 10× of BSA, 0.3 µL of MgCl₂ (50 mM), 1.1µL of distilled water, and 0.3 µL of each primer (10 pmol) in a 'GeneAmp PCR system 2700' thermocycler (Applied Biosystems). Bidirectional sequencing reactions were conducted using the Bigdye™ terminator cycle sequencing kit

(Applied Biosystems) using the automatic sequencer ABI3100. Forward and reverse sequences were edited and aligned with Clustal X (Thompson et al., 1997) implemented in MEGA version 6 (Tamura et al., 2013). Variable positions were revised by eye, and only high quality sequences were considered.

We generated a neighbour-joining tree using Mega version 6 (Tamura et al., 2013) by using the consensus sequence of mtDNA cytochrome oxidase II and

cytochrome b (n = 33 sequences, 668 informative positions). The tree was based on Kimura 2-parameters (K2P) (Kimura, 1980) with the rate of variation among sites being modelled with a gamma distribution (shape parameter = 1). The confidence probability of each interior branch was multiplied by 100.

Results

All five samples we collected were morphologically identified as *I. senegalensis*. Figure 1 shows the male anal appendages in the posterior view of an immature (A) and a mature male (B), both from Tenerife, and samples of *I. senegalensis* from Namibia (C) and *I. saharensis* from Morocco (D) for comparison.

For the DNA analysis, after deleting gaps and missing data, we obtained a total of 660 informative positions in the final dataset. The resulting neighbour joining tree (fig. 2) grouped our sample of *Ischnura* species in two main clusters. In the first cluster, one branch included all species of the *I. elegans* group and *I. fontaineae*. The second cluster includes all the samples of *I. senegalensis*, irrespective of their geographical origin. Therefore, our morphological identification was confirmed by the analyses of DNA sequences.

Discussion

The five Canarian samples were similar to the samples of *I. senegalensis* from Namibia although they were larger. The easiest way to distinguish between *I. saharensis* and *I. senegalensis* is the different orientation of lower appendages, which are clearly divergent in *saharensis* (as is typical of the *Ischnura elegans* group, to which it belongs; Dumont, 2013; see also below) and convergent in *senegalensis* (fig. 1).

The neighbour-joining tree (fig. 2) of the eight *Ischnura* species showed two main clusters, with all species of the *I. elegans* group (*I. elegans*, *I. genei*, *I. graellsii* and *I. saharensis*) and *I. fontaineae* in the first cluster. However, not all species formed monophyletic groups in our analysis. This is expected for taxa which have recently diverged, and is consistent with the hybridization processes between *I. elegans* and *I. graellsii* in Southern Europe (Sánchez-Guillén et al., 2011), *I. graellsii* and *I. saharensis* in Morocco (Sánchez-Guillén et al., 2014a), and *I. elegans* and *I. genei* in the Tyrrhenian Islands Elba and Giglio (Sánchez-Guillén et al., 2014a). The second group of the first cluster included all *I. senegalensis* samples, those from Namibia and Japan, and also from the Canary Islands. Finally, the second cluster included *I. pumilio* samples from the Azores and Germany, and *I. asiatica* from Japan. Our results are consistent with the tree constructed by Dumont (2013) using cytochrome oxidase I and ITS DNA fragments. *Ischnura pumilio* and *I. elegans* appear separated in the two main clades that form the genus, and *I. elegans*, *I. genei*, *I. graellsii*, and *I. saharensis* appear as closely related species.

In a recent paper, Peels (2014) presented photographs of *Ischnura* individuals from the Canary

Islands, morphologically identified as *I. senegalensis*. He also provided useful characters that allow the two *Ischnura* species found in the islands to be separated. Our research highlights the importance of voucher specimens in taxonomy and ecology (Corbet, 2000). Had no specimens been captured by Håkan Lindberg in 1949, studied by Valle (1955) and preserved in a museum, their identity would never have been confirmed. We agree that restraint should be exercised when collecting insects, or other animals, and that photography is preferable for identification purposes. This is indeed the safe approach for some species, particularly when the number of possible species is low (Peels, 2014). For instance, the first record of *Erythemis vesiculosa* for the Galapagos islands is based on an unpublished picture examined by expert taxonomists (Muddeman, 2007). Photo-identification is also possible for species that have a unique appearance, like *Ischnura nursei*, and Indian species recently found in the United Arab Emirates, but even in this case a voucher specimen was collected (Feulner & Judas, 2013). However, as photographs can not be used to extract DNA or to conduct a detailed study of morphology, they are of limited use in science. We do not know where *I. senegalensis* came from, although it likely colonized from northwestern Mauritania where the nearest populations of the species are found (Boudot et al., 2009). This question might be answered by a detailed genetic analysis, including samples from different geographical regions. Again, voucher specimens would be needed to do so.

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