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Rapid Communication

First report of the non-native webspinner *Embia* cf. *savignyi* Westwood, 1837 (Embioptera: Embiidae) in the Canary Islands with descriptions of its cytogenetic and morphological characteristics

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Abstract

Specimens of webspinners found on Fuerteventura Island (one of the Canary Islands) were provisionally identified as *Embia* cf. *savignyi* Westwood, 1837 (Embioptera: Embiidae). *Embia savignyi* is native to northeastern Africa and the population reported from the Canary Islands is introduced. The report provides location data, photographs of specimens, a description of cytogenetic characteristics, a DNA barcode, and a description of the morphological intrapopulation variability. A morphologically based identification key for Canary Island webspinners, composed of three alien species, is also provided.

Key words: Spain, introduced species, alien species, chromosomes, 18S rDNA, telomere

Introduction

Webspinners (Insecta: Embioptera) are small, elongate insects known for their ability to spin silk via glands in their specialized front legs. The insects use the silk to form domiciles or galleries on tree trunks, rock surfaces, soil, and other substrates (Ross 2000). Webspinners, which form an order that includes about 400 described species, are among the least studied insect groups (Miller 2009; Miller et al. 2012; Maehr et al. 2020). Webspinners usually live hidden in the soil or in crumbling wood (Ross 2000). Morphological identification is difficult, especially if adult males cannot be found, and a barcode library is not yet available. Because they occur in the soil, webspinners are easily transported with seedlings, potted plants, or other agricultural commodities (Ross 1966; Nozaki et al. 2018). The occurrence of non-native species is therefore not surprising, although they are rarely recorded because they are frequently overlooked and difficult to identify. Given that a single introduction may result in a population with a limited gene pool, such a population could over time become distinct from other populations (Ross 1966).

To date, only two species of webspinners have been recorded in the Canary Islands, and both are non-native and were introduced by human activities (Arechavaleta et al. 2010). Given the relatively rich fauna of Northwest Africa with habitats that are very similar to those on the Canary Islands (Ross 1966), it is surprising that no native species have been recorded on the islands. Natural spread to volcanic islands across the sea, however, is probably difficult for webspinners.

Webspinners usually feed on dead plant material and only rarely cause significant damage to plants (Ross 1970, 2000), although in new environments they could expand their food range and become pests (Ross 1970). *Oligotoma nigra* (Hagen, 1866), for example, was introduced from the Indian subcontinent (Ross 1966) to Israel, where it causes economic damage to avocado trees (*Persea americana* Miller, 1768) and mature fruits on peach trees (*Prunus persica* (L., 1753)); in one vineyard in Israel, this species of webspinner covered clusters of grapes with silk and excrement, and its feeding on grapes reduced their quality (Argaman and Mendel 1991). It follows that the introduction of any alien species of webspinner requires attention because the introduced species could switch to and damage new food sources. Webspinners that were thought to be insignificant and of no economic importance in the area of natural origin might become significant agricultural pests in areas where they are introduced (Argaman and Mendel 1991).

Here, we provide the report of the presence of the webspinner *Embia* cf. *savignyi* Westwood, 1837 in the Canary Islands. We also present photographs of the habitus of the species, information on its distribution, a key to the species of webspinners on the Canary Islands, a DNA barcode that will facilitate *Embia* cf. *savignyi* identification, and a description of the standard characteristics along with a description of its karyotype. We currently have available only basic information about the chromosomes of eight species of webspinners (see White 1976). Despite these limited data, the karyotypes seem to be well differentiated and useful for the taxonomic application.

Materials and methods

In 2019, one of the authors (F. Štáhlavský) detected a colony of webspinners composed of 20 to 30 nymphs under the bark of a dead pine tree at Costa Calma, Fuerteventura (Spain). Nymphs were collected, and a colony was reared in the laboratory to obtain adults for morphological identification. The colony was kept in 1 L plastic box in 22 °C, provided with crushed leaf litter and fed with ground grains (cereal), small amount of vegetables (leaf lettuce, cucumber) and flake fish food.

Morphological identification

Adult specimens were fixed and stored in 96% ethanol. For observation of morphological structures, specimens were soaked in 10% KOH at room

temperature for 1 h, washed with distilled water, observed, and then returned to 96% ethanol for storage. Observations and dissections were carried out with an Olympus SZX7 stereomicroscope. Body parts were dissected and slide-mounted in Euparal (BioQuip Products, Inc., CA, USA) and were subsequently observed and documented using an Olympus CX41 microscope equipped with a CANON D1000 camera. Micrographs of 100–130 different focal layers of the same specimen were combined using Quick Photo Camera 2.3 software. Measurements are presented as means ± standard deviation. All images were edited and assembled into plates in Adobe Photoshop CS6 Extended. Morphological identifications were conducted using the keys of Ross (1966, 2006) and Davis (1940), and by comparisons of studied specimens with the illustrations of all described *Embia* species published in Ross (1966, 2006) and Fontana (2001, 2002). The morphological terminology used here follows that of these studies. Voucher specimens are deposited in the collections of the National Museum in Prague, Czech Republic (NMPC) and University of Ostrava, Czech Republic, and the Department of Zoology at the Charles University in Prague, Czech Republic.

Laboratory methods

Total genomic DNA was extracted from abdominal muscle tissue or legs of adults using the QIAamp DNA Micro Kit (QIAGEN, Hilden, Germany) following the manufacturer's protocol. Cytochrome *c* oxidase was amplified by polymerase chain reaction (PCR) with the universal pair of primers LCO1490/HCO2198 (Folmer et al. 1994). Standard PCR was conducted in 20-µl reaction volumes containing 1 µl of DNA template, 0.4 µM of each primer, 5x MyTaq Red PCR buffer, 0.5 U/µl of MyTaq™ Red DNA polymerase (Bioline Reagents, London, UK), 0.6 mg/ml Bovine Serum Albumin (New England Biolabs), and distilled water. The PCR cycling profile was as follows: 2 min at 94 °C for initial denaturation; followed by 35 cycles of 15 s at 94 °C, 15 s at 51 °C, 15 s at 72 °C; and a final extension step at 72 °C for 6 min.

The amplified DNA was purified using the GenElute PCR Clean-up Kit (Sigma-Aldrich, St. Louis, MO, USA) following the manufacturer's protocol. Sanger sequencing reactions were performed using an ABI3730XL DNA Sequencer by Macrogen Europe (Amsterdam, The Netherlands). The chromatograms were visually checked and manually edited where appropriate using Chromas v2.6.4 software (Technelysium, Brisbane, Australia). Sequence is deposited in GenBank (GenBank accession number: MW495852).

The cytogenetic analysis was based on the chromosome preparations made from gonads of freshly dissected specimens according to Šťáhlavský et al. (2005). The tissues were hypotonized in 0.075 M KCl for 20 min and were fixed in methanol: acetic acid (3:1) for at least 20 min. Finally, the

chromosomes were stained with 5% Giemsa in Sörensen phosphate buffer for 20 min. The 18S rDNAs were detected by fluorescent *in situ* hybridization (FISH) with the biotin-labelled probe obtained from the genomic DNA of *Henschoutedenia flexivitta* (Walker, 1868) following Šťáhlavský et al. (2018). The (TTAGG)n probe was labelled with biotin and was obtained from Macrogen Europe (Amsterdam, The Netherlands). The protocol for the FISH procedure followed Šťáhlavský et al. (2021), and the signals were detected by streptavidine-Cy3 (Jackson ImmunoRes. Labs Inc.). Chromosomes were examined and documented with an Olympus AX70 Provis microscope using an Olympus DP72 camera. Measurements of the diploid set length and the descriptions of chromosome morphology were based on examination of six mitotic metaphases for both sexes using ImageJ v1.45r software (https://imagej.net/) with the Levan plugin (Sakamoto and Zacaro 2009) (Supplementary material Table S2).

Material studied

***Specimens of Embia* cf. *savignyi* examined from Fuerteventura.** SPAIN, Canary Islands: Fuerteventura, Costa Calma, 28.1378028N; 14.2451786W, January 2019, under the bark of dead pine *Pinus canariensis* C. Smith ex Buch, 1825 grown in the hotel garden, F. Šťáhlavský collected, 5♂♂; 5♀♀ deposited in the National Museum in Prague, Czech Republic (NMPC), 18♂♂; 23♀♀ deposited in collection of University of Ostrava and 6♂♂; 5♀♀ were used for cytogenetic analysis, collection of Charles University, Prague (Table S1).

Comparative material studied. *Embia savignyi* Westwood, 1837, Neotype ♂: "G.R.F. Medani, H.W.Bedford, 22.12.22, Attracted by light, Sudan Govt. / Blue Nile, A185 / Embia savignyi, det. Friederichs 1936 / Embioptera, *Embia savignyi* Westw., Neotype ♂, Selected Consett Davis"; coll. Natural History Museum, London, United Kingdom (BMNH).

Results and discussion

Embia savignyi Westwood, 1837 had been described from Egypt based on figures of Savigny, the original of which was a primary type of species (Westwood 1837; Davis 1940). However, because Savigny's figures lack certain essential details (especially details of the male terminalia), Davis (1940) defined a neotype from Sudan. To date, *E. savignyi* has been reported from Egypt, Sudan, Ethiopia, Cameroon, Nigeria, Benin, Greece: Crete, Jordan, and Palestine: Gaza (Davis 1940; Ross 1966, 2006; Dallai et al. 2007) (Figure 1). Even though *E. savignyi* is the first known species of the order, very few specimens have been collected (Ross 1966), and therefore the variability is not adequately known and described. *Embia savignyi* is native in the Ethiopian region and seems to be centred in southern Sudan and western Ethiopia (Ross 1966, 2006). It entered the Gulf

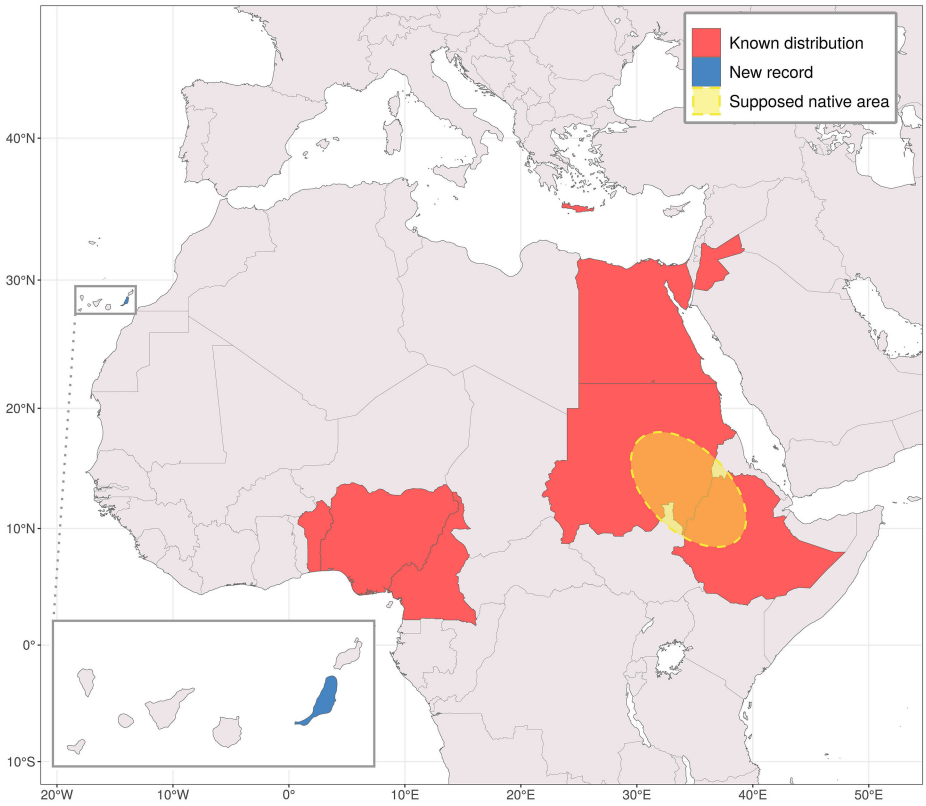

Figure 1. *Embia savignyi* Westwood, 1837 distribution: orange – supposed natural distribution of *E. savignyi*; red – known area of distribution; blue – studied non-native occurrence of *Embia* cf. *savignyi* on Fuerteventura Island (Canary Islands, Spain).

of Guinea and the Mediterranean region probably with the assistance of human commerce. Although this species has not been recognized as invasive at this time, the possibility that it affects other soil fauna cannot be ruled out.

Morphological identification and species concept of Embia savignyi

Specimens from Fuerteventura were identified by comparison of male terminalia with descriptions and illustrations in Ross (1966, 2006) and Fontana (2001, 2002), which contained all described *Embia* species. In the general shape of the head, the wing venation, and the basic morphology of the male terminalia, the specimens from Fuerteventura agree with the descriptions of *Embia savignyi* Westwood, 1837, published by Davis (1940) and Ross (1966, 2006), and with the studied neotype specimen from Sudan. There are, however, some minor differences in the shape of male terminalia not only between our specimens and the published descriptions, but also between the previously published descriptions (cf. Ross 1966, 2006 and Davis 1940). In addition, the descriptions by Ross (1966, 2006) do not fully agree with the neotype designated by Davis (1940), and the neotype specimen is imperfectly redrawn in the later publication (cf. Figure 2F and Figure 5 in Davis 1940). Shapes of male terminalia, especially the shape of the bend of processes of the right and the left hemitergites, seem to be variable. Specimens from Fuerteventura fall within the range of variability described and illustrated by Davis (1940) and it is close to the type of *Embia*

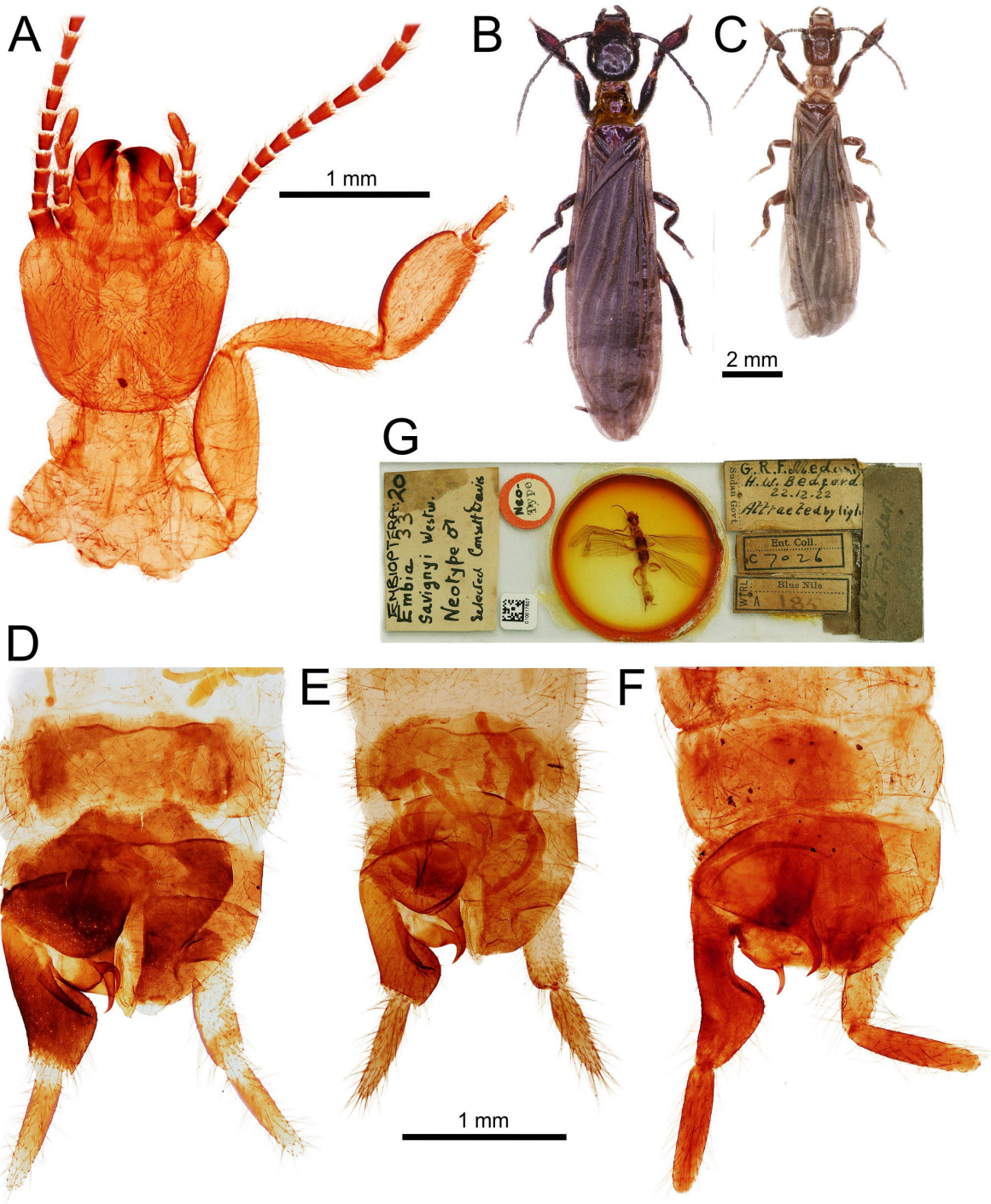

Figure 2. *Embia* cf. *savignyi* Westwood, 1837 (A–E) and neotype of *Embia savignyi* Westwood, 1837 (F, G): A, head and fore leg of *Embia* cf. *savignyi* male from Fuerteventura; B, black-colour form of male, length 13.5 mm; C, pale form of male, small specimen with length 9.8 mm; D, abdomen tip with terminalia of black form; E, abdomen tip with terminalia of pale form; F, abdomen tip with terminalia of *E. savignyi* neotype specimen from Sudan; G, microscopic slide with *E. savignyi* neotype male deposited in the Natural History Museum, London. Photo by Petr Kočárek.

socia Návas, 1929, which is in the synonymy with *E. savignyi* (Figures 13 and 15 in Davis 1940). According to Davis (1940), *E. savignyi* is quite variable, and it is possible that a species based on such a broad description of characteristics conceals additional cryptic species. Because the male terminalia of the specimens from Fuerteventura not fully agree with the studied neotype of *E. savignyi* (compare Figure 2D, E and F), we provisionally

refer to this population as *E.* cf. *savignyi*. Below, we describe and illustrate morphological characters important for future revision. We do not rule out that the broader concept of *E. savignyi* can hide a species complex, the taxonomy of which could be resolved by comparisons with material originating from the area of native distribution. Because of the described variability, the only way to distinguish the occurrence of interpopulation variability from the occurrence of different valid species is via molecular comparison. We found that specimens from the same colony were variable not only in size but also in basic coloration, which Ross (1966) considered an important identifying character (see below). Because no other population has been assessed for its DNA barcode, molecular comparison of populations from different parts of the range is currently impossible but should be conducted in the future.

Morphological description

Males. Body length 9.5–14.5 mm (11.92 ± 1.47 mm, n = 22). Males always winged. Generally light to blackish-brown in colour, with prothorax and pterothorax paler, legs brown; terminalia chestnut-brown with processes of left tergite and left paraproct dark amber, median flap whitish. Cranium broad, quadrate, sides subparallel; eyes small, interspace equal to four-five eye-widths; mandibles elongate, sides parallel, apical teeth strongly curled ventrad. Terminalia with left process very narrow, evenly tapered, abruptly curved to left at apical third; median flap narrow, longitudinally wrinkled, devoid of spiculation, acute; process of left paraproct very narrow, claw-like, resembling left tergal process, evenly arcuated ventrolaterad; ventral nodule of paraproct small, often conate; basal segment of left cercus broadly, obtusely rounded on inner side, not forming an abrupt lobe.

Females. Body length 12.5–16.0 mm (14.08 ± 1.24 mm, n = 28). Apterous, without distinctive specific characters, coloration similar to that of males, light-brown to blackish-brown.

Intrapopulation variability

Although morphological characters were stable across 22 males from the studied colony, there were significant differences in size and coloration. Ross (1966, 2006) described coloration of *E. savignyi* as light-brown or tan with head golden, prothorax paler than pterothorax, although Davis (1940) mentioned variability in basic colour from light-brown to dark-brown. The majority of *Embia* cf. *savignyi* specimens examined in the current study were blackish-brown and had an orange-red thorax (Figures 2 and 3), but some males and females (fewer than 5%) were light brown and has pale thoraxes (Figures 2 and 3). Therefore, the diversity of coloration within one colony suggests that colour is not useful for species determination; this is contrary to the claim of Ross (1966), who argued that *E. savignyi* males differ from males of all other Mediterranean species by having a pale coloration.

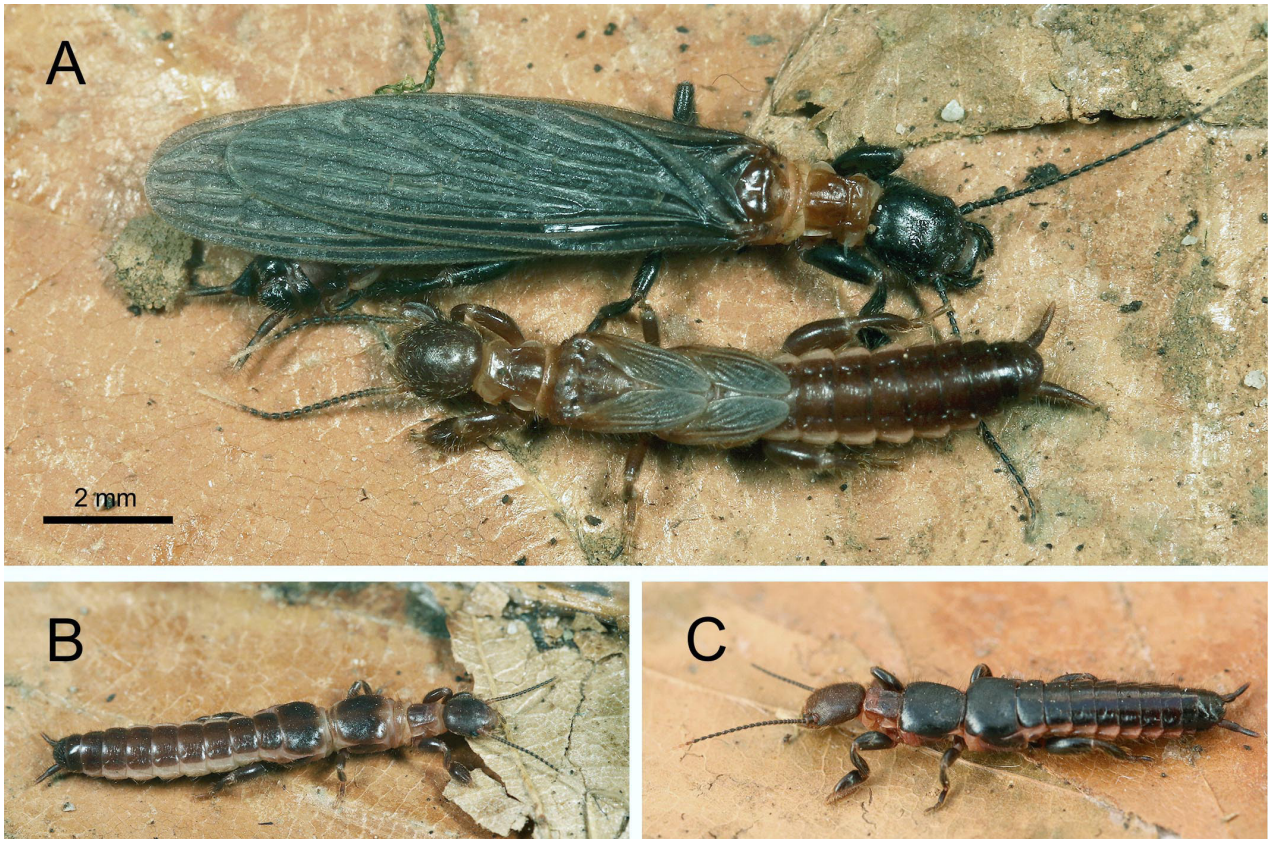

Figure 3. Habitus of live *Embia* cf. *savignyi* Westwood, 1837 from the culture that originated in Fuerteventura, Spain: A, black-colour form of adult male (above) and subadult male (below); B, adult female of pale-colour form; C, adult female of black-colour form. Photo by Petr Kočárek.

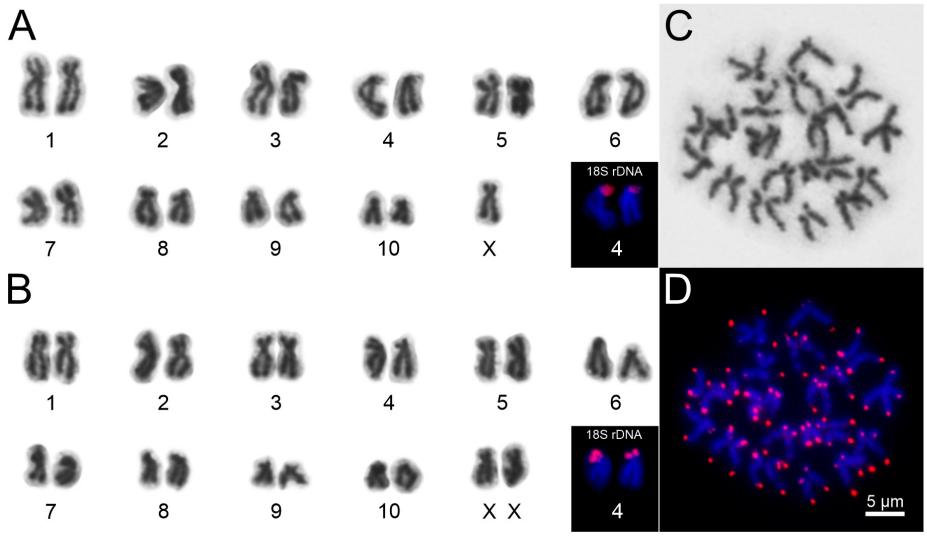

Figure 4. Chromosomes of *Embia* cf. *savignyi* Westwood, 1837: A, karyotype of the male based on mitotic metaphase (2n = 21); B, karyotype of the female based on mitotic metaphase (2n = 22); C, D, late mitotic metaphase of the male counterstained with Giemsa (C) and the same nuclei counterstained with DAPI after FISH with the (TTAGG)n telomere probe (red signal) (D). Insets show the chromosomes counterstained with DAPI after FISH with the 18S rDNA probe (red signal). Photo by František Šťáhlavský.

Cytogenetic characteristic

The diploid number of chromosomes is 21 in males and 22 in females (Figure 4). This number corresponds to the known range within Embioptera

(2n♂ = 19–23) (White 1976). The chromosomes of *Embia* cf. *savignyi* gradually decrease in length from 6.28% to 2.79% of the diploid set in males (from 5.64% to 3.03% in females). In contrast to other karyotyped species of webspinners, which mainly have metacentric chromosomes (White 1976), the males of *Embia* cf. *savignyi* have only three pairs of metacentric, one pair of submetacentric, and six pairs of subtelocentric autosomes. It should be noted that the arm ratios of some pairs are very close to the limits of specific morphological types of chromosomes, and that we identified small differences among the sexes (see Table S2).

The sex chromosome system in *Embia* cf. *savignyi* is X0. This system is also expected in all other species (White 1976). The sex chromosome X is proposed to be large and metacentric in previously examined species. The X chromosome of *Embia* cf. *savignyi*, in contrast, is subtelocentric (arm ratio 4.09) and is not very large (Figure 4). This indicates independent evolution and differentiation of the X chromosomes in webspinners. Application of FISH provides the first information about the number and position of major rDNA clusters in the Embioptera. We identified one pair of signals of 18S rDNA clusters that cover the whole short arm of chromosome pair number four. This low number of loci is the most frequent within the arthropods as well as in other animal groups (Sochorová et al. 2018). Application of the (TTAGG)n probe confirmed this telomeric motif in Embioptera. This telomeric motif is typical for all other polyneopteran orders apart from Dermaptera (Kuznetsova et al. 2020). We identified the (TTAGG)n signals entirely in the terminal positions of all chromosomes in *Embia* cf. *savignyi* (Figure 4).

List of Embioptera species recorded from the Canary Islands with distributional records:

Oligotomidae Enderlein, 1909

***Oligotoma saundersii* (Westwood, 1837)**
Distribution: Tenerife (Arechavaleta et al. 2010).

***Haploembia solieri* (Rambur, 1842)**
Distribution: Tenerife, Gran Canaria, Lanzarote, La Palma(?) (Arechavaleta et al. 2010).

Embiidae Burmeister, 1839

***Embia* cf. *savignyi* Westwood, 1837**
Distribution: Fuerteventura (this study).

Identification key to Embioptera found on the Canary Islands

1(2) Apterous species, only females (parthenogenetic populations) known from Canary Islands. Hind basitarus with two ventral papillae ....*Haploembia solieri* (Rambur, 1842)

2(1) Males alate, females apterous and morphologically indistinguishable between species. Hind basitarus with one ventral papilla.

3(4) Basal segment of male left cercus unlobed and not echinulated .... *Oligotoma saundersii* (Westwood, 1837)

4(3) Basal segment of male left cercus lobed and echinulated on inner surface.....*Embia* cf. *savignyi* Westwood, 1837

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Supplementary material

The following supplementary material is available for this article:

Table S1. Geo-referenced record data of *Embia* cf. *savignyi* presence in Fuerteventura, Spain.
Table S2. Chromosome relative lengths (%DSL) and arm ratio (AR).

This material is available as part of online article from:
http://www.reabic.net/journals/bir/2021/Supplements/BIR_2021_Kocarek_etal_SupplementaryMaterial.xlsx