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Description and biology of *Euborellia arcanum* sp. nov., an alien earwig occupying greenhouses in Germany and Austria (Dermaptera: Anisolabididae)

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Abstract

Greenhouses in botanical or zoological gardens are home to dozens of species of invertebrates that were introduced alongside plants or potting soil. Our study presents the description of an alien species of earwig, *Euborellia arcanum* **sp. nov.**, found in tropical greenhouses in Leipzig and Potsdam (Germany) and in Vienna (Austria), including information about its biology in breeding culture. The species was most likely introduced into Europe by way of plants or plant matter from Florida, but the region of its natural habitat is unknown. The sequence of the mitochondrial gene cytochrome c oxidase subunit I (COI) was also evaluated and added to GenBank as a DNA barcode for further identification.

Key words: Dermaptera, Anisolabididae, *Euborellia*, alien species, taxonomy, biology, nymphal development, description, new species, Germany, Austria, Europe

Introduction

Greenhouses in botanical or zoological gardens are home to dozens of invertebrate species that were introduced into the environment along with plants or potting soil. These invertebrates, which can survive for multiple generations, generally come from tropical or subtropical areas and often spread to other greenhouses through the transfer of plant material or soil (Weidner 1974).

There are approximately 2000 species grouped in eleven families of earwigs in the world (Kocarek *et al.* 2013). Only a few of them, however, are synanthropes. These are mostly of tropical origin and spread with goods transported by land, ship or air (Nishikawa & Kusui 2008). In Europe, four alien species have been identified as occurring in the wild and in greenhouses and other buildings with constant temperature. These are the oviparous species *Nala lividipes* (Dufour, 1828), *Euborellia annulata* (Fabricius, 1787) (syn. *E. stali* (Dohrn, 1864)), and *E. annulipes* (Lucas, 1847), and the ovoviviparous species *Marava arachidis* (Yersin, 1860) (Harz & Kaltenbach 1976, Kocarek 2009, Rasplus & Roques 2010, Kocarek *et al.* 2015). *Euborellia annulipes* has been imported into many countries and can now be considered one of the most widely distributed member of the Dermaptera (Koppenhöfer 1994).

During research on the invertebrate fauna of Gondwanaland in the Leipzig Zoo, an unknown species of *Euborellia* sp. was discovered, and a subsequent search of greenhouses in Germany and Austria resulted in the detection of the same earwig species in Biosphere Potsdam and in a tropical house in Schönbrunn (Vienna). A detailed study of the male genital armature confirmed that it was an undescribed species. We describe the species here, including information about its biology in breeding culture. The mitochondrial gene Cytochrome C Oxidase subunit I (COI) was evaluated and added to GenBank as a DNA barcode for further identification.

Material and methods

Nomenclature and morphological terminology follow those used by Steinmann (1989b). Specimens of the new

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species were dry-mounted, examined, and photographed with an Olympus SZ61 stereomicroscope (20–40× magnification) equipped with an Olympus E-410 camera. For each specimen, 10–15 layers of focus from micrographs were combined with Quick Photo Camera 2.3 software. Penultimate sternites were dissected and mounted with methylcellulose glue on the same card as the specimen. The genital armature of male specimens was mounted in dimethyl hydantoin formaldehyde resin (DMHF, a water-soluble mounting medium) on the same label. Specimens of the type series have been deposited in the following collections:

NMPC—Národní muzeum, Prague, Czech Republic; RMLE—PHYLLODROM e.V—Rainforest Museum Leipzig, Germany; ZSM—Zoologische Staatssammlung München, Germany; SMNS—Staatlichen Museums für Naturkunde Stuttgart, Germany; SNMD—Senckenberg Natural History Collections, Dresden Museum of Zoology, Germany; PKCO—Petr Kocarek collection, Ostrava, Czech Republic; DMCO—Danilo Matzke collection, Leipzig, Germany

Total genomic DNA was extracted from abdominal muscle tissue with the DNeasy Blood & Tissue isolation Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. To amplify the segment of mitochondrial protein-coding gene COI using polymerase chain reaction, the universal pair of primers LCO1490/HCO2198 was used (Folmer et al. 1994). PCR was conducted in a 25–30 µl reaction volume containing: 2–3 µl DNA template, 0.3 µM each primer, 0.15 mM dNTP, 0.7 units of Taq polymerase, distilled water, 10X PCR buffer and 2.5 mM of MgCl2. The PCR cycling profile was as follows: 2.5 min at 94°C for initial denaturation, followed by 40 cycles of 30 s at 90°C, 1 min at 48°C, 1 min at 72°C and the final extension step at 72°C for 10 min. PCR products were purified with GenElute PCR clean-up kit (Sigma-Aldrich, St. Louis, MO, USA). Sequencing reactions were carried out using the ABI3730XL DNA Sequencer at Macrogen Corporation (Amsterdam, The Netherlands). Sequences obtained were deposited in GenBank (GenBank accession number KP019208).

Specimens of *Euborellia arcanum* **sp. nov.** used to establish the breeding culture were caught in Gondwanaland Leipzig (Germany), Biosphere Potsdam and Tropical House in Vienna Schönbrunn (Austria) in 2012–2013. The collection included adults and nymphs of 3rd to 5th instars. The living specimens were initially kept in pairs in transparent plastic containers (170×100×90 mm). After eggs were laid, the male was removed. Females stayed with the eggs and nymphs until the moult to second instar. Females were subsequently removed, and the nymphs continued to develop independently.

As substrates, coconut powder and palm pulp were usually used in combination. Temperature was maintained at 23–26°C and humidity at 65–70%. Food for the adults and nymphs was provided in the form of bananas pieces and dried *Gammarus*. The differentiation of the individual nymphal stages was based on two methods. Firstly, exuviae from the freshly moulted nymphs was observed. Exuviae could usually be associated with a discrete specimen because they derived from tubes occupied by the single nymph (see Matzke & Klass 2005). This method is not very effective from the 1st to 3rd nymphal stages, however, because the nymphs consume their exuviae shortly after moulting. Later, during stages 4 and 5, the entire cuticle, including the mandible, hardens for a longer time (1–4 hours), and therefore by this age, the exuviae remained intact. In the second method, the nymphs were regularly measured (HW: maximal width of the head included the compound eyes; BL: total body length from top of head to the top of the cerci), and the antennomeres were counted (Table 1). Many elements of nymph behaviour were documented with a Ricoh R10 photo camera (Ricoh, Tokyo) and a Sony a3000 video camera (Sony, Tokyo).

Systematics

Euborellia arcanum Matzke & Kocarek sp. nov.

(Figures 1–14)

Material examined. Holotype ♂, Germany: Sachsen, Leipzig, Zoo – Gondwanaland, 26.III.2013, Danilo Matzke leg. (coll. NMPC). Paratypes: same label as the holotype, 1 ♀ (coll. NMPC); 1 ♂, 1 ♀ (coll. PKCO), 1 ♂, 1 ♀ (coll. DMCO), 1 ♂, 1 ♀ (coll. SNMD), 1 ♂ (coll. RMLE), 1 ♂ (coll. ZSM), 1 ♂ (coll. SMNS); Austria: Wien, Tiergarten, Schönbrunn, Regenwaldhaus, 20.VI.2013, Jonathan Neumann leg. 1 ♂ (coll. NMPC).

Description. Body blackish brown, shiny; antennae blackish brown with the exception of the typically 2–3 yellow antennomeres in the span of antennomeres12–15 (13–15 in holotype); legs yellowish; forceps reddish brown. Cuticle punctured, shiny; tegmina and wings entirely absent. Total body length without forceps: ♂♂: 14.9–17.6 mm, holotype 15.7 mm; ♀♀: 15.4–17.2 mm. Length of forceps: ♂♂: 3.1–3.9 mm, holotype 3.2 mm; ♀♀: 3.2–3.8 mm.

Male. Head (Fig. 1) same width as length; postfrontal and coronal sutures fine but distinct; frons convex; posterior margin of the head feebly emarginated at the middle. Eyes blackish brown, length approximately 0.75 times that of the head behind the eyes. Antennae with 21 antennomeres (in holotype antennae incomplete, with 19 antennomeres); antennomere 1 long, narrowed basally, widened terminally, as long as antennomeres 2–4 combined; antennomere 2 transverse, wider than long; antennomere 3 longer than antennomere 4. Antennomeres 2–6 cylindrical, other antennomeres conical. All antennomeres pubescent.

Pronotum (Fig. 1) smooth, a little longer than wide, parallel-sided; anterior margin nearly straight; lateral margins straight; posterior margin convex. Median sulcus fine but distinct. Meso- and metanotum transverse, wider than long, smooth, mesonotum posteriorly convex, metanotum broadly emarginate. Median sulcus visible on mesonotum and metanotum. Sternal plates typical for genus. Tegmina and wings entirely absent. Legs comparatively long, uniformly yellowish; femora stout; tibiae covered with thick and fine setae; length of metatarsomere 1 nearly equal to metatarsomeres 2 and 3 combined.

Abdomen (Fig. 1) sparsely punctate. Tergites (with the exception of the ultimate tergite) convex, somewhat widened posteriorly; lateral glandular folds visible on tergites 3–4. Tergites 7–8 with a low blunt lateral longitudinal ridge, not developed in some specimens. Ultimate tergite transverse, sides convex, slightly narrowed posteriorly, and slightly depressed medially, with a visible median longitudinal furrow; lateral longitudinal ridge rugose, posterior margin in middle slightly concave. Penultimate sternite narrowed posteriorly, with rounded, weakly emarginated posterior margin (Fig. 2). Pygidium flat. Male forceps asymmetrical, right branch more curved in distal fourth than left branch; both branches subcontiguous and stout at base, gradually tapering apically, nearly straight in basal two-thirds, followed by a slight curve inwards; apices gently hooked, inner margin finely crenulated, dorsally trigonal in basal half, depressed posteriorly.

Genitalia (Figs. 3–6) with short parameres, 1.2 times longer than wide, broadened in middle and narrowed apically, external margin convex, internal margin gently emarginated in basal fourth. Longer genital lobe, 1.8 times longer than length of paramere, with two apical, weakly sclerotized denticulated pads (Fig. 5). Virga very long, approximately 2.5 times longer than the entire body (in holotype 4.6 cm). Tip of the virga and seminal vesicle as seen in Fig. 6.

Female. (Figs. 7–9). Comparable to male in most characters except penultimate sternite is narrowed posteriorly, with posterior margin broadly rounded (Fig. 8); female forceps have simple and straight contiguous branches (Fig. 9).

Eggs. (Fig. 10). White, smooth, ellipsoid, 1.2 mm length and 0.95 mm width.

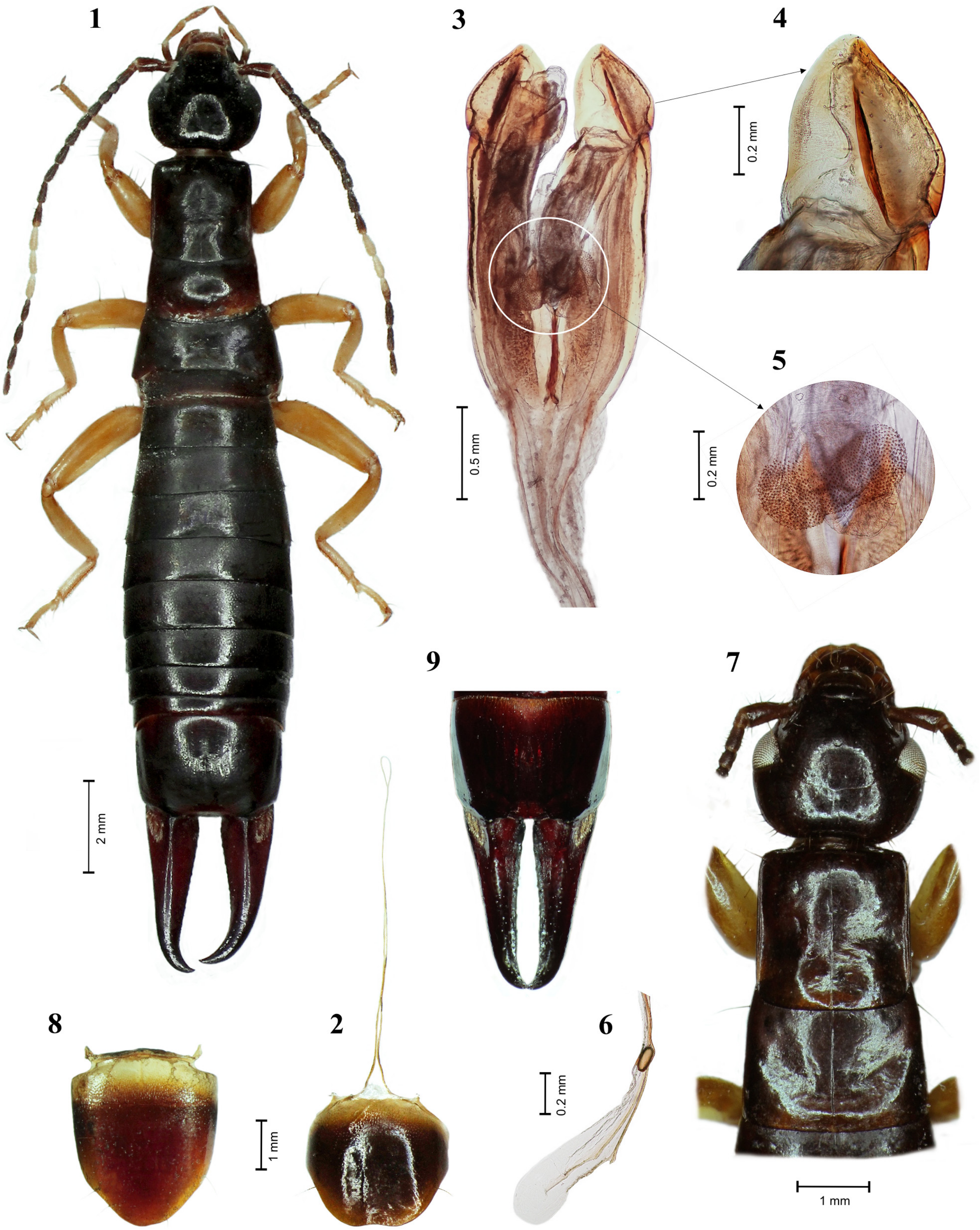
Nymphs. 1st instar (Figs. 11–15) light brown to brown, head darker and shiny, mandibular palps light to yellow-brown, antennae light brown except antennomere 7 white or yellowish, legs light brown to brown, femora dark-striped, last tergite and forceps reddish brown. Head as wide as long, postfrontal and coronal sutures distinct. Pronotum and metanotum dorsal to median sulcus. Nymphs of instars 2–5 with many characteristics of 1st instar nymph, but differin in body size and number of antennomeres (Table 1). In addition, the order of the white or yellowish antennomere differing: 2nd instar, antennomere 10; 3rd instar, 12 or 13; 4th instar, 13 or 14; 5th instar, 15 or 16.

TABLE 1. Measures and enumeration of *Euborellia arcanum* **sp. nov.** at individual nymphal stages.

Characters	Instar					
	1	2	3	4	5	Adult
Number of measured specimens	24	13	8	12	10	15
Head width HW [mm]	0.9	1.0	1.3	1.5–1.6	2.0	2.7
Body length BL [mm]	4–5	6–8	9–10	11–12	15–18	20–26
Number of antennomeres	8	12	15	17	19	19–21

Differential diagnosis. *Euborellia arcanum* **sp. nov.** is an entirely apterous species and differs from all other apterous species of *Euborellia* in the combination of the following characters: pronotum longer than wide, rectangular, with parallel sides; uniformly yellow legs; characteristic male genitalia with parameres short, slightly longer than broad, with convex external lateral margin and concave internal margin in basal fourth; genital lobe with two apical, weakly sclerotized denticulated pads. Nearctic species *Euborellia peregrina* (Mjöberg, 1904) and

Euborellia antoni (Dohrn, 1864) are similar but differ in shape of the pronotum (widening posteriorly) and shape of the male forceps and genitalia.



FIGURES 1–9. *Euborellia arcanum* **sp. nov. 1.** Habitus of male holotype. **2.** Male penultimate sternite, ventral view. **3.** Male genitalia. **4.** Right paramere of male genitalia. **5.** Detail of denticulated pads on the tip of genital lobe. **6.** Tip of virga with seminal vesicle. **7.** Female head and pronotum. **8.** Female penultimate sternite, ventral view. **9.** Ultimate tergite and forceps of female.

FIGURES 10–15. *Euborellia arcanum* **sp. nov. 10.** Female with clutch of eggs. **11.** 1st- instar nymph. **12.** 2nd-instar nymph. **13.** 3rd-instar nymph. **14.** 4th-instar nymph. **15.** 5th-instar nymph. All photographs from breeding culture.

Etymology. The name "*arcanum*" is derived from Latin, meaning "the mystery" or "the secret", and reflects an unknown country of origin as well as manner of introduction into Europe.

Distribution. Unknown. Occurrence in European greenhouses is alien.

Biology of *Euborellia arcanum* sp. nov.

Occupying habitats, burrowing behaviour. *E. arcanum* **sp. nov.** lives in underground galleries in tubes and chambers of varying lengths and shapes; burrowed under stones and trunks; or in moist soil. The species is active at night, when it leaves the underground galleries and searches for food on the soil surface. Nymphs of instars 2–5 show a similar pattern of burrowing behaviour as adults.

Mating, oviposition and hatching. Copulation outside the galleries has not been observed and appears to be confined to the underground galleries. Mating can occur immediately after moulting to the adult stage. Sperm from a copulation is stored in a receptaculum seminis, and the sperm of a single male can fertilize several clutches of eggs laid over time (see Günther & Herter 1974). Males of *E. arcanum* **sp. nov.** have anextraordinarily long virga with an extended tip, which is used in the Anisolabididae to remove rival sperm of the preceding copulation from the spermatheca (see Kamimura 2014 and Discussion). Approximately 16 days after copulation, females build a breeding chamber and begin laying eggs. Females lay 35 to 65 eggs in each batch. The eggs are regularly cleaned, and unfertilized or damaged eggs are eliminated (eaten) by the female. After hatching, females care for the nymphs for the next 8–12 days. From the moult up to the 2nd nymphal stage, nymphs leaving the breeding chamber and start to live independently from the mother. After 43–55 days, the female usually lays the next clutch of eggs. Exceptionally, as in one case, females may lay a 3rd clutch of eggs. In another case, a clutch contained 20–30 eggs, but no nymphs had hatched after 44 days.

Postembryonic development. The nymphs of *E. arcanum* **sp. nov.** undergo five developmental stages, wherein instars 1–3 are relatively short compared instars 4 and 5 (Table 2). In abreeding culture with temperatures of 23–26°C, postembryonic development, from hatch to adult moult, lasted 98–293 days (Table 2). The nymphs of the individual developmental stages are morphologically almost indistinguishable but can be separated by the size and number of antennal segments (Tab. 1). After becoming independent at the second developmental stage, nymphs build their own burrows and live individually inside the tubes. During development, nymphs are very agile and can also be observed outside the galleries during the day.

Feeding behaviour. In breeding culture, nymphs and adults fed on dried *Gammarus* sp., apples and banana pieces. It was observed that the food is not usually eaten immediately, but that the earwigs retracted a small piece of food into the tube and ate it there undisturbed. This behaviour is independent of trophic pressure from other nymphs because it was also observed in nymphs breeding in isolation. Cannibalism was rare, but the earwigs often fed on conspecific carcasses. In addition, cannibalism was observed at lower humidity (<65%) or under conditions of high population density in the container.

TABLE 2. Duration of the individual nymphal stages and duration of development from hatching to the adult moult (in days).

Origin	n	1	2	3	4	5	Total
Biosphere Potsdam	30	17–21	7–13	11–26	21–119	89–114	145–293
Gondwanaland, Leipzig	30	22–41	10–14	6–22	28–67	32–74	98–218

Discussion

Anisolabididae is a widespread and cosmopolitan family, containing predominantly wingless earwigs with dark and non-contrasting coloration. One specific characteristic of Anisolabididae is the male genital armature bearing two genital lobes, with one lobe directed distally and the second directed basally (Steinmann 1989b, Srivastava 1999). Based on recent phylogenetic studies, the family appears to be monophyletic (Haas 1995, Jarvis *et al.* 2005, Tworzydlo *et al.* 2010, Kocarek *et al.* 2013), but these authors included only a few species from the Anisolabidinae in their studies. Anisolabididae is subdivided into 13 subfamilies (*sensu* Srivastava 1999) and comprises approximately 400 species in 31 genera (Steinmann 1989; Srivastava 1999). Subfamily Anisolabidinae, to which the described species belongs, comprises 277 species in 13 genera (Anisyutkin 1998a,b, 2004, Srivastava 1990, 1993a, 1999, 2003a,b, Nishikawa 2008, Kocarek 2011a,b). The species are difficult to distinguish based on external morphology because of the uniform habitus and relatively large variability (Steinmann 1989b). The generic classification is based exclusively on the shape of the male genitalia, particularly the shape and size of the parameres (Srivastava 1999). *Euborellia* (Burr, 1910) is a widespread cosmopolitan genus encompassing primarily small earwigs that are usually dark with non-contrasting coloration. This genus currently has 54 identified species, of which approximately 10 are Palearctic (Steinmann 1989a,b, Anisyutkin 1998a, Srivastava 1993b, 1999, 2003b, Kocarek 2011a, b). *Euborellia annulipes* (Lucas, 1847) is the only cosmopolitan species.

Current knowledge of anisolabidine biology is based on only a few species studied in breeding culture, such as *Anisolabis maritima* (Bonelli, 1832), *A. littorea* (White, 1846), *Euborellia cincticollis* (Gerstaecker, 1883), *E. plebeja* (Dohrn, 1863), *E. annulata* (Fabricius, 1793), and *E. annulipes* (Lucas, 1847) (Klostermeyer 1942, Giles 1953, Herter 1960, Knabke & Grigarick 1971, Baijal & Srivastava 1974, Matzke & Klass 2005, Nonci 2005). All species studied, including *E. arcanum* **sp. nov.,** pass through five developmental stages and show similar patterns of biology. However, the feeding behaviour observed in this study, in which food is retracted into an underground tube, is specific to *E. arcanum* **sp. nov.**

Males of *E. arcanum* **sp. nov.** have an extraordinarily long virga, more than 2.5 times longer than the entire body and likely one of the longest among all earwigs (see Kamimura 2014). Conspicuously elongated genitalia are found sporadically in Spongiphoridae and Anisolabididae and are used for the removal of rival sperm from the preceding copulation in the spermatheca (Kamimura 2014). The mechanism of sperm removal and the significance of such behaviour had been studied in *E. plebeja* (Dohrn, 1863) and *Mongolabis brunneri* (Dohrn, 1864). The males of these species insert the virga into the spermatheca without ejaculating and then extract the rival sperm using a fringe-like projection on the virgal tip while simultaneously ejaculating semen (Kamimura 2000, 2014). Both species studied are highly promiscuous, and, due to multiple matings, their offspring show mixed paternity (Kamimura 2005, van Lieshout & Elgar 2011).

By commerce, earwigs are frequently imported into Europe via plants and goods, as documented by Weidner (1974), who studied the importation of Dermaptera into Hamburg. The spread between botanical gardens and other greenhouses is likely facilitated by the trade and exchange of seedlings and other plant material. Thigmotactic and sciophilous earwigs usually live in potting soil, and their eggs are laid in wet soil. In Europe, alien earwigs in human habitats have been documented in Germany, United Kingdom, the Netherlands, Sweden, and Ukraine (Harz & Kaltenbach 1976, Brindle 1977, Joost & Klausnitzer 1986, Wallaschek 1998, Borisch 2002, Heller & Haas 2013). The most frequently observed synanthropic earwig is *E. annulipes* (Kocarek *et al.* 2015).

According to tropical greenhouse employees, the majority of the plants in our study were imported from Florida. This was particularly true of the Biosphere Potsdam where almost all the plants originated in Florida (J. Neumann, pers. comm.). We can therefore assume that *E. arcanum* was introduced to Europe along with plant material from South Florida´s greenhouses or nurseries. Because the only native species of *Euborellia* in Florida seem to be *E. ambigua* (Borelli, 1906) (Hebard 1921, Steinmann 1989a, 1989b), the source population is probably also alien, and the region of origin remains undetermined. The most probable region of origin could be Neotropical, due to the distribution of the morphologically similar and likely related species *E. peregrina*. In the past, two species of *Euborellia*, *E. janeirensis* (Dohrn, 1864) and *E. peregrina*, were accidentally transported with plant material to Europe from South America, but these species did not become established (Weidner 1974).

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