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The worrying arrival of the invasive Asian needle ant *Brachyponera chinensis* in Europe (Hymenoptera: Formicidae)

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Invasive alien species are considered one of the main causes of the current biodiversity crisis (Pyšek *et al.* 2020). At least 200 ant species have been introduced outside their natural range by humans and some of them have become invasive (Wong *et al.* 2021). The Asian needle ant, *Brachyponera chinensis* (Emery, 1895), until recently included under the genus *Pachycondyla* genus (see Schmidt & Shattuck 2014), has undergone an impressive range expansion in the last 80 years thanks to human-mediated introductions (Guénard *et al.* 2018). Its Asian range at present includes coastal regions of mainland China, Taiwan, the Korean peninsula and Japan, while its introduced range includes the USA where it has been established at least since the 1930's and is now present in 17 states. More recently, *B. chinensis* was recorded in Russia and Georgia along the eastern coast of the Black Sea (Guénard *et al.* 2018). The fate of an invasive population in northern New Zealand is currently unknown (Brown 1958).

While most successful tramp and invasive ants are generalist species belonging to the Formicinae, Dolichoderinae and Myrmicinae subfamilies, *B. chinensis* belongs to the subfamily Ponerinae. As most ponerines, and unlike typical invasive ants, it is mainly a predatory species with a preference for termites, although in its invasive range it has broadened its diet to include other invertebrate preys (Bednar & Silverman 2011; Suehiro *et al.* 2017). In the USA, *B. chinensis* invades native old forest habitats and had a strong negative effect on most native ant species (Guénard & Dunn 2010; Suehiro *et al.* 2017). Ecological cascade effects of its activity have been documented and include, at least, the disruption of ant-seed dispersal mutualisms (Rodriguez-Cabal *et al.* 2012). Furthermore, due to its functional sting and the properties of the venom it injects, this species has been identified as an emerging public health threat in the USA, causing significant allergic reactions (Guénard *et al.* 2018; Nelder *et al.* 2006).

Brachyponera chinensis is considered a taxonomically challenging species, and past confusion over its distinction from congeneric species still hinders a full understanding of its distribution in Asia (Guénard *et al.* 2018). It is considered to be part of an unresolved species complex, characterized by high intraspecific morphological variation (Yamane 2007; Yashiro *et al.* 2010). The revision carried out by Yashiro *et al.* (2010), mainly based on Japanese and Taiwanese material, distinguished three species using molecular and morphological data: *B. chinensis*, *B. nakasujii* Yashiro *et al.*, 2010, and *B. luteipes* (Mayr, 1862), whose ranges considerably overlap.

Regarding Europe, the only existing record is a reported interception in Hamburg (Germany), on plants shipped from Japan, which dates back to the year 1900 (reported as *Ponera solitaria* by Forel (1900)). However, given the taxonomic confusion reigning at that time, the identity of the species recorded by Forel (1900) is uncertain.

In this paper, we present the first confirmed record of *B. chinensis* for Europe, initially suggested by morphological characteristics, and then confirmed by genetic analysis (DNA barcoding). A single specimen of an unidentified male belonging to the genus *Brachyponera* was collected while attracted to a streetlight on the 3rd of July 2020 by one of the authors (VG) in Torre Annunziata (Naples, Italy), in a residential area about 1 km from the town harbour, at the coordinates 40.758824, 14.434152 (uncertainty ±10 m, 32 m a.s.l.). The specimen is stored at the Butterfly Diversity and Evolution Lab, Institute of Evolutionary Biology (CSIC-UPF) (Barcelona, Spain) under the voucher code MM21B056a1.

A first morphological investigation revealed that the specimen differed from any native Euro-Mediterranean ponerine genera and matched *Brachyponera* instead by features such as the ventral cuticular processes of the metasternum and

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petiole (Figure 1c). Moreover, the male specimen corresponded to *B. chinensis* by its light coloration and mandible shape (Yashiro *et al.* 2010). However, like in most ant genera, *Brachyponera* taxonomy is mostly based on workers, with detailed male-based keys missing and males are undescribed for at least half of its 23 described species (Bolton 2022). As a result, an accurate and definitive identification on the basis of morphological features only was not possible.

Four legs of the specimen were removed for genetic analysis and sent to the International Barcode of Life project (iBOL) (deWaard *et al.* 2008). A DNA-barcoding (mitochondrial gene cytochrome c oxidase subunit I, *COI*) sequence of 658 bp was obtained using the primers LepF1 and LepR1 (deWaard *et al.* 2008) and submitted to GenBank (accession number OM604749). For the genetic identification, we retrieved a total of 17 sequences of five *Brachyponera* species from GenBank: *B. chinensis* (accession numbers GQ264540, GQ264547, GQ264550, GQ264554, GQ264561, GQ264562, GQ264565, GQ264566, GQ264570 from Yashiro *et al.* 2010 and MT800254, MT800255, MT800256 from Park *et al.* 2020), *Brachyponera luteipes* (Mayr, 1862) (a.n. GQ264575, GQ264582, GQ264583 from Yashiro *et al.* 2010), *B. nakasujii* (a.n. GQ264583 from Yashiro *et al.* 2010), *Brachyponera nigrita* (Emery, 1895) (a.n. GQ264596 from Yashiro *et al.* 2010) and *Brachyponera obscurans* (Walker, 1859) (a.n. EF609925 from Smith & Fisher 2009).

Sequences were edited and aligned with Geneious 2020.2.4 (www.geneious.com) and a neighbour-joining tree was built with the same program, using *Ectomomyrmex javanus* Mayr, 1867 as outgroup (a.n. GQ264573 from Yashiro *et al.* 2010). A haplotype network of *B. chinensis* sequences was built with the program TCS 1.21 (Clement *et al.* 2002) and later edited with tcsBU (Múrias dos Santos *et al.* 2016) and Adobe Illustrator CC 2019. The genetic analysis confirmed the single male as *B. chinensis* with high support (99% bootstrap value) (Figure 1d). The *COI* haplotype found in Italy is the same previously reported in the USA, suggesting a possible origin from this country or a common source of introduction (Figure 1e).

Besides the potential problematics related to the use of barcoding for ant species delimitation (e.g. Schifani *et al.* 2021) and identification (e.g. Blatrix *et al.* 2020), here this technique helped us determining a potentially established population of the highly invasive species *B. chinensis*, based on the taxonomic framework by Yashiro *et al.* (2010).

As many other soil invertebrates, ants are often accidentally introduced in new environments due to the ongoing globalization and in particular to the plant trade (Pyšek *et al.* 2020). Their frequent introduction in private gardens or greenhouses can facilitate their recording when the species are particularly charismatic and easy to spot (e.g. through citizen science, Mori *et al.* 2021), but it can also make the field surveys harder to be carried out. The latter one is probably the case for *B. chinensis* in Torre Annunziata (Naples, Italy): an area of 300 m of radius around the site where the specimen was found, as well as the area of the harbour, have been surveyed multiple times from 2016 to 2021 and no other individual belonging to this species have been found. More surveys, perhaps employing specific traps, will be useful to further verify the presence of nests. The fact that we collected a swarming male points to three potential concerns: a) At least one nest is already in an advanced stage and the species may have already overcome the typical "lag phase" after introduction and during establishment, preceding the increase-phase marking spread (Pyšek *et al.* 2020). This is problematic considering that the high inbreeding tolerance of *B. chinensis* may allow a few foundresses to give birth to an entire population (Eyer *et al.* 2018). b) Since the dispersal capabilities of *B. chinensis* are undocumented, and it may disperse by independent foundations, including through pleometrosis (Benoit Guénard pers. comm.), it is hard to locate the nest of origin and eradicate it. In fact, the nest of origin may be in hardly accessible private areas and the colony (or colonies) may have time to spread while remaining undetected. c) A queenless colony may have been introduced and males were produced by the workers, capable of laying haploid eggs in the *B. chinensis* complex (Gotoh & Ito 2008).

The Mediterranean basin hosts an increasing number of exotic species, but most of them have remained limited to indoor or at least urban environments (Schifani 2019). Climate change may also play a significant role for the establishment of *B. chinensis* in Italy (Bertelsmeier *et al.* 2013). However, the success of *B. chinensis* in North American forest habitats suggests that this species could potentially invade European natural habitats and particularly forests, likely affecting native communities. On the other hand, its establishment in urban areas may constitute a public health concern (Nelder *et al.* 2006). We call for action at the presumably early stage of this new potentially harmful biological invasion and propose that a detailed survey of a wide area around Torre Annunziata is conducted.

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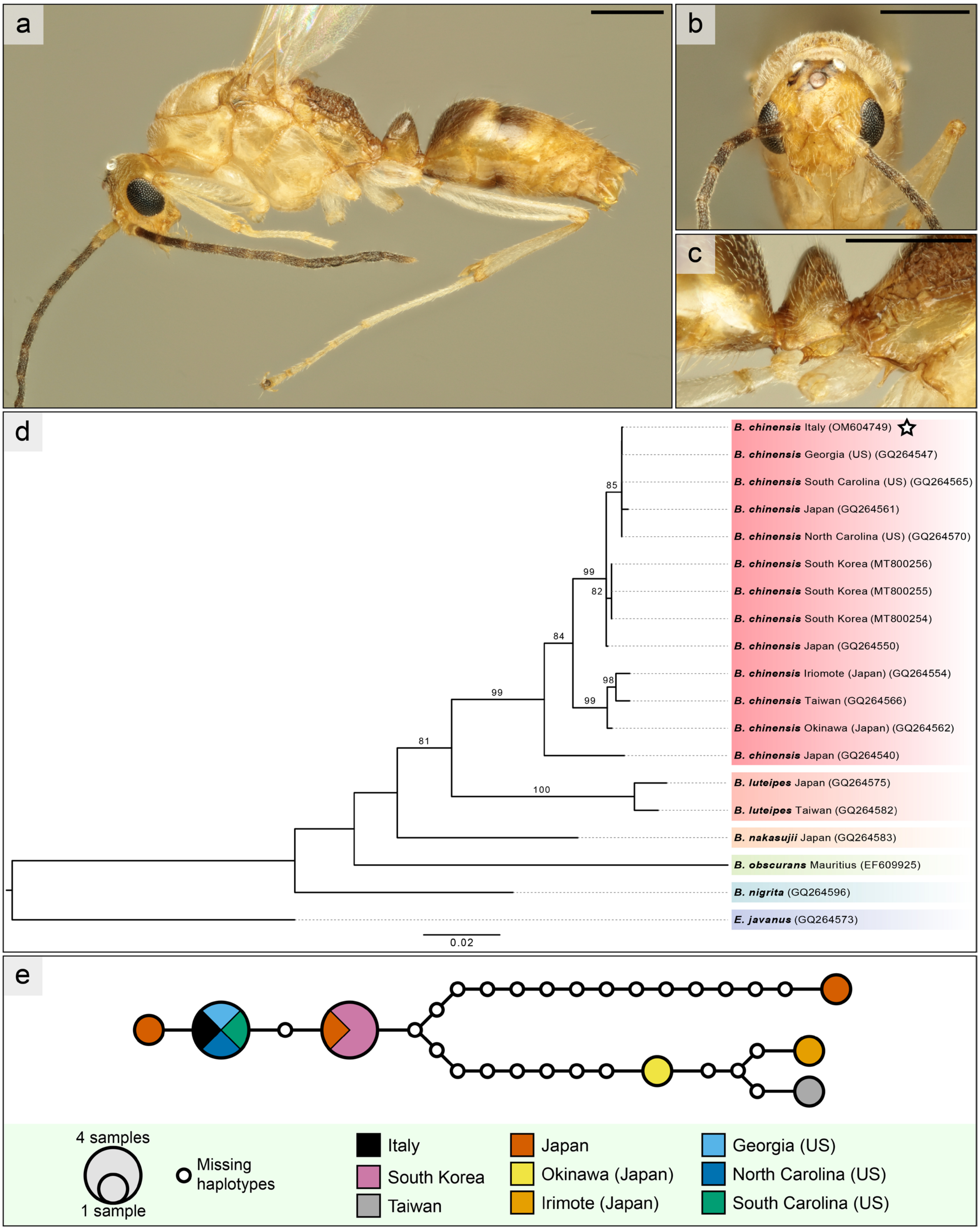

FIGURE 1. Lateral view (a), frontal view of the head (b) and a close-up lateral view of the petiole (c) of the specimen of *Brachyponera chinensis* collected and sequenced. d) Neighbour-joining tree based on mitochondrial *COI* sequences, the new record is indicated with a star, bootstrap values above 75 are shown on the branches and the species names, countries and GenBank accession numbers are indicated in the tip labels; e) Haplotype network of *B. chinensis COI* sequences, colours indicate the sample origin and sizes of the circles the number of samples.

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