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First record of *Thaumastocoris peregrinus* in Portugal and of the neotropical predator *Hemerobius bolivari* in Europe

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Abstract

The *Eucalyptus* pest *Thaumastocoris peregrinus* Carpintero et Dellape, (Hemiptera Thaumastocoridae) was found in a *Eucalyptus* arboretum in Lisbon, Portugal, in April 2012. This is the first report for this species in Western Europe. Separate surveys were conducted to assess the geographical distribution, host plant susceptibility and natural enemies of *T. peregrinus*. To ascertain the geographical distribution of *T. peregrinus* surveys were conducted between May and June 2012 at 53 sites in central and southern Portugal. *T. peregrinus* was present in only three sites, which were all located in Lisbon and surrounding areas suggesting an introduction pathway through the harbors or the airport in this coastal city. Of the 30 *Eucalyptus* species present in Lisbon's *Eucalyptus* arboretum, 14 were confirmed as infested by *T. peregrinus* during the first survey in April 2012. In August 2012 the host range had increased to 19 *Eucalyptus* species, revealing an expansion phase. We report the first record of *Hemerobius bolivari* Banks (Neuroptera Hemerobiidae), a native of South America, preying on *T. peregrinus* nymphs. This is the first record of *H. bolivari* in Europe and we hypothesized that this predator may have reached Europe together with its prey.

Key words: biological invasion, bronze bug, *Eucalyptus*, sentinel forest, *Hemerobius bolivari*.

Introduction

Eucalyptus species have been planted worldwide and represent 10% of all planted forests (FAO, 2001). A large part of the *Eucalyptus* plantations are concentrated in South America (Brazil and Chile) and Asia. During recent decades an increasing number of eucalypt pests, originating in Australia, have been recorded to threaten eucalypt plantations worldwide (Paine *et al.*, 2011). As a result, severe reductions in productivity of eucalyptus plantations for commercial purposes have been estimated as, for example, in the case of *Gonipterus platensis* Marelli infestations (Reis *et al.*, 2012).

A recent survey of pests on eucalypts growing in the arboretum of the Instituto Superior de Agronomia (ISA), Lisbon, Portugal, in March 2012, revealed a new hemipteran pest attacking *Eucalyptus camaldulensis* Dehnh. This insect was identified as *Thaumastocoris peregrinus* Carpintero et Dellape (Hemiptera Thaumastocoridae).

T. peregrinus, native to Australia, is commonly known as the bronze bug. This small, sap-feeding insect has a broad range of hosts all belonging to Myrtaceae (Noack *et al.*, 2011). Outside its native range *T. peregrinus* was found in South Africa in 2003 (Jacobs and Neser, 2005), Argentina in 2005 (Noack and Coviella, 2006), Uruguay and Brazil in 2008 (Martínez and Bianchi, 2010; Wilcken *et al.*, 2010), Chile in 2009 (Ide *et al.*, 2011), Malawi, Kenya and Zimbabwe (Hurley *et al.*, 2011), and, more recently, in Italy in 2011 (Laudonia and Sasso, 2012) and New Zealand in 2012 (Sopow and Bader, 2012).

This study aimed to: i) analyze the insect's geographical distribution in Portugal and deduct possible introduction pathways; ii) analyze its host range and implications for its economic impact and iii) analyze possible natural enemies and their potential for biological control.

Materials and methods

Identification of *T. peregrinus*

Several adult specimens of the insect were collected from *E. camaldulensis* trees growing in ISA's arboretum and brought to the laboratory for identification based on adult morphology (under stereoscopic microscope, 40x) and genitalia (microscope observation at 100x). The genitalia was diaphanized in 10% KOH for 48 h at room temperature conditions, and mounted in Hoyer's medium on glass microscope slides for examination and photography under microscope. Identification to family was made using the key provided in Schuh and Slater (1995). Identification at species level was established using the key published by Noack *et al.* (2011) and was based primarily on male genitalia characters.

Susceptibility of *Eucalyptus* species

Based on the presence or absence of eggs, nymphs and adults on the leaves, susceptibility to *T. peregrinus* was compared among 30 *Eucalyptus* species planted in the ISA's arboretum in 2007 (table 1).

Four trees, about 3-5 m high, from each eucalypt species were surveyed in both spring (19 April) and summer (6 August) of 2012. From each of the selected trees, four branches, at each cardinal direction (N, S, E and W) from the crown and at the observer height (1.5-1.8 m), were examined using a head-mounted magnifying glass (4.8x magnification). Sampled branches were checked thoroughly for the presence of eggs, nymphs and adults of *T. peregrinus*. Host species susceptibility was classified by the incidence of *T. peregrinus*, at all life stages, and based on the following criteria: i) not susceptible, if not present; ii) low incidence level, whenever present in less than 25% of the leaves; and iii) high incidence level when present in more than 25% of the leaves.

Survey of natural enemies

From May to June 2012, surveys were conducted weekly to evaluate the presence of natural enemies of *T. peregrinus*. To this end, four trees of *E. camaldulensis*, the most highly infested tree species in the arboretum, were inspected visually. From each tree, four selected branches were thoroughly searched in order to find predators of *T. peregrinus.* Any possible predator collected at the larval stage was brought to the laboratory and fed with nymphs and adults of *T. peregrinus* until they reached adult stage. Posterior identification was made based on adult traits.

Geographical distribution of *T. peregrinus*

In order to estimate the geographical distribution of *T. peregrinus* in Portugal, a survey comprising of 53 sites, located in the central and southern areas of the country (an area of approximately 800 km^2), was undertaken (figure 1). This survey was conducted between May and June 2012. Sample sites were selected from available eucalypt plantations and trees present along roads, which mainly consisted of *E. camaldulensis* and *E. globulus*. At each site, four trees were evaluated for the presence of *T. peregrinus* using the methodology described above for the susceptibility evaluation.

Results

Identification of *T. peregrinus*

The following gross morphological characters of the adult specimens of this insect resulted in its identification as *Thaumastocoris* as described by Noack *et al.* (2011): "small, between 2-3mm in length; body strongly dorsoventrally compressed and elongate; eyes pedicellate; conspicuous elongate mandibular plates; conspicuous tibial teeth; and elongate *fossula spongiosa*" see figure 2A, 2B. *Thaumastocoris* species are difficult to identify to species level because the differences in their gross morphology are subtle; therefore we further used male genitalia characteristics (figure 3) to confirm species identification following genitalia description by Noack *et al.* (2011).

Table 1. *Eucalyptus* species surveyed in the Lisbon arboretum with absence (−), low infestation, i.e. less than 25% of leaves with presence (+) or high infestations, i.e. more than 25% of leaves with presence (++) of *T. peregrinus* in August 2012.

Eucalyptus species	Incidence
E. camaldulensis Dehnh.	++
E. pauciflora Sieber ex Sprengel	++
E. viminalis Labill.	++
Corymbia citriodora (Hook.)K.D.Hill et L.A.S.Johnson	+
C. maculata (Hook.)K.D.Hill et L.A.S.Johnson	+
E. botryoides Sm.	+
E. cypellocarpa L.A.S.Johnson	+
E. globulus Labill.	+
E. grandis W. Hill ex Maiden	+
E. macarthurii Deane et Maiden	+
E. nitens (Deane et Maiden) Maiden	+
E. occidentalis Endl.	+
E. ovata Labill.	+
E. pilularis Sm.	+
E. pulverulenta Sims	+
E. resinifera Sm.	+
E. rudis Endl.	+
E. saligna Sm.	+
E. tereticornis Sm.	+
E. cinerea F. Muell. ex Benth.	−
E. crebra F.Muell.	−
E. goniocalyx F.Muell. ex Miq.	−
E. meliodora A.Cunn. ex Schauer	−
E. nicholii Maiden et Blakely	−
E. perriniana R. Baker et H.G. Smith	−
E. polyanthemos Schauer	−
E. propinqua Deane et Maiden	−
E. regnans F. Muell.	−
E. robusta Sm.	−
E. sideroxylon A. Cunn. ex Woolls	−

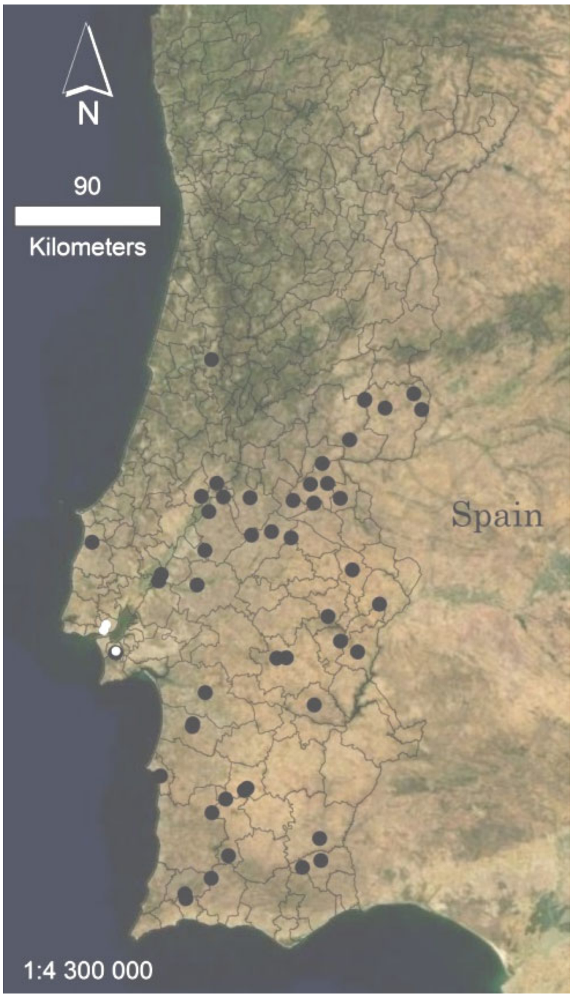

Figure 1. Sites surveyed for detecting the presence of *T. peregrinus* in Portugal. Circles in black and white represent the absence or presence of *T. peregrinus*, respectively.

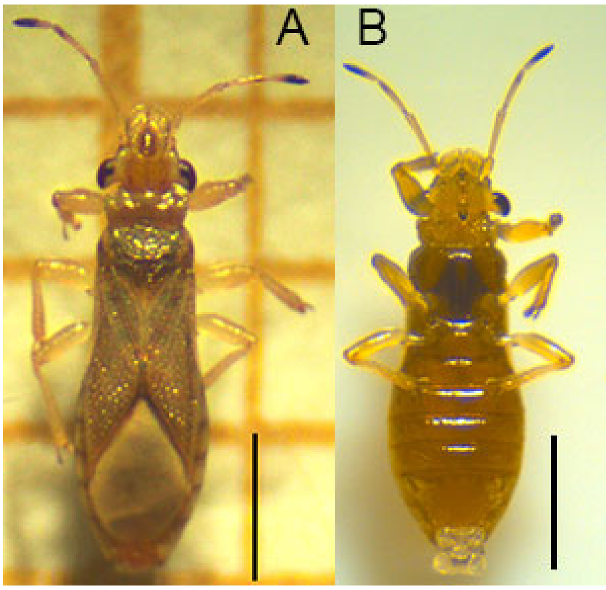

Figure 2. *T. peregrinus* adult female: A: dorsal side; B: ventral side. Scale bar 1 mm.
(In colour at www.bulletinofinsectology.org)

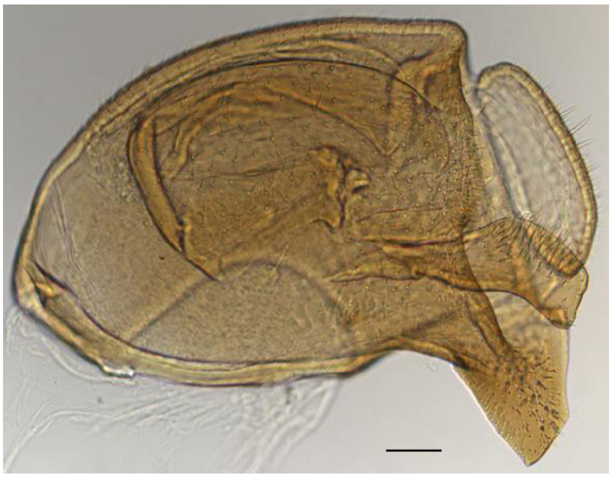

Figure 3. *T. peregrinus* male terminalia of specimens collected in the ISA's arboretum. Scale bar 64 µm.
(In colour at www.bulletinofinsectology.org)

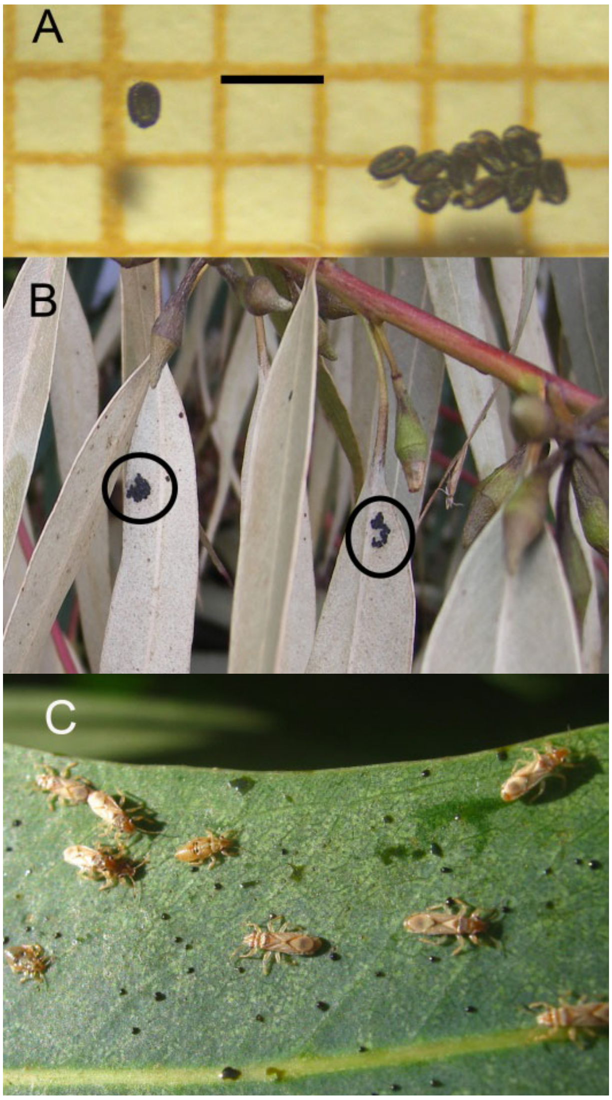

Figure 4. Presence of different developmental stages of *T. peregrinus* on eucalypts leaves and observation of adults and eggs under binocular magnifying glass - A: egg and egg cluster. Scale bar 1 mm; B: egg clusters; C: Colony with nymphs and adults and 'oil like' pattern.
(In colour at www.bulletinofinsectology.org)

Susceptibility of *Eucalyptus* species

The initial survey, conducted in April 2012, established that 14 of the 30 *Eucalyptus* species growing in the arboretum were attacked by *T. peregrinus*. There appeared to be an increase in the insect's host range, since 19 species were confirmed as attacked in the subsequent August survey (table 1). Out of the 19 species, a high incidence of attack was registered for three *Eucalyptus* species: *E. camaldulensis*, *E. viminalis* and *E. pauciflora* (table 1). In particular, *E. camaldulensis* was found heavily attacked with more than 50% of the leaves with signs (eggs, nymphs, adults and /or with insect excreta) of the insect´s presence. *T. peregrinus* egg clusters (figure 4A, 4B) were also mostly observed in *E. camaldulensis* trees, as well as aggregation of nymphs and adults (figure 4C). An 'oil like' pattern (figure 4C) could also be observed on leaf surfaces. This pattern is due to the insect excreta (Barbosa *et al.*, 2009). The adults were found to have aggregate pattern on leaves and disperse quickly when disturbed.

Survey of natural enemies

Two late instar larvae of a hemerobiid (Neuroptera) were found preying on *T. peregrinus* nymphs present on *E. camaldulensis*. In laboratory, the larvae were reared with nymphs and adults of *T. peregrinus* until adult stage. Adult specimens were identified as *Hemerobius bolivari* Banks (Neuroptera Hemerobiidae) using a key of this Neotropical species (Monserrat, 1996). No other predators were found near the colonies of *T. peregrinus*.

Local distribution of *T. peregrinus*

From the 53 sites surveyed, *T. peregrinus* was only detected in two sites in Lisbon (the ISA's arboretum and the Lisbon Zoo) and in Peninsula de Setubal, near Lisbon (figure 1). The species was absent in the other 50 sites covering the centre and south of Portugal.

Discussion

Originating from Australia, *T. peregrinus* has dispersed over three continents in the past 10 years. This worldwide spread of *T. peregrinus* is presumably a result of the international trade of eucalypts wood and plants. Since 2011, this species is included in the European and Mediterranean Plant Protection Organization (EPPO) Alert List (EPPO, 2012). To the authors' best knowledge, the present study provides the first record of *T. peregrinus* in Iberian Peninsula and second record in Europe after Italy (Laudonia and Sasso, 2012).

The species was detected in Lisbon and Peninsula de Setúbal, but not in any of the inland sites surveyed, suggesting that one or both of these two coastal cities could be point of entry of this pest species. Thus, the introduction of this species in Portugal through terrestrial transport seems less likely than through air and/or sea. Transport overseas has facilitated the transport of goods over long distances, but also makes that many species may easily hitchhike on these goods and subsequently cross natural barriers that would otherwise be difficult to surpass by natural dispersion (Liebhold and Tobin, 2008). The increased in trade transport over the past decades has also increased the chance of introducing and consequent establishment of invasive species (Hulme, 2009).

South America is the main supplier of the *Eucalyptus* wood commerce to Europe (UNECE and FAO, 2005; 2012) and may thus represent a threat for the introduction of new eucalypt pest species into Europe. It is plausible that *T. peregrinus* entered Portugal from South America and that this introduction might be independent of the introduction reported in Italy. Future research based on DNA bar-coding may aid in revealing the source of *T. peregrinus* introduced in Portugal, as previous successfully used for other invaded regions (Nadel *et al.*, 2010).

This recent invasion adds another hemipteran pest species of eucalyptus with Australian origin to the four species previously reported in the Iberian Peninsula: *Ctenarytaina eucalypti* Maskell, in 1971 (Azevedo and Figo, 1979), *Ctenarytaina spatulata* Taylor, in 2002 (Valente *et al.*, 2004), *Glycaspis brimblecombei* Moore, in 2005 (Valente and Hodkinson, 2009) and *Blastopsylla occidentalis* Taylor, in 2009 (Pérez-Otero *et al.*, 2011).

Species may colonize new territories by different means (anthropogenic or by natural dispersion) and, in most cases, are only detected when the population is established and begin to cause damage. Early detection is therefore considered a valuable tool in the management of invasive species (Liebhold and Tobin, 2008). This may be a way to prevent the spread of a new species and to control them through eradication processes, like in agricultural systems using susceptible species (e.g. trap crop) to attract and eliminate pest species (Shelton and Badenes-Perez, 2006). In the present case, the *Eucalyptus* arboretum in Lisbon proved to be a valuable tool in detecting the arrival of a new pest in much the same way sentinel forests are used (Britton *et al.*, 2010).

Of the 30 *Eucalyptus* species studied in the arboretum, about 60% were attacked by *T. peregrinus* (table 1). This number is possibly conservative, as between the first and subsequent surveys, April and August respectively, the number of eucalypt species attacked increased. This indicates that *T. peregrinus* is possibly still increasing in population and broadening its host range, which, in turn, could lead to an increase in the number eucalypt species attacked.

E. globulus is the principal species used by the Portuguese pulp industry (ICNF, 2013). Although *E. globulus* trees examined in the arboretum were not as affected by *T. peregrinus*, the susceptibility of this eucalyptus species to infestation by *T. peregrinus* has previously been documented (Wilcken *et al.*, 2010; Noack *et al.*, 2011). This is of great concern if *T. peregrinus* continues to increase in population and expands its geographical range since it could affect *E. globulus* plantations in Portugal and subsequently lower productivity of the National pulp production industries.

We found strong aggregation behaviour of *T. peregrinus* followed by dispersion when disturbed. These traits, together with the symptoms of infested leaves, ease the detection of *T. peregrinus*. The above pattern behavior further suggests a possible alarm pheromone. This is in agreement with previous observations that both males and females possess a glandular type organ that they quickly extrude when disturbed, accompanied with the release of volatile compounds (Martins *et al.*, 2012).

During the spring/summer surveys, we discovered a neuropteran, *H. bolivari*, predating on *T. peregrinus* nymphs. In the laboratory, the larvae of this insect were fed exclusively with *T. peregrinus* and developed to its adult stage, confirming its status as a predator. *H. bolivari* is native to South America and widely distributed from Mexico to Argentina (Monserrat, 2004; 2008). In Brazil the predator was found associated to plantations of coffee (*Coffea arabica* L.), maize (*Zea mays* L.) and mate (*Ilex paraguariensis* St. Hill.), being its morphology described by Lara and Freitas (2003). To our best knowledge, this is the first time that *H. bolivari* is reported as a predator of *T. peregrinus* and the first time that this species is detected in Europe. This finding suggests *T. peregrinus* is also preyed by this predator in its South America distribution range, corroborating the hypothesis of a common South American origin. Other natural enemies of *T. peregrinus* previously reported in South America include the neuropteran *Chrysoperla externa* (Hagen) (Barbosa *et al.*, 2010), the pentatomid *Supputius cincticeps* Stal (Souza *et al.*, 2012) and the entomopathogenic fungus *Zoophthora radicans* (Bref.) A. Batko (Mascarin *et al.*, 2012). In Australia, two Hymenoptera egg parasitoids have been confirmed: *Cleruchoides noackae* Lin et Huber and *Stethynium* sp. Enock (Hymenoptera Mymaridae) (Noack *et al.*, 2009).

H. bolivari requires further study to ascertain its potential as a biological control agent.

The introduction of alien (invasive) species is principally intermediated by human activity. *T. peregrinus* was most probably introduced in Portugal via the importation of eucalypt logs from a Latin American country. This species is established in several countries of the southern hemisphere (outside its native range, Australia) and since 2011 it has also started to colonize the northern hemisphere. It was first detected in Italy in 2011 and in Portugal through the present study in 2012. In the next years, if no control measures are successfully undertaken, the pest will undoubtedly invade surrounding countries, as was previously observed in South America, where this pest species spread in Argentina, Uruguay and Brazil, with high impacts on eucalypts plantations (Wilcken *et al.*, 2010).

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