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# DNA bar-coding reveals source and patterns of *Thaumastocoris peregrinus* invasions in South Africa and South America

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**Abstract** *Thaumastocoris peregrinus* is a recently introduced invertebrate pest of non-native *Eucalyptus* plantations in the Southern Hemisphere. It was first reported from South Africa in 2003 and in Argentina in 2005. Since then, populations have grown explosively and it has attained an almost ubiquitous distribution over several regions in South Africa on 26 *Eucalyptus* species. Here we address three key questions regarding this invasion, namely whether only one species has been introduced, whether there were single or multiple introductions into South Africa and South America and what the source of the introduction might have been. To answer these questions, bar-coding using mitochondrial DNA (COI) sequence diversity was used to characterise the populations of this insect from Australia, Argentina, Brazil, South Africa and Uruguay. Analyses revealed three cryptic species in Australia, of which only *T. peregrinus* is represented in South Africa and South America. *Thaumastocoris peregrinus* populations contained eight haplotypes, with a pairwise nucleotide distance of 0.2–0.9% from seventeen locations in Australia. Three of these haplotypes are shared with populations in South America and South Africa, but the latter regions do not share haplotypes. These data, together with the current distribution of the haplotypes and the known direction of original spread in these regions, suggest that at least three distinct introductions of the insect occurred in South Africa and South America before 2005. The two most common haplotypes in Sydney, one of which was also found in Brisbane, are shared with the non-native regions. Sydney populations of *T. peregrinus*, which have regularly reached outbreak levels in recent years, might thus have served as source of these three distinct introductions into other regions of the Southern Hemisphere.

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## Introduction

Commercial *Eucalyptus* forestry serves as a major source of fibre and wood production in many parts of the world (Turnbull 1999). By 2006, some 496,000 hectares of productive commercial forestry land in South Africa had been planted to non-native *Eucalyptus* (Forestry South Africa 2006). This resource is seriously threatened by alien invasive pests and pathogens (Wingfield et al. 2008; Wingfield et al. 2001). Accidental introductions and impacts of insect pests of trees appear to be increasing due to global trade and travel (Brockerhoff et al. 2006; Wingfield et al. 2008). Understanding the origin and patterns of these introductions is an important component of efforts to reduce them in the future.

The *Eucalyptus* pest *Thaumastocoris peregrinus* (Hemiptera: Thaumastocoridae), is a small (2–4.5 mm) insect belonging to the Thaumastocoridae, a family of true bugs first described by Kirkaldy (1908). The Thaumastocoridae includes three sub-families; namely the Thaicorinae from Thailand with one extant genus, the South American Xylastodorinae represented by two genera and six species and the Thaumastocorinae with four genera and 13 species (Cassis et al. 1999; Heiss and Popov 2002; Slater 1973). The latter group are phytophagous sap feeding insects from Australia, with only one genus and species occurring in Southern India (Drake and Slater 1957). The Thaumastocorinae have a wide host range including species of *Acacia, Agonis, Banksia, Dryandra, Elaeocarpus, Eucalyptus*, *Melaleuca, Schizomeria* and *Xanthorrhoea*, whereas the Xylastodorinae feed exclusively on Palmae (Carpintero and Dellapé 2006; Cassis et al. 1999; Hill 1988; Jacobs and Neser 2005; Kumar 1964; Slater 1973).

The genus *Thaumastocoris* included five species and was established in 1908 when Kirkaldy described *Thaumastocoris australicus* from specimens collected in Bundaberg, Queensland (Carpintero and Dellapé 2006; Kirkaldy 1908). Very little research had been conducted on *Thaumastocoris* prior to 2002 when *T. australicus* became a major pest on planted *Eucalyptus* trees in Sydney (Noack 2002; Noack and Coviella 2006). This pest was first encountered in South Africa in 2003 and in 2005, it was found causing significant damage to plantation *Eucalyptus* spp. in the Gauteng, Mpumalanga and North West provinces (Jacobs and Neser 2005). Interestingly, around this time, a new species *T. peregrinus* (Carpintero and Dellapé 2006) was described from specimens collected on *Eucalyptus* spp. planted in the Buenos Aires province of northern Argentina. Carpintero and Dellapé (2006) speculated that *T. peregrinus*, classified only from specimens collected in Argentina, had been introduced into that country from Australia. The anterolateral tubercles on the anterior lobe of the pronotum in *T. peregrinus* are characteristic and these structures were also noted on the invasive *Thaumastocoris* sp. thought to represent *T. australicus* in South Africa (Carpintero and Dellapé 2006).

It is now believed that the initial reports of *T. australicus* outbreaks in Australia, Argentina and South Africa (Jacobs and Neser 2005; Noack and Coviella 2006) were incorrect and were in fact caused by the newly described species *T. peregrinus* (Carpintero and Dellapé 2006; A. Noack, unpublished data). The initial misidentification of *T. peregrinus* in South Africa, Australia and Argentina highlights the difficulty in differentiating between morphologically similar species of *Thaumastocoris*. This can have implications for understanding spread, collection of potential biological control organisms and other species-specific control strategies (Bickford et al. 2007). A potential solution to overcome such problems is to use DNA sequence data for the mitochondrial bar-coding gene, cytochrome *c* oxidase subunit I (COI), for species identification (Hebert et al. 2003a, b, 2004). Such data also provide a measure of divergence amongst geographically distinct populations (Brown 2004).

Since December 2003, *T. peregrinus* has become one of South Africa's most significant *Eucalyptus* pests. It is currently distributed throughout South Africa and has moved northwards in Africa, reaching Zimbabwe in August 2007 and Malawi in June 2008. Similarly, *T. peregrinus* was reported as having become established in Argentina in April 2005 and it was recorded as established in Uruguay and Brazil in January and June 2008, respectively (Carpintero

and Dellapé 2006; Noack and Coviella 2006; M. J. Wingfield and C. F. Wilcken, unpublished data). Typical symptoms of infestation include initial reddening of the canopy leaves on the north to north eastern side of a compartment (Fig. 1). Subsequently, the foliage changes to a reddish-yellow or yellow-brown colour. This is coupled with some leaf loss and the visible abundance of adults, nymphs and black egg capsules usually clustered in high numbers (Fig. 1). During severe infestations, loss of leaves leads to severe canopy thinning and this sometimes results in branch dieback (Fig. 1).

In this study, we use COI bar-coding sequences of *T. peregrinus* from native populations in Australia, and compare these with those of specimens from South African and South American *Eucalyptus* plantations. Using these data we considered whether only one species has been introduced into exotic *Eucalyptus* plantations of the Southern Hemisphere. We further attempted to determine the number and

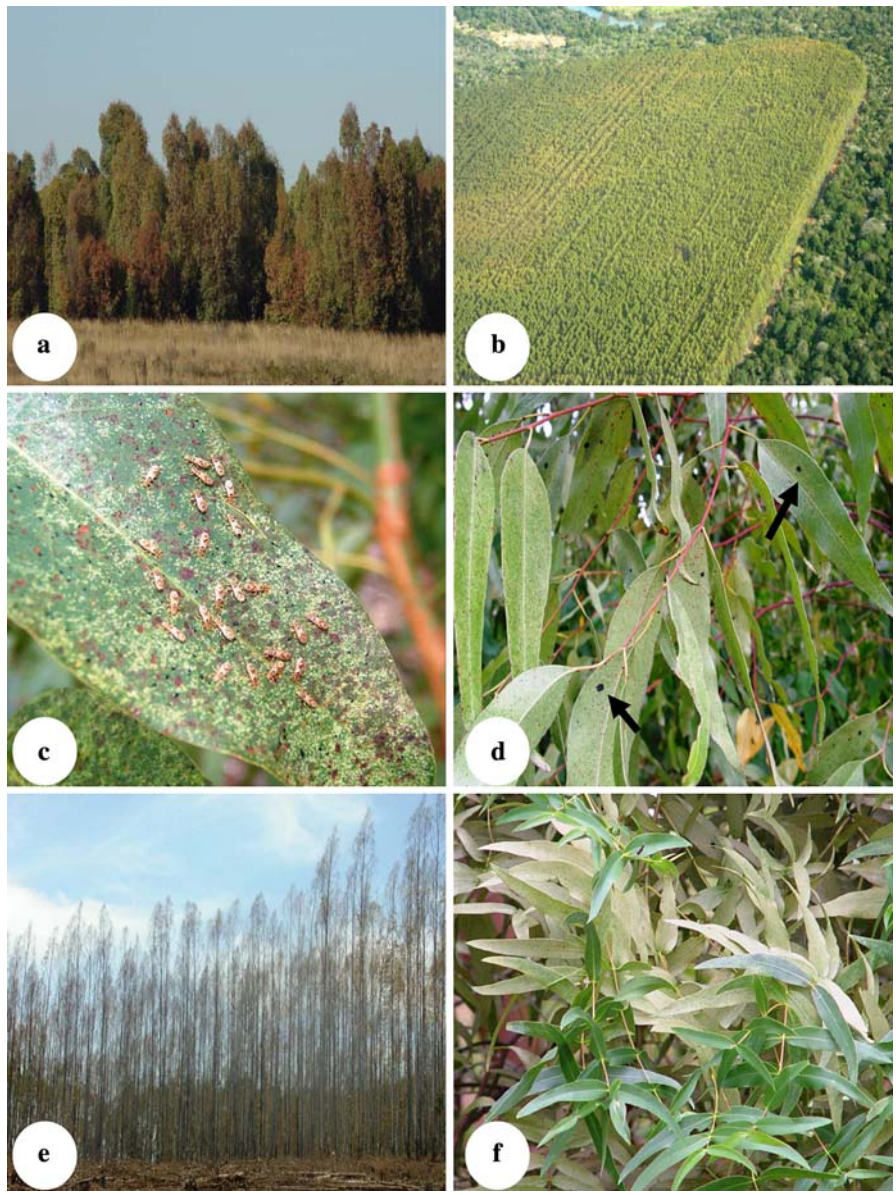


**Fig. 1** Symptoms of *Thaumastocoris peregrinus* infestations in several *Eucalyptus* plantations: **a** initial reddening of the canopy leaves. **b** Progression of infestation severity coupled with foliage changing to a yellowish colour. **c** Feeding *T. peregrinus* adults. **d** Clusters of black egg capsules on infested leaf surfaces. **e** Severe infestation resulting in the loss of canopy leaves. **f** Re-sprouting leaf shoots that are un-infested by *T. peregrinus*, with older leaves from a previous infestation

pattern of introductions into South Africa and South America and considered where the introductions might have originated.

## Materials and methods

### Host range, infestation and distribution

In South Africa, the host range and distribution of *T. peregrinus* was determined by field sampling and plantation surveillance over an 18-month period from February 2006 to July 2007. Assessments of infestations were carried out throughout South Africa, although they were strongly focussed on commercially grown *Eucalyptus* in plantations in the Limpopo, Mpumalanga and KwaZulu-Natal provinces. The surveys were undertaken in collaboration with forestry companies that form part of the Tree Protection Co-operative Programme (TPCP) (www.fabinet.up.ac.za/tpcp). Only 26 *Eucalyptus* species that were found to support several generations (life stages) of adults, nymphs and eggs of *T. peregrinus* were listed as being susceptible.

### Insect collection

*Thaumastocoris peregrinus* individuals are gregarious with many adults and nymphs typically occurring on the same leaves. At least five adults and/or nymphs from several infested trees at an infested site were randomly collected to maximise the diversity sampled. Adults and nymphs were placed in labelled containers filled with 100% ethanol for subsequent DNA analysis.

In South Africa, thirty-one sites ($n = 432$ individuals) were sampled three times between 2006 and 2008. In South America, two sites in Argentina ($n = 14$), three sites in Brazil ($n = 15$) and a single site in Uruguay ($n = 10$) were sampled between 2007 and 2008. In Australia, *Thaumastocoris* specimens were collected in and around three urban centres, namely those of Southeast Queensland, Perth and Sydney, in addition to surrounding rural areas. In May 2008, one site ($n = 7$) was sampled in a rural area within the Brisbane region, in addition to the four sites ($n = 20$) in the Perth region. In Sydney, where *T. peregrinus* is a major pest on planted *Eucalyptus*, samples were collected from twelve sites ($n = 40$) between 2001 and 2002, whereas five sites ($n = 37$) were sampled in May 2008.

### DNA extraction and sequencing

Total genomic DNA was extracted from 657 adult *T. peregrinus* individuals using PrepMan® Ultra Sample Preparation Reagent (Applied Biosystems, California, USA). Mitochondrial COI gene PCR amplifications were initially undertaken using the primer pairs C1-J-2183 and TL2-N-3015 (Simon et al. 1994). From resulting sequences, a new set of primers was developed to increase efficiency using the software Primer 3 (Rozen and Skaletsky 1998) and Net Primer (PREMIER Biosoft International). A 547 base pair fragment of the mitochondrial COI gene was amplified using PCR with this newly developed primer pair Tp2390F (5′ACCCGAGCAT ACTTTACTTC) and Tp2937R (5′ATTGTGGCTCGTTTTGATA).

PCR reactions included 2 µl of genomic DNA (50–100 ng/µl), 10× PCR buffer (Roche Diagnostics), 3 mM $MgCl_2$, 1 mM dNTP's, 0.4 µM of each primer, 1 U Taq polymerase with 12 µl of Sabax water to bring the total volume of the reaction to 25 µl. The PCR cycling regime was as follows: initial denaturation at 95°C for 1 min followed by 30 cycles of 30 s at 95°C, 45 s at 48°C, 45 s at 72°C, and a final elongation for 10 min at 72°C. The PCR products were cleaned using the 3 M NaOAc (pH 4.6) and Ethanol ($CH_3CH_2OH$) precipitation method prior to being visualized under UV on an Ethidium Bromide stained 2% agarose gel.

A total of 215 specimens from 22 locations in South Africa ($n = 73$), two locations in Argentina ($n = 14$), three locations in Brazil ($n = 15$), one location in Uruguay ($n = 10$) and 22 locations in Australia ($n = 103$) were sequenced. Sequencing reactions were performed in 10 µl with 4 µl of cleaned PCR product, 0.5 µM of the same primers used for the PCR, 2 µl 5× sequencing buffer and using the BigDye® Terminator v3.1 Cycle sequencing Kit (Applied Biosystems, Warrington, UK). PCR conditions were 25 cycles of 10 s 95°C, 5 s at 48°C, 4 min at 60°C. PCR products were purified by precipitation using 3 M NaOAc (pH 4.6) and Ethanol ($CH_3CH_2OH$) followed by vacuum drying and sequencing using the ABI Prism™ 3100 Genetic Analyzer (Applied Biosystems). Representative sequences were deposited in GenBank (FJ623760–FJ623773).

### mtDNA sequence analyses

Sequence alignments were done using Vector NTI 9.1 (Invitrogen Corporation, 2004). Phylogenetic and distance analyses were conducted using MEGA version 4 (Tamura et al. 2007). A pairwise distances matrix for haplotypes and species was produced using the Kimura 2-parameter model and a neighbour joining analysis was conducted for phylogenetic reconstruction. One thousand bootstrap replicates were done to assess the statistical support of the nodes in the phylogenetic tree.

The haplotype diversity was determined using DnaSP ver. 4.5 (Rozas et al. 2003). A parsimony haplotype network with 95% statistical support was obtained using TCS 1.21 (Clement et al. 2000) to determine the relationship among *T. peregrinus* haplotypes.

### RFLP analyses

Sequences obtained from South African samples were analysed for variation in Restriction Enzyme (RE) sites. The two haplotypes found in South Africa differed at a restriction site for the enzyme *Alu*I. PCR's using the same primers and conditions as described above were conducted on an additional 432 samples, which were then digested using the restriction enzyme *Alu*I. RFLP reactions were performed in 10 µl reactions with 5 µl of cleaned PCR product, 1 µl of 10× Buffer Tango$^{TM}$ (Fermentas Inc., Canada) and 10 units of *Alu*I restriction enzyme. The RFLP reaction was performed at 37°C for 1 h, 20 min at 60°C. The RFLP PCR product was visualized under UV on an ethidium bromide stained 2% agarose gel.

## Results

### Host range, infestation and distribution

Between 2005 and 2006 *T. peregrinus* spread throughout South Africa, infesting 26 *Eucalyptus* species (Fig. 2; Table 1). By mid 2006, it was found as far north as the Soutpansberg, Limpopo province, ±400 km north of Pretoria (since August 2005) and as far south as Cape Town, Western Cape province, ±1,450 km south of Pretoria (since May 2006) (Fig. 2). An infestation was first reported from the Southern coastal Zululand region, KwaZulu-Natal province in February 2006 (±600 km south east of

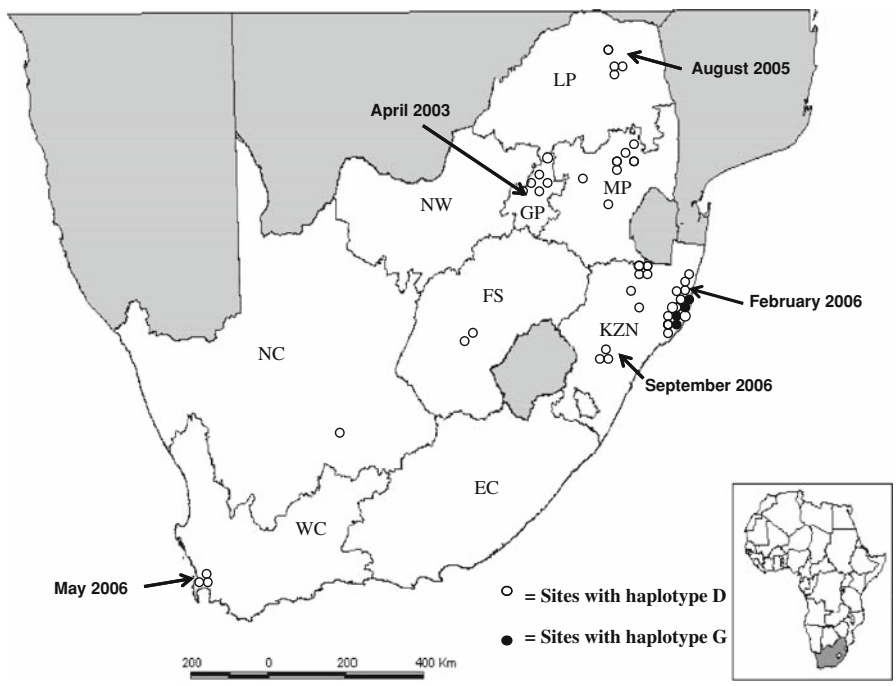


**Fig. 2** Map of South Africa showing the confirmed infestations and date of first reported occurrences of *Thaumastocoris peregrinus*. Sequence and RFLP data indicate the existence of two COI haplotypes. The provinces are labelled as follows Western Cape (WP), Eastern Cape (EC), Northern Cape (NC), Free State (FS), KwaZulu-Natal (KZN), Mpumalanga (MP), Limpopo (LP), Gauteng (GP) and North West provinces (NW)

**Table 1** *Eucalyptus* species infested by *Thaumastocoris peregrinus* in South Africa

| Genus | Sub genera | Section | Species |
|---|---|---|---|
| *Eucalyptus* | *Corymbia* | *Septentrionales* | *citriodora* |
| | | | *henryi* |
| | | | *maculata* |
| | *Eucalyptus* | *Cineraceae* | *pauciflora* |
| | *Symphyomyrtus* | *Adnataria* | *sideroxylon* |
| | | | *paniculata* |
| | | | *argophloia* |
| | | *Exsertaria* | *tereticornis* |
| | | | *camaldulensis* |
| | | *Latoangulatae* | *botryoides* |
| | | | *longirostrata* |
| | | | *punctata* |
| | | | *grandis* |
| | | | *saligna* |
| | | *Maidenaria* | *nicholii* |
| | | | *dunnii* |
| | | | *smithii* |
| | | | *macarthurii* |
| | | | *globulus* |
| | | | *nitens* |
| | | | *dorrigoensis* |
| | | | *scoparia* |
| | | | *viminalis* |
| Eucalyptus hybrids | | | |
| | *Eucalyptus grandis* × *camaldulensis* | | |
| | *Eucalyptus grandis* × *nitens* | | |
| | *Eucalyptus grandis* × *urophylla* | | |

Classification of *Eucalyptus* according to Brooker (2000)

Pretoria), with numerous other infestations reported further north along the coast in March 2006 (Fig. 2). More recent new infestations were reported from the interior Midlands region of KwaZulu-Natal in September 2006 (Fig. 2).

## mtDNA analysis

Mitochondrial DNA (mtDNA) sequence analysis for *Thaumastocoris* specimens collected from 22 localities in Australia and 28 introduced localities in the South Africa and South America revealed three distinct clades differing by 6.7–8.7% (Fig. 3). One clade represents specimens collected exclusively from the Perth region of Western Australia, whereas a second clade contained specimens from the Sydney region. The remaining clade included specimens that were collected in Brisbane and Sydney (Australia), as well as Argentina, Brazil, South Africa and Uruguay. Specimens from this latter clade were confirmed by A. Noack (unpublished data) as *T. peregrinus*.

The *T. peregrinus* clade was further analysed for haplotype diversity for 186 *T. peregrinus* individuals from 22 locations in South Africa, two locations in Argentina, three locations in Brazil, a single location in Uruguay and 17 locations in Australia. Pairwise genetic nucleotide distance calculations ranged between 0.2 and 0.9%, characterised by nine polymorphic nucleotide sites. The haplotype diversity sampled from Australian *T. peregrinus* populations ranged between 0.52 and 0.75 with the highest diversity obtained from the Sydney (2001–2002) region and the lowest in Brisbane (2008) region (Table 2). Two haplotypes (B and F) were found to occur exclusively within Brisbane region in comparison to four haplotypes (A, C, E and H) found to occur only within the Sydney region (Table 2). Haplotype D was shared among the two Australian regions whereas haplotype A was dominant and present only in the Sydney region (Table 2). Three haplotypes found amongst Australian samples (haplotype A, D and G) were also found in the non-native populations of *T. peregrinus* in Argentina, Brazil, Uruguay and South Africa. Haplotype A, was found to occur within South America whereas two haplotypes, D and G were found in South Africa (Table 2). The relationship among COI haplotypes as represented by the haplotype network (Fig. 4), indicate that the dominant haplotypes within the Australian population are those of haplotype A and D (Fig. 4).

## RFLP analyses

Two mtDNA COI sequence haplotypes were identified amongst 432 specimens from thirty-one locations in South Africa using the restriction enzyme *Alu*I, thus allowing for easy screening of the population for the two distinct haplotypes. A dominant haplotype ($n = 399$) was observed throughout South Africa across several climatic regions, including both summer and winter rainfall patterns (Fig. 2). A second haplotype ($n = 33$) was found only in three locations within the subtropical, summer rainfall area of the

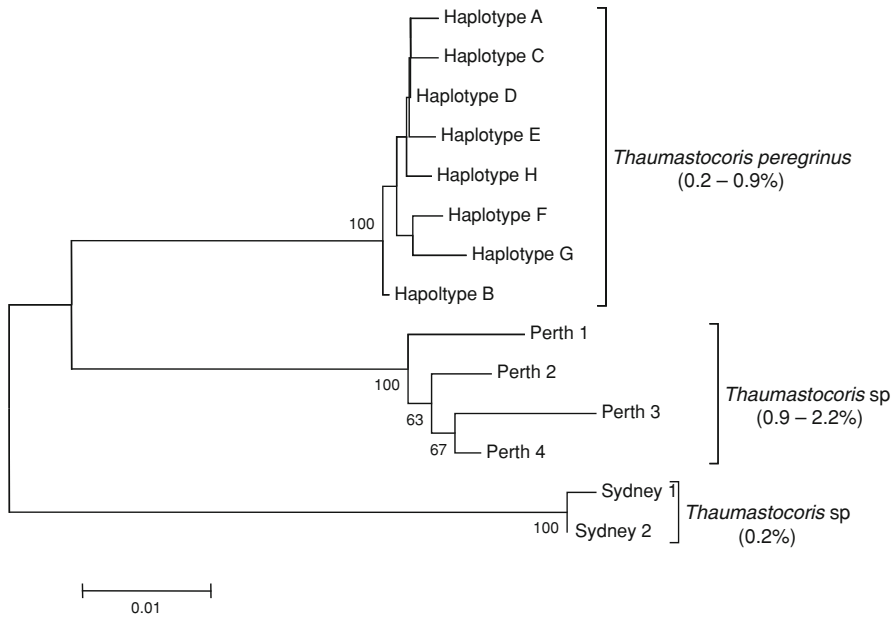


**Fig. 3** A neighbour joining tree constructed using the Kimura 2-parameter distance model, between *Thaumastocoris* species and their associated haplotypes based on mtDNA COI data. Bootstrap values are presented at the nodes with the range of genetic divergences shown in brackets

**Table 2** *Thaumastocoris peregrinus* mtDNA COI haplotypes: the number of sequenced *T. peregrinus* individuals (*N*) and the number of each haplotype ($H_{A–H}$), haplotype diversity (±SD) for each of the populations sampled

| | | Haplotypes | | | | | | | | Haplotype diversity (*h*) |
|---|---|---|---|---|---|---|---|---|---|---|
| Populations | *N* | $H_A$ | $H_B$ | $H_C$ | $H_D$ | $H_E$ | $H_F$ | $H_G$ | $H_H$ | |
| Sydney (2001–2002) | 35 | 12 | – | – | 11 | 1 | – | 7 | 4 | 0.7513 ± 0.0347 |
| Sydney (2008) | 36 | 16 | | 1 | 10 | – | – | 2 | 7 | 0.7032 ± 0.0463 |
| Brisbane (2008) | 7 | – | 1 | – | 5 | – | 1 | – | – | 0.5238 ± 0.2086 |
| South Africa | 62 | – | – | – | 46 | – | – | 16 | – | 0.3892 ± 0.0538 |
| South America | 32 | 32 | – | – | – | – | – | – | – | 0 |

coastal Zululand region of northern KwaZulu-Natal (Fig. 2). This second haplotype appeared only in samples collected subsequent to March 2006 where it was present at one site. Eight months later (November 2006) it had spread along the coast to two adjacent sites where it now co-occurs together with the dominant haplotype.

## Discussion

Surveys and collections done as part of this study, confirmed the establishment of *T. peregrinus* in regions of the Southern Hemisphere between 2003 and 2006. Extreme long range dispersal by air travel from urban centres in Australia, where outbreaks of this pest have occurred over the past 10 years, are suggested as being the most likely initial dispersal mechanism of *T. peregrinus* into South Africa and South America. The establishment and spread of *T. peregrinus* across South Africa and South America appears to have been through stratified dispersal (Lockwood et al. 2007), encompassing both long and short distance modes of dispersal (Liebhold and Tobin 2008). Long range dispersal mechanisms are principally thought to be through human mediation and wind (Pasek 1988; Smith et al. 2007), which would result in the spread and establishment of numerous isolated *T. peregrinus* populations, as was noted to occur within South Africa in 2006 (Fig. 2). Once established these isolated populations are expected to grow in size and eventually coalesce

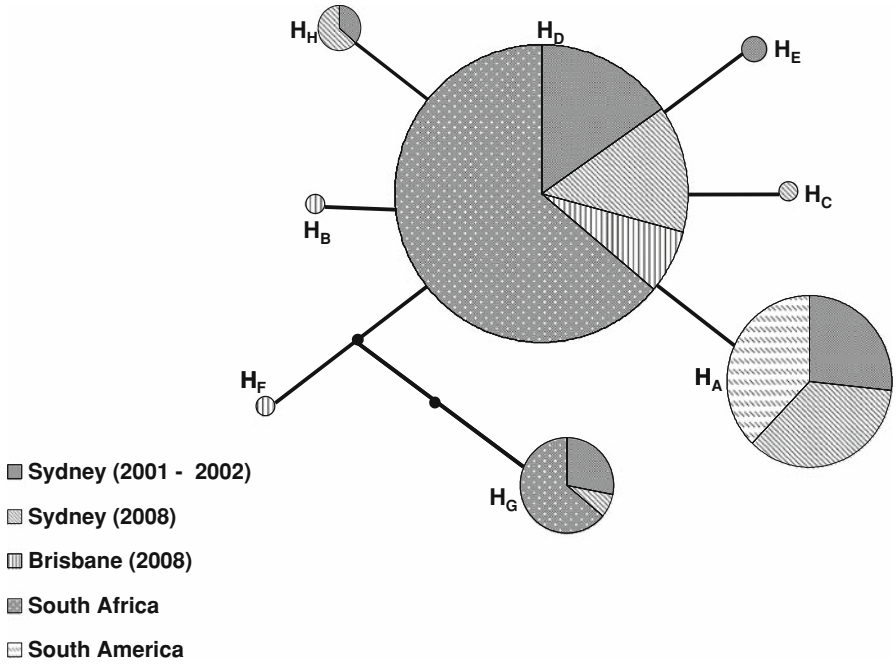


**Fig. 4** The haplotype distribution and network of 186 *Thaumastocoris peregrinus* COI sequences. A haplotype network of the eight haplotypes. Haplotype frequencies are represented by the area of the circles. Each line corresponds to a mutational step. Black circles represent haplotypes not observed during sampling. Different patterns represent different populations

with neighbouring populations. This is in comparison to the situation if only short distance dispersal mechanisms had occurred (Liebhold and Tobin 2008; Lockwood et al. 2007).

*Thaumastocoris peregrinus* has a wide host range attacking at least 30 *Eucalyptus* species and three common commercial hybrids. In South Africa, 26 species including all commercially grown *Eucalyptus* are susceptible to attack (Table 1) (Jacobs and Neser 2005). In Australia, 14 *Eucalyptus* species are affected with *E. scoparia* and *E. nicholii* being most susceptible to infestation (Noack and Coviella 2006; A. Noack, unpublished data). In comparison, South American *T. peregrinus* infestations have been reported on seven *Eucalyptus* species (Carpintero and Dellapé 2006; Noack and Coviella 2006). The wide host range on *Eucalyptus* supports the establishment, survival and reproduction of *T. peregrinus*. This varied level of polyphagy is not uncommon and has been observed for other members of *Thaumastocoris* and the Thaumastocorinae sub family of the Thaumastocoridae (Cassis et al. 1999; Kumar 1964; Slater 1973).

Analysis of the bar-coding COI sequences revealed three phylogenetically distinct clades, which most likely represent three distinct *Thaumastocoris* species. The *Thaumastocoris* species could be distinguished based on their levels and pattern of mtDNA sequence divergence. Variation between clades were high (6.7–8.7%) and in the range typical of species divergence, while that within the groups was low (0.2–2.2%), as would be expected within species (Hebert et al. 2004). These samples were subsequently analysed morphologically, confirming the distinction of the three *Thaumastocoris* species, of which two appear to be undescribed species (A. Noack; unpublished data). One of these undescribed species was found to co-occur with *T. peregrinus* in Sydney, whereas the third species was found only in Perth, Western Australia. The clade identified as *T. peregrinus* confirmed that the *Thaumastocoris* in Sydney and Brisbane is the same species occurring in Argentina, Brazil, South Africa and Uruguay. This demonstrates the value of using COI as a bar-coding region to explore species diversity of *Thaumastocoris*.

Bar-coding not only revealed cryptic species diversity in *Thaumastocoris*, but it was also useful in determining a potential source and the pattern of invasion of *T. peregrinus* in South Africa and South America. *T. peregrinus* samples from 17 localities in Australia yielded eight mitochondrial COI haplotypes. The COI haplotype diversity of *T. peregrinus* was highest within the Sydney region, with similar diversities obtained for two sampling events. Over

the past 7 years (2001–2008) the haplotype diversity in Sydney has thus remained relatively constant, indicating the absence of evolutionary forces such as drift, immigration or genetic bottlenecks that would result in temporal changes in population structure (Roderick 1996; Sakai et al. 2001). This is despite the urban environment and presence of parasitoids that might affect new introductions from other areas, or lead to bottlenecks when control programs result in populations crashing.

Of the three *T. peregrinus* haplotypes (B, D and F) sampled in the Brisbane region, two (haplotype B and F) were unique to that area. This is not surprising because there is a substantial geographic distance, where native populations of the pest would be expected to occur, between Brisbane and Sydney. The overlapping haplotype (D) is also one of the most frequent haplotypes in Sydney, and possibly reflects movement of the pest between these regions. Further sampling within the Brisbane region would, however, be necessary to make more meaningful comparisons between the Sydney and Brisbane populations.

Three of the haplotypes (A, D and G) of *T. peregrinus* in Australia were also found among the non-native *T. peregrinus* specimens from Argentina, Brazil and Uruguay (haplotype A) and South Africa (haplotype D and G). These haplotypes were also shown to be the most common during outbreaks in the Sydney region during 2001–2002, prior to their discovery in South Africa (since 2003) and Argentina (since 2005). The occurrence of only these three haplotypes (A, D and G) outside of Australia, indicates that a small number of individuals, were most likely introduced into South Africa and South America. The lower haplotype diversity present in the non native populations of *T. peregrinus*, are in agreement with a recent review indicating that 80% of genetic diversity studies have shown lower genetic diversity in invasive as opposed to native populations (Puillandre et al. 2008).

The presence of distinct haplotypes in South Africa and South America also indicates that the insect has not moved between these continents, but that they more likely represent separate introductions from Australia. These multiple introductions reflect the apparent ease with which *T. peregrinus* has been introduced into new environments, and the threat of new introductions in future. The haplotypes A and D that are most common in Sydney (from 2001 to 2002 and 2008 collections) are also those found in South Africa (D) and South America (A). This indicates that the Sydney area is the most likely origin of the other southern hemisphere introductions although an introduction from the Brisbane region to South Africa is also possible. The most common haplotypes in a source population are those that are more likely to be sampled and to eventually spread (Ficetola et al. 2008; Roderick 1996; Sakai et al. 2001) and this also appears true for *T. peregrinus* in the present study.

Two *T. peregrinus* mtDNA haplotypes were found to occur in South Africa. The dominant haplotype D was found throughout South Africa spanning all climatic regions, whereas the second haplotype G was only found within the summer rainfall area of KwaZulu-Natal. This second haplotype appeared only in samples found at a single site in March 2006. After several months, it was detected further along the coast at sites where it now occurs together with the dominant haplotype D. The locality of the first report of this second South African haplotype and its subsequent spread into new areas after its initial discovery (May 2006), suggests that this haplotype represents a second independent introduction of the pest into South Africa from the Sydney population. Based on their distribution, possible ports of entry for specimens representing the two haplotypes are the O. R. Tambo International Airport in Gauteng and the harbour at Richards Bay in Northern KwaZulu-Natal.

Bar-coding revealed three separate introductions of *T. peregrinus* from Australia, most likely via extreme long distance dispersal in a short period of time. It is interesting to note that the introductions into South Africa and South America (2003–2005) coincide in time with the outbreaks of the pest in Sydney that have occurred regularly during the past 10 years. Large population build-ups in the Sydney region are suggested to have resulted in the separate unconnected introductions of this pest into South African and South American non-native *Eucalyptus* plantations. Because *T. peregrinus* populations remain high in Sydney, further introductions and a greater diversity is expected to appear in *T. peregrinus* populations in South Africa and South America in coming years. Results of this study have highlighted the threat of outbreaks of pests in urban centres where the large numbers of insects can increase the chances of their accidental movement to other urban centres in a

country or elsewhere in the world. These cosmopolitan centres are often hubs linking transport systems between different countries and continents and they should be considered important sources of new introductions.

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