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Interception of exotic ants and survey of the ant fauna at Gaoming Port, China

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ABSTRACT. Globalization of trade and tourism has promoted the dispersal of exotic animals and plants around the world. Quantifying the invasion of exotic insects resulting from overseas trade is essential for national and international risk assessments. Seaports are important hubs for the arrival of exotic insects and a pathway for the invasion of alien species. Analyzing the interception data of exotic species at seaports and investigating the surrounding areas can improve our understanding and limit the risks of biological invasions. In this study, we examined the diversity of ant species associated with different shipping commodities and the origin of exotic ants arriving at Gaoming Port, China, during 2014–2017. In addition, we surveyed the ant communities at the port using bait and pitfall traps. We found 609 records of ants that were intercepted from foreign imports over a four years period, with most originating from Southeast Asian countries. *Dolichoderus thoracicus* and other species belonging to this genus were the most frequently intercepted species, accounting for 35.79% of interceptions. *Solenopsis* spp., *Anoplolepis gracilipes* and several other species presenting significant risks to ecosystems, agriculture, forestry and human health were also intercepted on several occasions. Field surveys showed that *Solenopsis invicta* was the dominant ant species in the seaport ant community, which also harbored other known tramp species, such as *Tapinoma melanocephalum* and *Paratrechina longicornis*. This study indicates that there is high probability of the introduction of foreign ants through seaports. Strengthening quarantine work and monitoring at and near ports is helpful for reducing the risks of the establishment and spread of exotic ants during the early phases of invasion associated with ports.

Keywords Risk analysis; Exotic ants; Port investigation; Prevention and control
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INTRODUCTION

International trade is flourishing and currently accounts for a large share of the gross domestic product and income in many countries. However, it is also a primary driver of biological invasions, and thousands of exotic and invasive species currently hitchhike through trade networks (Bright 1999). The introduction and establishment of alien insects is an accelerating problem worldwide due to increased trade (Early et al. 2016). Exotic ants have received significant attention around the world due to their severe economic impacts on agriculture and the environment. Previous estimates indicate that no fewer than 150 species of ants have been transported to new regions as a result of global trade (McGlynn 1999). Shipping consignments at ports are important means for the entrance of exotic ants to new areas since these consignments account for 90% of global trade. Several examples illustrate how numerous exotic ant species have been detected at seaports and in association with other trade pathways. For example, in New Zealand and Australia, sea containers were identified as a potential entry pathway for ants and other insects (Stanaway et al. 2001; Nendick et al. 2006; Ward et al. 2006), with approximately 65% of exotic ants detected in New Zealand transiting through empty sea containers and commodities, such as root crops originating from Pacific Islands (Nendick et al. 2006). Exotic ants are often introduced by means of the transport of waste paper, wood packaging carrying a commodity, seedlings, fruits and other imported goods or containers, as well as via passenger luggage carried through ports (Stanaway et al. 2001; Ward et al. 2006). Following introduction, exotic ants can negatively affect the local environment, biodiversity, human health and public safety and cause economic losses, with invasive ants in China and the United States reported to cause the greatest economic losses (Perrings *et al.* 2005; Hulme 2009; McGeoch *et al.* 2010; Paini *et al.* 2016). For instance, invasive ant species can prey on bees, flower-visiting insects and fruits, which ultimately affects agricultural production and causes significant economic loss (Cole *et al.* 1992). Huang *et al*. (2010) found that the seeds of several plants damaged by the Red Imported Fire Ant, *Solenopsis invicta* Buren, showed no or very low germination. At the same time, exotic ants, such as *Anoplolepis gracilipes* (Fr. Smith), and other species often form supercolonies in their introduced ranges and can rapidly impact local biodiversity by reducing the ecological carrying capacity (Abbott 2006). For instance, local ant species richness was reduced by 33 and 46% as a result of *S. invicta* invasion in Beiliu, Guangxi, and Shenzhen, Guangdong, respectively (Shen *et al*. 2007; Wu *et al*. 2008). In addition, some exotic ants, such as *Monomorium pharaonis* (Linnaeus), can carry a variety of pathogenic bacteria and contaminate food (Edwards and Baker, 1981). These ants can cause skin irritation and allergy after stinging, which may disturb infants, and stinging by *S. invicta* can cause severe shock or even death following strong allergic reactions (Edwards 1985; Williams & Moak 1999; Kemp *et al.* 2000; Wang *et al.* 2018). Therefore, preventing the introduction and establishment of exotic ants can be perceived as the most important way to avoid these problems. The prevention of ant contamination at entry points into a country and accurate estimates regarding their arrival time and number are also required to develop risk assessments for specific trade pathways and for future decision making in regard to the management of exotic ants in newly colonized areas. Future decision making requires more focus on the ways humans facilitate the transport of exotic ant species to new areas. Therefore, the current study was conducted to provide statistics on the taxonomic diversity and origins of exotic ant species that were unintentionally transported via trade through Gaoming Port over a four years period (2014 – 2017). Gaoming Port handles a large number of international consignments and shipping containers, thus offering great risks of the introduction of exotic insect species in China Furthermore, the assessment of the diversity of ants near the port was another objective of this study to determine ant species residing on and/or adjacent to the Port. The results of this study represent the first step in establishing a solid basis for quarantine and monitoring measures at the port.

MATERIALS AND METHODS

Study sites

The study was conducted at Gaoming Port, which is located in Guangdong Province, the most populous province in China and a hub for international trade and tourism. This region has a humid subtropical monsoon climate, with a minimum monthly average temperature of 10 °C in January and a maximum monthly average temperature of 29 °C in July. It includes two cargo ports and one passenger port, namely, Gaoming Shichu Port (22.896632 N, 112.908935 E) located on the Xi River, Pearl River Port (22.876007 N, 112.914689 E) and Gaoming Passenger Port (22.894 N, 112.907094 E) (Fig. 1). The southern portion of Gaoming Shichu Port is a barren grassland, while its northern area is adjacent to Linggui Park in Gaoming District; the northern region of this Park is also adjacent to Gaoming Passenger Port. Pearl River Port is approximately 2 km south of Gaoming Shichu Port. At all three ports, trees such as *Mangifera indica* L., *Ficus microcarpa* L.f., and *Broussonetia papyrifera* (Linnaeus), weeds such as *Eleusine indica* (L.), *Digitaria sanguinalis* (L.), *Rhynchelytrum repens* (Willd.), *Setaria viridis* (L.), *Bidens pilosa* L., *Celosia argentea* L., *Ageratum conyzoides* L., *Polygonum plebeium* R. Brown, and *Tridax procumbens* L. and the turf grass *Cynodon* sp. grow around the buildings.

Table 1. Exotic ant species and number intercepted from different shipments at Gaoming Port during 2014 – 2017

Ant species intercepted	Number of interceptions				
	2014	2015	2016	2017	Total
DOLICHODERINAE					
Dolichoderus thoracicus		17	122		139
Dolichoderus sp.			79		79
Unidentified genus		172	5		177
DORYLINAE					
Unidentified genus	1				1
FORMICINAE					
Formica sp.				1	1
Anoplolepis gracilipes		29	4		33
Camponotus japonicus			4		4
Camponotus sp.		58	9	20	87
Paratrechina sp.	1	8		1	10
Polyrhachis dives			1		1
Polyrhachis armata			1		1
Polyrhachis sp.			1		1
Unidentified genus	2			1	3
MYRMICINAE					
Myrmecina sp.		1			1
Solenopsis geminata	2	1			3
Solenopsis invicta	1	2		2	5
Crematogaster rogenhoferi	1	23	4		28
Unidentified subfamilies	7		27	1	35
Total	15	311	257	26	609

Interception of ants at Gaoming Port

Quarantine personnel conducted on-site inspections of goods, containers and passenger luggage entering Gaoming Port for a period of four years (2014 – 2017). The consignments varied in quantity, size, shape, and commodity, and 0.02% – 0.2% of the consignments were randomly selected and sampled for further inspection. The data for each sample include information on the country of origin and the type of commodity involved. The inside and outside surface of containers were inspected, including packaging, such as plastic wrapping, cardboard boxes, foodstuffs and other types of wrapping. Ants within the cargo were isolated from the contained goods, tarpaulins were used to cover the commodities to restrict the movement of ants, and then the ants were collected. The collected ants were stored in 75% ethyl alcohol at room temperature. Morphological identification was carried out under a microscope (Olympus SZ61) to determine the species of ants intercepted using the key by Zhou (2001) and confirmed by Dr. Zhilin Chen, an ant taxonomist at Guangxi Normal University. Voucher specimens were deposited at the laboratory of the Gaoming Office of Foshan Customs.

Diversity of ants at Gaoming Port

Methods of ant collection

A field survey was conducted to collect foragers of different ant species at the three selected ports to determine ant species residing on and/or adjacent to the Port. During the mid-period of each month from September to December 2017, bait traps and pitfall traps were used to collect ants. All traps were installed in the planted areas near the buildings at the ports. We established three plots with an area of 800 m^2 at each port, and the distance between plots was 50 m. A total of 120 pitfall traps and 120 bait traps were deployed over the entire survey period.

The pitfall traps were constructed of plastic centrifuge tubes (17 mm diameter × 120 mm long) filled with one-third volume of ethyl alcohol (45%) as a preservative. Each trap was inserted into the soil by digging to create a hole such that the upper rim of the centrifuge tube was at the soil surface. The gaps around the opening were filled with soil and leveled after insertion. A total of 30 pitfall traps were deployed in random locations during each sampling period, with ten traps in each plot and a distance of 10 m between them. The traps were collected after 24 h, and then the ants were preserved in 75% ethyl alcohol and labeled.

For the bait traps, 30 ml transparent plastic vials (71 mm × 71 mm) containing ham sausage slices (10 mm diameter) and several drops of honey were placed horizontally on the ground and out of direct sunlight. Ten bait traps were deployed in random locations 10 m apart in each plot between 8:00 and 18:00 h. Ants were collected at any time 30 to 60 minutes after deployment, preserved in 75% ethyl alcohol, and labeled.

Statistical analysis

A chi-square test was used to compare the numbers of individuals and species of ants intercepted in association with different years, geographical sources and carriers. Data from the ant community investigation from September to December were pooled for each plot and tested for normality with the Shapiro-Wilk test and for homogeneity of variances with Levene's test.One-way analysis of variance was used to compare the number of different ant species among ports, and Tukey's post hoc test was used for multiple comparisons. The difference in the number of individuals captured using bait traps and pitfall traps was analyzed with paired-sample t-tests. Microsoft Excel 2010 and SPSS 17.0 were used to process data and draw graphics.

RESULTS

Ant interception at the port

Total intercepted ants

From 2014 to 2017, 58,853, 54,460, 56,563 and 49,244 consignments were imported at Gaoming Port, respectively, among them 79215 were

Table 2. Geographic origin of exotic ants intercepted at Gaoming Port during 2014 – 2017.

Geographic source	Number of consignments inspected	Number of interceptions	% of interceptions per inspection	% of total interceptions
Southeast Asian countries or regions	**58981**	**526**	**0.89%**	**86.38%**
Malaysia	3643	417	11.45%	68.47%
Hong Kong	26776	78	0.29%	12.82%
Thailand	20655	20	0.10%	3.28%
Macao	5904	6	0.10%	0.99%
India	1282	2	0.16%	0.33%
Indonesia	268	2	0.75%	0.33%
Vietnam	453	1	0.22%	0.16%
Australian countries	**17419**	**72**	**0.41%**	**11.82%**
Australia	17419	72	0.41%	11.82%
European countries	**395**	**6**	**1.52%**	**0.99%**
UK	395	6	1.52%	0.99%
American countries	**2420**	**5**	**0.21%**	**0.81%**
Canada	2410	3	0.12%	0.49%
Panama	5	1	20%	0.16%
Bolivia	5	1	20%	0.16%
Total	**79215**	**609**	**0.77%**	**100%**

Table 3. Carriers associated with intercepted ants at Gaoming Port from 2014-2017

Carriers	Number of consignments inspected	Number of interceptions	% of ants found on particular goods	Overall % of intercepted ants
Fruits	2138	413	19.3%	67.8%
Waste hardware	49570	124	0.25%	20.4%
Waste plastics	16081	40	0.24%	6.6%
Wooden boards	543	14	2.57%	2.3%
Containers	2012	13	0.64%	2.1%
Raw cotton	68	3	4.41%	0.5%
Wood logs	14	1	7.14%	0.15%
Chinese mesona herbs	59	1	1.69%	0.15%

inspected and resulting in a total of 609 ant interceptions from imported goods, with individuals belonging to four subfamilies and 12 genera and from which we identified eight species and nine morphospecies during the whole period. These included four species of Myrmicinae, nine species of Formicinae , three species of Dolichoderinae and one species of Dorylinae. *Dolichoderus thoracicus* (Smith) was the most frequently collected species, with 139 interceptions (22.82%), followed by 33 interceptions of *A. gracilipes*, 28 interceptions of *Crematogaster rogenhoferi*

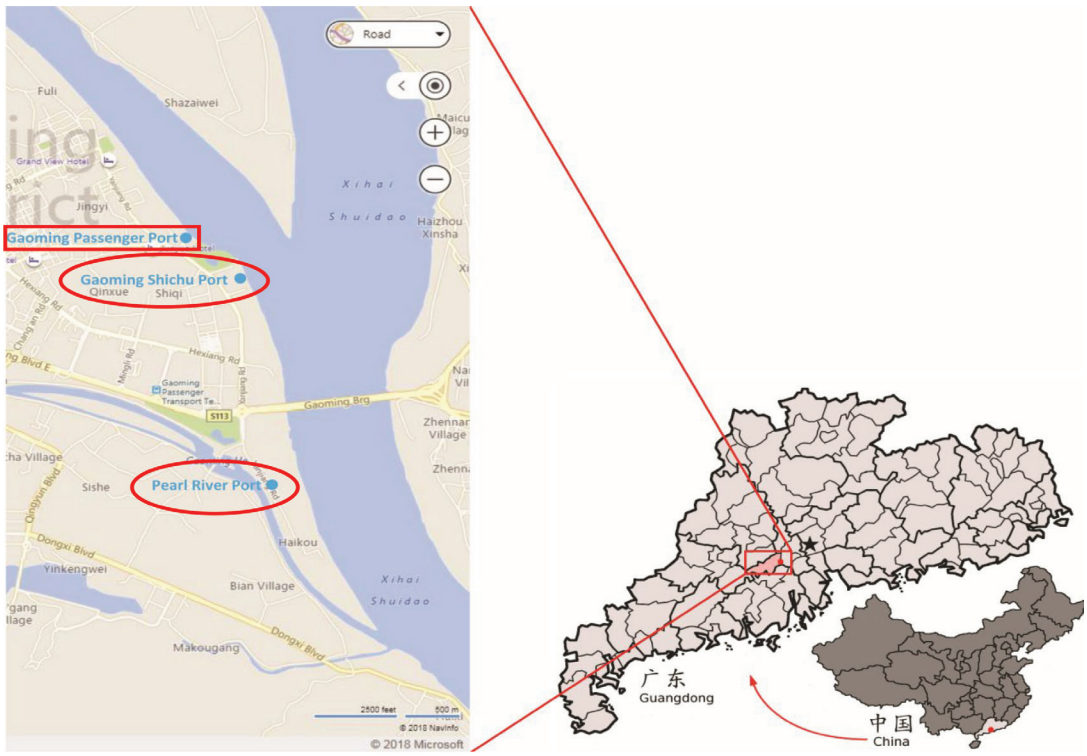
Mayr, five interceptions of *S. invicta* and three interceptions of *S. geminata* (Table 1). In terms of species richness, the number of Formicinae species intercepted was significantly higher than that of the other three subfamilies: Dolichoderinae, Myrmicinae and Dorylinae ($\chi^2 = 432.56$, $df = 3$, $p = 0.03$). A significant difference in the total number of ant interceptions per year was also observed ($\chi^2 = 466.015$, $df = 3$, $p < 0.001$), with a maximum observed in 2015.

Geographic origins of the intercepted ants

The number of ant interceptions significantly differed with respect to the geographic origin of the goods ($\chi^2 = 1242.698$, $df = 3$, $p < 0.001$). Most of the ant species and individuals intercepted originated from Southeast Asian countries (86.38%). Malaysia accounted for most (68.47%) of the total interceptions, followed by Hong Kong (12.82%), with other countries accounting for 5.09%. The percentages of interceptions originating from European (0.99%) and New World (0.81%) countries were the lowest (Table 2). Number of consignments inspected coming from European countries was very less (395), but the percentage of interception per inspection from these countries was higher (1.52%) compared to Southeast Asian and other countries. Among Southeast Asian countries, higher number of interceptions per inspection were from the consignments coming from Malaysia followed by Indonesia and Hong Kong. All the *S. invicta* records intercepted over the past four years were originating from Hong Kong.

Association of intercepted ants with different goods in shipments

The main carrier goods associated with the intercepted ants were fruits, waste hardware, waste plastics, containers, wooden boards, logs, raw cotton and Chinese mesona herbs. The interception of ants associated with fruits, waste hardware and waste plastics accounted for 67.8%, 20.4%



(maps from Wikipedia (https://zh.wikipedia.org/wiki/) and Bing Maps)

Fig. 1. Maps of ant survey sites near Gaoming Port

and 6.6% of the total interceptions, respectively (Table 3), and the number of interceptions differ significantly among goods carrying them (χ^2 = 1551.034, *df* = 7, $p < 0.001$). Consignments containing fruits, followed by those with wooden logs, were more likely to be associated with transported ants than other goods. Among the imported fruits, 99.5% of the intercepted ants were associated with imported mangosteen (*Garcinia mangostana*). The imported mangosteen was the main carrier good associated with *D. thoracicus* (90.6%) and *A. gracilipes* (81.8%) (Fig. 2).

Ant fauna at Gaoming Port

Seven species of ants belonging to three subfamilies and six genera were collected at the three ports of Gaoming Port using bait and pitfall traps.

Among all ports, and independently of the sampling method used, *S. invicta* was the most abundant species retrieved, with its abundance significantly higher than that of any other ant species collected (pitfall traps: Gaoming Shichu Port: $F_{6,14} = 92.184$, Pearl River cargo port: $F_{6,14} = 137.275$, Gaoming passenger port: $F_{6,14} = 51.998$; bait traps: Gaoming Shichu Port: $F_{6,14} = 1292.445$, Pearl River cargo port: $F_{6,14} = 2833.502$, Gaoming passenger port: $F_{6,14} = 1192.349$; all $p < 0.001$). The number of individuals captured using bait traps was significantly higher than that captured with pitfall traps (t = -28.897, *df* = 2, $p = 0.001$). The species abundance did not significantly differ between the two methods used to capture the ants. Four species, *Nylanderia bourbonica, N. flavipes*, *Pheidole parva* and *Paratrechina longicornis,* were collected at all the ports sampled, while *Tapinoma melanocephalum* (Fabricius) was collected only at the Gaoming Shichu and Gaoming passenger ports, and *Dolichoderus thoracicus* was only collected at the Pearl River cargo port (Fig. 3a,b).

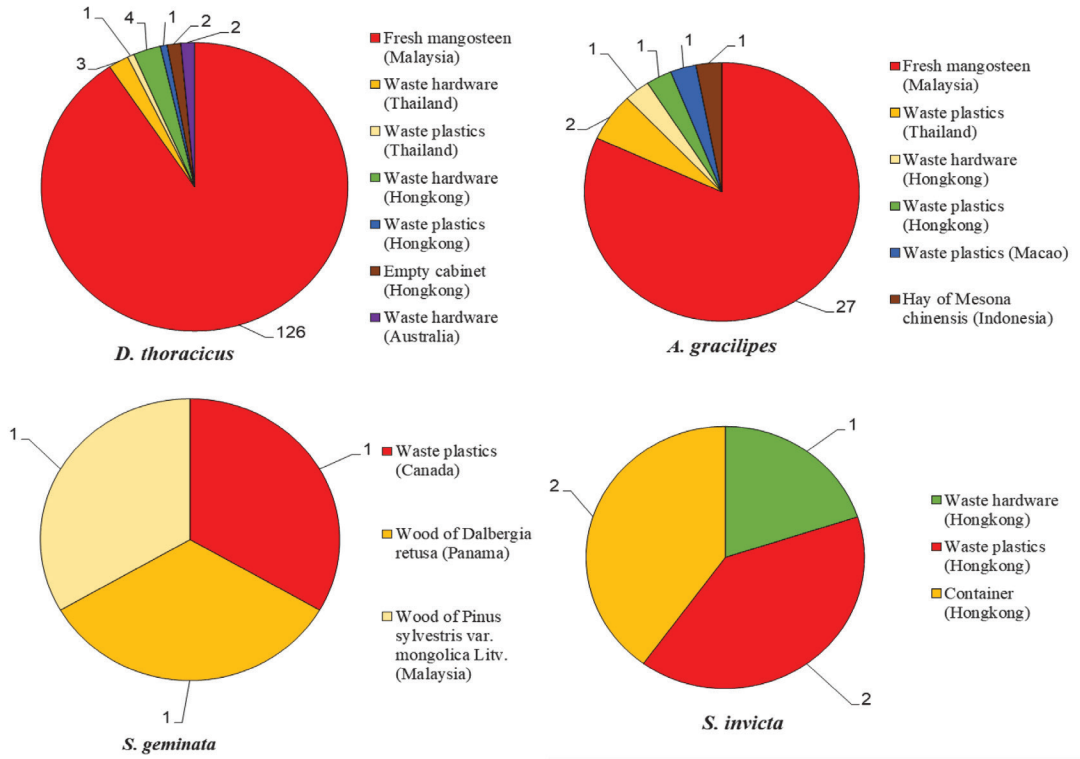


Fig. 2. Distribution of four types of harmful ants among different carrier goods (numbers on each image indicate the number of ants intercepted from each carrier)

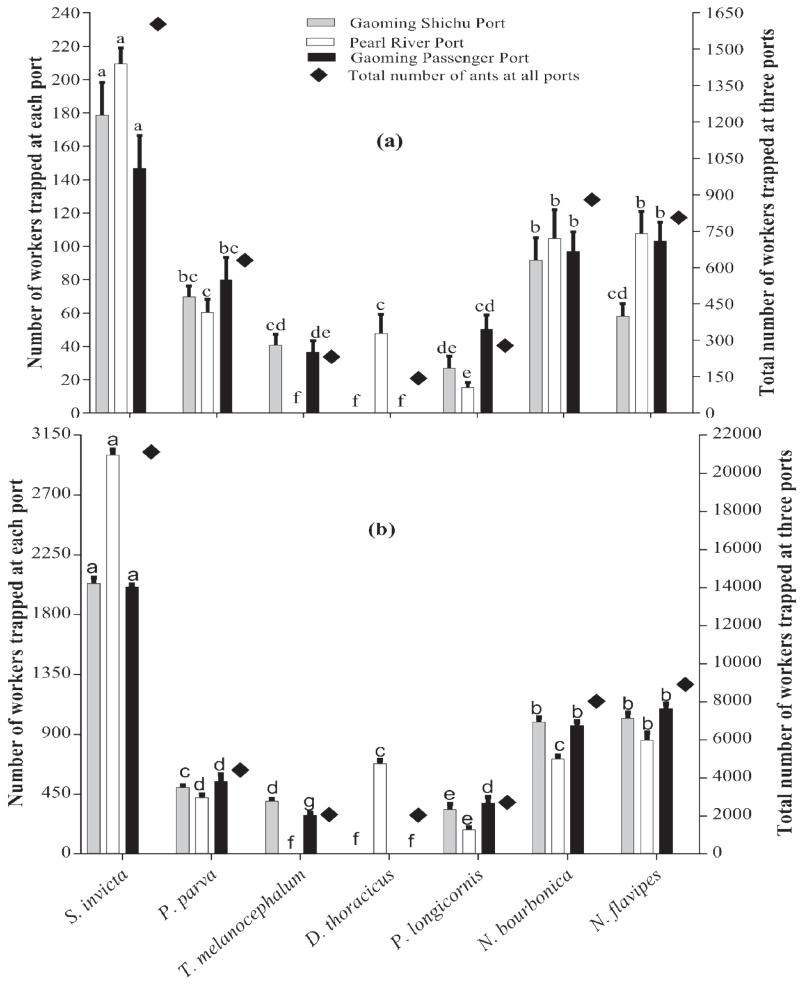


Fig. 3. Ant species and total number of individual ants collected from three ports using pitfall (a) and bait traps (b)

DISCUSSION

Although the total number of consignments imported at Gaoming Port was similar each year from 2014 to 2017 (~55,800 ± 4100), the interception of ants in association with the different consignment carriers varied significantly among years. These differences may be associated with the variation in traded goods across the different years.

Higher number of inspected consignments were from Southeast Asia, and these countries were the main source of the ants intercepted during our study. There are likely to be a variety of reasons for this. First, Southeast Asian countries are located in the biodiversity-rich tropics; therefore, goods originating from these countries have a higher probability of containing exotic taxa (Myers *et al.* 2000). Second, these countries are closer to Guangdong province, and the transport time is shorter, which reduces the probability of mortality among the carried ants during transportation. Third, the shipments coming from Southeast Asian countries mostly contain fresh fruits, such as mangosteen. Ants, such as *D. thoracicus*, can build nests in mangosteen and obtain water and nutrients by feeding on the fruits. This enhances the probability of the transport of ants with these consignments, their intermingling with goods and their survival during transportation (Pellizzari & Porcelli 2014). Although the number of inspected consignments originating from

European countries were limited, the probability of ants being intercepted was higher in these consignments compared to South Asian and other countries.

Among the intercepted ants, *D. thoracicus* was the most frequently intercepted species during quarantine inspection, and fruit was its main carrier, likely due to its nesting habits in orchards (Pellizzari & Porcelli 2014). Three major global invasive and harmful species, *S. invicta*, *S. geminata* and *A. gracilipes* were also detected but the number of interceptions of these species was relatively small. It should be noted that all the interceptions of *S. invicta* originated from consignments from Hong Kong. These consignments mostly contained waste plastic and hardware material. Previous contact with the ground or removal from the ground, where fire ants are most abundant, might be the reason for the interception of these ants from these consignments. Therefore, understanding the global distribution, association with plants and biological characteristics of exotic ants could be helpful for their effective interception during quarantine inspection at ports.

Surveys of the diversity of ants at the ports showed that *S. invicta*, *N. bourbonica* and *N. flavipes* were dominant in terms of abundance. Higher abundance of these ants may be due to the presence of favorable habitats near the ports. For example, disturbed habitats and beaches near the ports can favors *N. bourbonica* and *S. invicta*. Both species prefer to live in disturbed areas and in the upper zones of beaches (Stiles 1998, Wetterer *et al*. 2007; Deyrup 2017). Similarly, the presence of parks and trees near the ports can favor *N. flavipes*, which usually prefers to live in the soil associated with grasslands and trees (Yamaguchi 2005; Pecarevic *et al*. 2010). Among these species, *S. invicta* has been reported to replace local ant species with overlapping or even similar niches, resulting in a decrease in biodiversity in invaded areas (Morrison 2002; Wetterer *et al*. 2006). The frequent transport of these ants in imported goods may also be another reason for the large number of exotic near ports. Our survey results indicate that the habit of nesting in orchards of *D. thoracicus*, which was frequently intercepted during sampling, facilitates the movement of this species through consignments carrying fruits. Thus this species was frequently detected in consignments carrying fruits.. Previous studies also showed that most exotic ant species are disturbance specialists and are therefore particularly well adapted to urban environments (Silverman 2005; Tschinkel 2006; Sanford *et al*. 2009). Their presence near ports might indicate introduction via trade or that they have colonized the urban habitats surrounding ports independently from a different source (Yamaguchi 2005; Sakamoto *et al.* 2016). In addition, our field survey was carried out from September to December, which may limit the number of ant species collected due to lower foraging activity in this season (Vogt *et al*. 2003; Toft 2010). Furthermore, the methodology we implemented might also have affected the results, e.g., the size of pitfall tubes and the concentrations of ethyl alcohol may also affect collection results (Sheikh *et al*. 2018).

Border control is the major line of defense for impeding the establishment and spread of exotic ants (Bacon *et al.* 2012). The results of the current study have important implications for the understanding of secondary spread and invasion of ants. Based on our interception data and port investigation results, we can assert the following: 1) Ants are dispersed through goods being transported through ports, which thus represents a potential source of introduction. However, to support this conclusion, further research is needed to distinguish between the interception of workers and that of queens/gynes, which can establish new populations around the port. 2) Among the goods, fruits and wooden logs were the major carriers of ants; therefore, specific attention and inspection of consignments containing these types of goods is needed. 3) The conditions surrounding ports are potentially favorable for the establishment of newly dispersed invasive species (*S. invicta*, *P. longicornis*) and some known for their affinity to disturbance (*T. melanocephalum*, *N. bourbonica*). Monitoring schemes to regulate accidental transport of transferred species will be more effective if we have information about ant origins and spreading habits. Therefore, it is necessary to pay more attention to the form of commerce at port quarantine. Transport of fruits and wooden material were the main carriers of ants. Therefore, care should be taken while transporting such material. Furthermore, quarantine

authorities and researchers should collaborate to carry out continued monitoring of areas near the ports. This would be helpful to prevent accidental transport of invading ants and for the detection of the initial stages of invasion and thus help with early eradication.

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