



Morphological and DNA Barcode Identification of the *Delottococcus confusus*, (Hemiptera: Pseudococcidae), an invasive alien species to China.

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Abstract: This study was initially carried out based on an unidentifiable mealybug in the fresh-cut flowers bought in the market. Using morphological and DNA barcoding techniques, the mealybug was finally identified as *Delottococcus confusus*. Native to South Africa, it is the main pest of *Proteaceae* plants and has been banned from entry in many countries due to its damage to citrus. As of 2021, the area planted with citrus in China has exceeded 2,617,333 hectares. Therefore, the invasion of *Delottococcus* species will seriously threaten the development of the citrus industry in China. Mealybugs are small in size and tend to be hidden, making them very easy to spread via the fresh-cut flowers of *Proteaceae* plants. It's imperative to strengthen the quarantine of nursery stock, cut flowers and fruits to prevent pests from entering China and harming the ecological safety of agriculture and forestry.

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1. Introduction

On Father's Day 2021, the author accidentally found a mealybug in the fresh-cut flowers, which was finally identified as *Delottococcus confusus* by morphological characteristics and DNA barcoding. At present, nine species of mealybugs in this genus (Mill D.R. and Giliomee J.h., 2011) have been reported. They are major pests of the family Proteaceae and some cash crops such as citrus (Millar I.M., 2002). According to China's National Bureau, the planting area of citrus in China reached 2,617,333 hectares in 2019, with an output of 45.85 million tons, and citrus became the most popular fruit in China (Li, 2021). Because of mealybugs are small and have high reproductive rates, the invasion of *Delottococcus species* will severely threaten the development of the citrus industry in China, further affecting citrus exports, as well as poverty alleviation through industry development and citrus branding. *Delottococcus confusus* is currently recognized as an entry quarantine pest by Japan and Korea and as a

regulated pest by the European and Mediterranean Plant Protection Organization by reason of its invasiveness. Mealybugs are small in size and tend to be hidden, making them very easy to spread via the fresh-cut flowers of *Proteaceae* plants. It's imperative to strengthen the quarantine of nursery stock, cut flowers and fruits to prevent pests from entering China and harming the ecological safety of agriculture and forestry.

In this paper, the distribution, host and morphological characteristics of the pest were introduced, and a key identification of approximate species was prepared for the reference of plant quarantine personnel.

2. Material and methods

2.1 Sample collection

The mealybugs in *Leucospermum nutans* bought on the market were collected (Table 1). The sample was preserved in 95% ethyl alcohol and kept in a refrigerator at -20°C for standby use.

Table 1. Mealybugs used in this study and COI or 28S sequences used for analysis

No.	Species	GenBank no.	
		mDNA COI	28S rDNA
1	<i>Dysmicoccus neobrevipes</i>	KY373089.1	AY427323.1
2	<i>Dysmicoccus brevipes</i>	KP875974.1	AY427321.1
3	<i>Dysmicoccus lepelleyi</i>	KY372729.1	KX015065.1
4	<i>Ferrisia virgata</i>	MN901462.1	AY179454.1

5	<i>Maconellicoccus hirsutus</i>	KY372938.1	KY211356.1
6	<i>Phenacoccus solenopsis</i>	MG437496.1	KJ461274.1
7	<i>Phenacoccus solani</i>	KP692629.1	KP692379.1
8	<i>Planococcus citri</i>	JF714198.1	KY211353.1
9	<i>Planococcus lilacinus</i>	KY373178.1	KY211352.1
10	<i>Planococcus minor</i>	MT707315.1	KY211346.1
11	<i>Pseudococcus longispinus</i>	KY372655.1	MG866179.1
12	<i>Pseudococcus baliteus</i>	KU056834.1	KY211337.1
13	<i>Pseudococcus cryptus</i>	KP692670.1	KY211358.1
14	<i>Pseudococcus comstocki</i>	KP692667.1	KY211360.1
15	<i>Pseudococcus jackbeardsleyi</i>	KP981087.1	KX015052.1
16	<i>Delottococcus confusus</i>		this study
17	<i>Delottococcus confusus</i>	KP771952.1	KP771927.1
18	<i>Delottococcus confusus</i>	KP771953.1	KP771931.1
19	<i>Delottococcus aberiae</i>	/	JF714185.1
20	<i>Delottococcus aberiae</i>	/	JQ651344.1
21	<i>Coccus hesperidum</i>	KY085153.1	MK53322.1

2.2 DNA extraction and slide preparation

To ensure consistency between the mealybug slide specimen for morphological identification and the template genomic DNA for PCR amplification, the non-destructive genomic DNA extraction method of Ye et al. (2016) was used as the reference for the extraction of total DNA in this study. The specific extraction steps were as follows: the mealybug preserved in 95% ethanol alcohol was cleaned with sterile water three times and placed under the dissecting microscope. A small incision was made in the center of the posterior surface of the cephalothorax, approximately 1/4 or less of the length of the polypide, using a dissecting needle knife. The polypide was then placed in a 1.5ml centrifuge tube containing 20µL Proteinase K, which was then placed in the metal bath for digestion overnight at 56°C. The next day, the centrifuge tube was removed using a lifting ring and placed on a concave dish for the preparation of slide specimen. The remaining digestion solution was used for extraction of mealybug genomic DNA. QIAGEN DNeasy Blood & Tissue kit DNA was used to extract monocephalic mealybug genomic DNA according to the requirements of the specification. The BioDrop DUO UV Spectrophotometer was used to measure the concentration and mass of the mealybug DNA, which was then stored in a refrigerator at -20°C for standby use.

The retrieved specimens were prepared for slide mounting and vouchering according to the method of Williams D.J. (2004). Morphological identification was done using relevant published keys for scale insects.

2.3 PCR amplification, detection, and sequencing

The *COI* region was amplified using the primers PcoF(CCTTCAACTAATCATAAAAA TATYAG) and LepR (AAACTTCTGGATGTC

CAAAAAATCA) (Tang et al., 2019), and the 28S-F (AGAGAGAGTTCAAGAGTACGTG) and 28S-R (TTGGTCCGTGTTTCAAGACGG G) (He et al., 2011) were used to amplify the 28S region. 25µL PCR reaction system: 12.5µL TakaRa Premix TapTM Version 2.0 plus dye, 1µL (10µmol/L) of forward and reverse primer respectively, 2µL (10-30ng) of template, and 8.5µL of sterile ddH₂O. Amplification procedure: 94°C 4min; 94°C 40s, 51°C 40s, 72°C 50s, with 35 cycles; 72°C 10min. The PCR products were electrophoresed in 1.2% agarose gel (voltage: 150V; electrophoresis time: 25min) and then placed on the gel imager to observe the results. PCR products were entrusted to BGI Genomics for purification and bidirectional sequencing. Bidirectional sequence assembly was performed on the obtained sequences using BioEdit version 7.0.5 and proofread manually to obtain the mDNA *COI* sequence and the rDNA 28S sequence of mealybugs. The sequences obtained were compared with those released by GenBank to verify the consistency between the molecular detection results and the morphological identification.

The homologous sequence of rDNA 28S of *Delottococcus confusus* and the approximate species *Delottococcus aberiae* was downloaded from GenBank. The GenBank accession numbers were KP771931.1, KP771927.1, JQ651344.1, and JF714185.1, respectively. The homologous sequence of mDNA *COI* of *Delottococcus confusus* was downloaded and the Genbank accession number was KP771952.1. The mDNA *COI* homologous sequences of similar species of *Delottococcus confusus* have not been found. GenBank accession numbers corresponding to the *COI* or 28S sequences used for this analysis are indicated in Table 1.

2.4 Data analysis

MEGA 6 was used to perform the comparative analysis on the assembled sequences. *Coccus hesperidum* was used as an outgroup to construct a phylogenetic tree by Neighbor-joining (NJ) based on the Kimura-2-parameter model, and the genetic distance was calculated. The Bootstrap Method was used to determine the relative bootstrap value of the evolutionary branches of the phylogenetic tree, with a bootstrap coefficient of 1000.

3. Results

3.1 Morphological identification

Body of adult female is gray (Figure 1), with four pairs of lateral filaments, posterior-most pair is longest. Feeding occur on the undersides of leaves. Morphological characteristics of female adults (Figure 2):

Dorsum. 18 pairs cerarius, with sclerotic anal lobe cerarius. The trilocular pores are distributed in rows in each segment, and the single pores are around the trilocular pores. Oral-rim tubular ducts, abundant over most of surface, are distributed in rows in the abdominal segments, with 24 and 14 on abdominal segments V and

VII respectively, and usually absent in medial areas of thorax, with one or more ducts near position of cerarius 13 (C_{13}) and frontal cerarius. The dorsal setae are variable, with the longest one on the 7th abdominal segment, 28 μ m in length. The lobe setae are 230 μ m in length, which is about 1.9 times the diameter of the anal ring.

Venter. Antennae has 8-segmented and apical segment usually partially divided. Trilocular pores and the single pores are distributed in rows in each segment. Multilocular disk pores are mainly distributed in segments V-VIII, and present on anterior margins of segments VII and VIII, rarely segment IV, usually absent from thorax and submarginal pores. Oral-rim tubular ducts smaller than on dorsum, with 21 on each side of body from anterior spiracle to segment II, and there is usually one duct on head near of frontal cerarius. There are two sizes of oral-collar tubular ducts, and the large ones are distributed in groups near body margin, and the small ones present in medial and mediolateral areas of thorax and abdomen, without oral-collars near C_{12} and C_{13} . Hind femur lacks transparent pores, but hind tibia has. Hind tibia and hind tarsus are 315 μ m and 112 μ m long respectively, at a ratio of 2.8.



Figure 1. *Delottoccus confusus* found in *Leucospermum nutans*

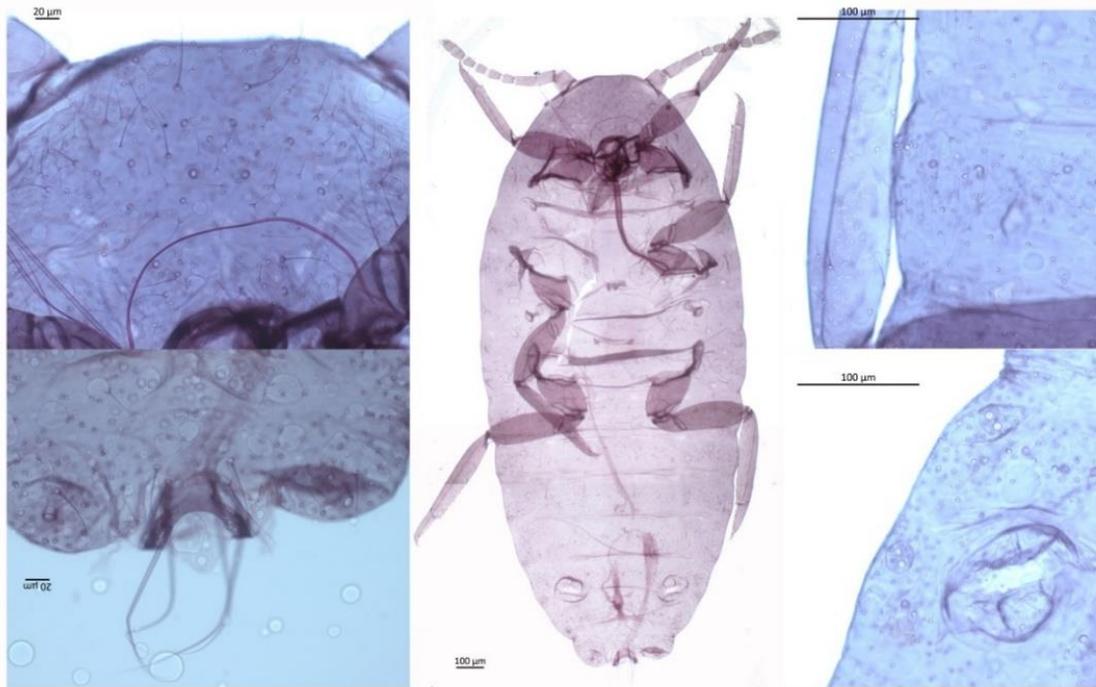


Figure 2. Slide figure of *Delottococcus confusus* (photographed by Zhao Lang)

3.2 Comparison with similar species

Morphological feature of *Delottococcus*: antennae 8-segments; 9-18 pairs of cerarius, with more than 2 conical spines of each cerarius in the abdomen as well as accessory seta; anal lobe on the venter has an anal bar; the ostiole on the dorsum is distinct; with 6 anal ring hairs; without circulus on the venter; the disc pores are usually distributed on the venter, with trilocular pores; the multilocular disc pores are usually distributed on the middle venter, often without a sub-margin; well-developed feet, without teeth under the lower surface of the claw; the hind tibia has translucent pores, but the hind coxa has; there are oral-rim tubular ducts, with an indistinct periphery, and the oral-rim tubular ducts on the venter are smaller than those on the dorsum. The key to species of *Delottococcus* (adult females) is as follows (Cox J.M. and Ben-Dov. Y., 1986; Mill D.R. and Giliomee J.h., 2011):

- 1 Translucent pores present on hind femur.....2
- Translucent pores absent from hind femur.....7
- 2 Without oral-collar tubular ducts laterad of anterior spiracle.....3
- With one or more oral-collar tubular ducts laterad of anterior spiracle.....4
- 3 The translucent pores of the hind femur are less than 50, and the 3rd segmental venter lacks multilocular pores.....*D. phylicus*

- The translucent pores of the hind femur are more than 50, and the 3rd segmental venter has multilocular pores.....*D. confusus* (in part)
- 4 The anterior margin of the 4th or the 5th abdominal segment lacks multilocular pores, and the multilocular pores at the thorax are more than 10.....5
- The anterior margin of the 4th or the 5th abdominal segment has multilocular pores, and the multilocular pores at the thorax are more than 10.....*D. millari*
- 5 Multilocular pores absent form submarginal areas of abdominal segments.....6
- Multilocular pores present in submarginal areas of some abdominal segments*D. quaesitus*
- 6 Thoracic and head cerarian setae not unusually elongate; usually with 15–18 pairs of cerarii.....*D. aberiae*
- Some of thoracic and head cerarian setae unusually elongate; with less than 15 pairs of cerarii.....*D. proteae*
- 7 With fewer than 20 multilocular pores in submarginal areas of abdomen.....8
- With more than 20 multilocular pores in submarginal areas of abdomen *D. euphorbiae*
- 8 The translucent pores of the hind tibia are less than 70, and the tibia is not swollen.....9
- The translucent pores of the hind tibia are more than 70, and the tibia is conspicuously swollen.....*D. confusus* (in part)

9 Antennal length about 500µm or more; 20–26 setae on hind femur; cerarian setae on head and thorax noticeably more conical than dorsal setae.....

.....*D. trichiliae*

Antennal length usually about 400µm or less; 13–20 setae on hind femur; some cerarian setae on head and thorax slender, similar in shape to dorsal setae.....*D. elisabethae*

3.3 Molecular biological identification

DNA barcode identification was carried out simultaneously in this study to further prove the accuracy of the result of morphological identification. Without affecting the preparation of the mealybug slide, DNA was subject to non-destructive extraction and PCR Amplification. Based on mtDNA *COI* and

rDNA *28S* gene sequences, the phylogenetic trees were built using the Neighbor-Joining Method to identify the *Delottococcus confusus*.

3.3.1 Analysis of *COI* and *28S* gene sequences

The electrophoresis result shows that the amplification of universal primers for mtDNA *COI* and *28S* gene sequences of the mealybug yielded the monospecific bands (Figure 3). The PCR products were purified, sequenced, and spliced, and the size of an amplified band of *COI* genes was 649 bp and that of an amplified fragment of *28S* genes was 340 bp. The comparison of such sequences with that of the *Delottococcus confusus* in the GenBank indicated that the homology was above 99%. Thus, the insect could be identified as *Delottococcus confusus*.

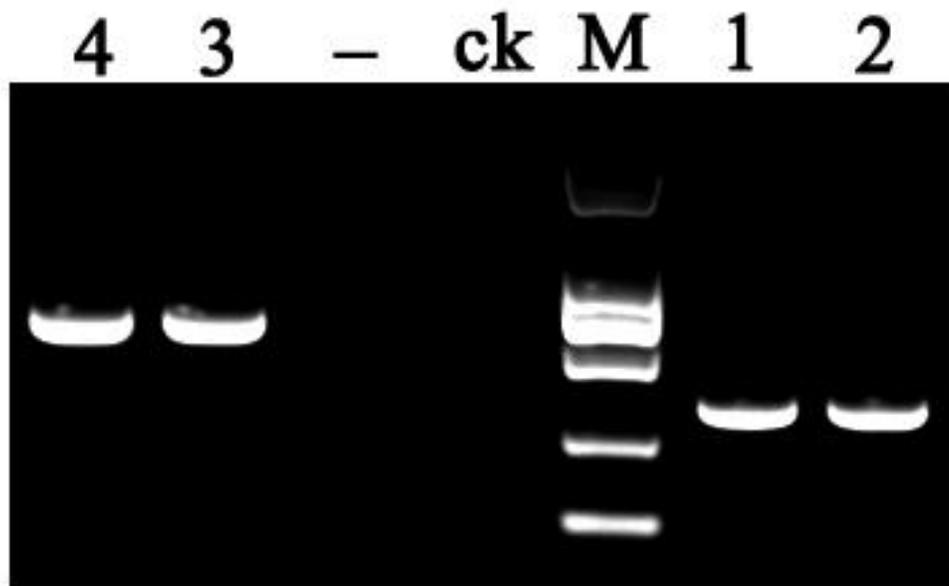


Figure 3. PCR amplification and electrophoretogram

M: DL 2000 marker; CK: blank control; —: negative control; 1 and 2: amplification using primers 28S-F/28S-R; 3 and 4, amplification using primers PcoF/LepR.

3.3.2 Phylogenetic analysis of mealybugs based on *COI* and *28S* genes

The mtDNA *COI* and rDNA *28S* gene sequences, as well as the homologous sequences in the GenBank were clustered with other populations of the family Pseudococcidae in Table 1, with *Coccus hesperidum* as the outgroup to build phylogenetic trees using the NJ Method (Figure 4 and Figure 5). The results show that the phylogenetic trees of mealybugs based on *COI* and *28S* genes were similar and both of them could help distinguish *Delottococcus confusus* discovered in the *Leucospermum nutans*. Moreover, the phylogenetic trees can be clustered into one branch with the

homologous sequences in the GenBank. Then, the morphological results have been proved to be accurate.

The results in Table 2 show that the mealybug specimen of the *Leucospermum nutans* keeps the closest genetic distance from the *Protea magnifica* (KP771931.1) and *Leucadendron argenteum* (KP771927.1) from South Africa, i.e., 0.009, and keeps a genetic distance of 0.023 from the *Delottococcus aberiae* of the same genus. Results in Table 3 show that the mealybug specimen of the *Leucospermum nutans* maintains the closest genetic distance from the *Leucadendron argenteum* (KP771952.1) from South Africa, i.e., 0.003.

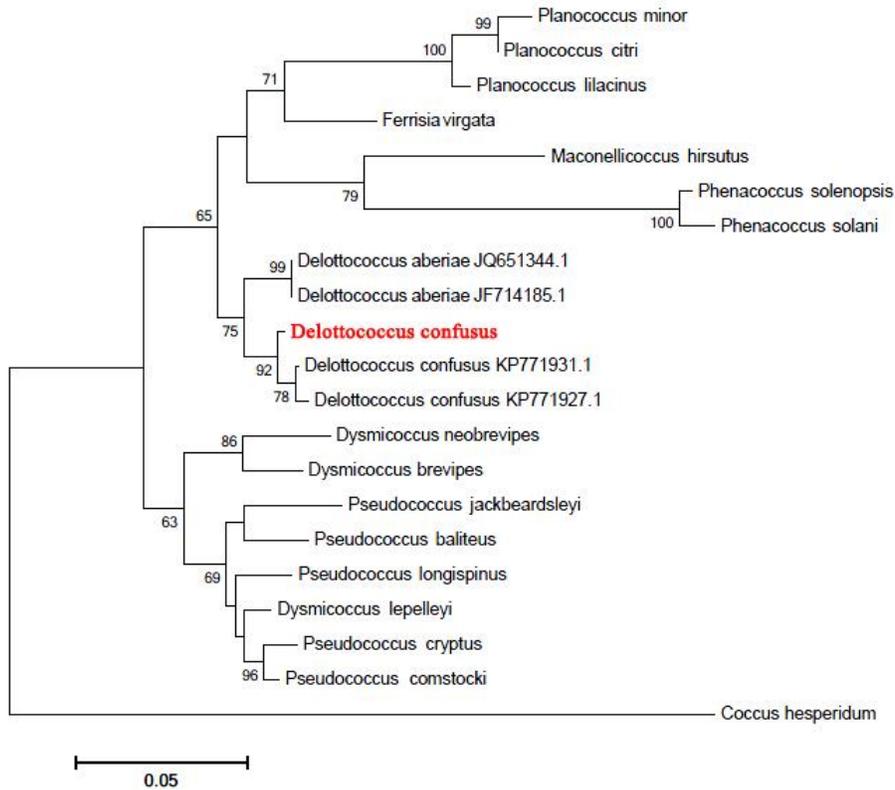


Figure 4. NJ phylogenetic tree based on 18 species of rDNA 28S sequences

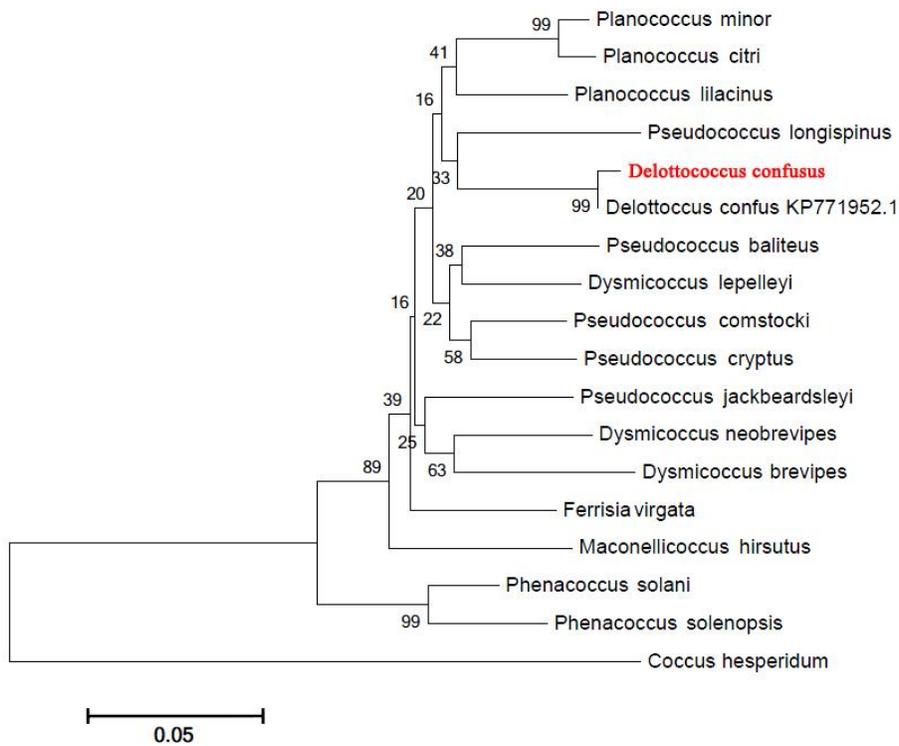


Figure 5. NJ phylogenetic tree based on 17 species of mtDNA COI sequences

Table 2. Genetic distances of 28S gene sequences of 18 species of mealybugs

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
1	<i>Ps.ongispinus</i>																					
2	<i>Ps.jackbeardsleyi</i>	0.052																				
3	<i>Ps.cryptus</i>	0.033	0.052																			
4	<i>Ps.comstocki</i>	0.028	0.047	0.014																		
5	<i>Ps.baliteus</i>	0.047	0.047	0.047	0.042																	
6	<i>Pl.minor</i>	0.146	0.176	0.169	0.163	0.157																
7	<i>Pl.ilacinus</i>	0.124	0.152	0.146	0.140	0.146	0.028															
8	<i>Pl.citri</i>	0.135	0.164	0.157	0.152	0.146	0.009	0.018														
9	<i>Ph.solenopsis</i>	0.200	0.194	0.226	0.220	0.227	0.187	0.164	0.175													
10	<i>Ph.solani</i>	0.207	0.213	0.233	0.226	0.234	0.193	0.170	0.181	0.014												
11	<i>Ma.hirsutus</i>	0.154	0.154	0.172	0.166	0.173	0.188	0.165	0.176	0.148	0.154											
12	<i>Fe.virgata</i>	0.093	0.103	0.114	0.109	0.098	0.092	0.087	0.082	0.170	0.176	0.136										
13	<i>Dy.neobrevipes</i>	0.072	0.093	0.077	0.072	0.092	0.169	0.152	0.163	0.218	0.225	0.153	0.130									
14	<i>Dy.lepellei</i>	0.028	0.052	0.023	0.019	0.028	0.157	0.146	0.146	0.226	0.233	0.172	0.103	0.067								
15	<i>Dy.brevipes</i>	0.072	0.072	0.067	0.057	0.072	0.169	0.157	0.157	0.211	0.218	0.158	0.113	0.042	0.057							
16	<i>De.confusus</i> KP771931.1	0.077	0.093	0.093	0.088	0.088	0.108	0.092	0.097	0.170	0.176	0.120	0.077	0.083	0.088	0.093						
17	<i>De.confusus</i> KP771927.1	0.082	0.093	0.098	0.093	0.093	0.113	0.097	0.102	0.170	0.176	0.120	0.077	0.088	0.093	0.093	0.005					
18	<i>De.aberiae</i> JQ651344.1	0.083	0.088	0.088	0.093	0.093	0.119	0.097	0.108	0.147	0.152	0.109	0.082	0.083	0.093	0.082	0.033	0.033				
19	<i>De.aberiae</i> JF714185.1	0.083	0.088	0.088	0.093	0.093	0.119	0.097	0.108	0.147	0.152	0.109	0.082	0.083	0.093	0.082	0.033	0.033	0.000			
20	<i>De.confusus</i>	0.077	0.083	0.093	0.088	0.088	0.108	0.092	0.097	0.158	0.164	0.109	0.067	0.083	0.088	0.082	0.009	0.009	0.023	0.023		
21	<i>Co.hesperidum</i>	0.286	0.313	0.293	0.286	0.285	0.347	0.326	0.333	0.358	0.358	0.353	0.320	0.292	0.272	0.306	0.306	0.313	0.299	0.299	0.306	

Table 3. Genetic distances of COI gene sequences of 17 species of mealybugs

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
1	<i>Fe.virgata</i>																		
2	<i>Pl.minor</i>	0.091																	
3	<i>Ps.baliteus</i>	0.103	0.102																
4	<i>Dy.neobrevipes</i>	0.102	0.105	0.085															
5	<i>Ma.hirsutus</i>	0.109	0.120	0.102	0.112														
6	<i>Ph.solani</i>	0.122	0.135	0.124	0.124	0.133													
7	<i>Dy.brevipes</i>	0.103	0.118	0.105	0.091	0.127	0.126												
8	<i>Dy.lepellei</i>	0.098	0.085	0.073	0.087	0.107	0.129	0.103											
9	<i>Pl.ilacinus</i>	0.084	0.070	0.093	0.094	0.096	0.116	0.105	0.085										
10	<i>Ph.solenopsis</i>	0.131	0.146	0.141	0.131	0.150	0.054	0.148	0.144	0.137									
11	<i>Ps.longispinus</i>	0.102	0.096	0.100	0.122	0.120	0.141	0.129	0.098	0.105	0.156								
12	<i>De.confusus</i>	0.091	0.085	0.105	0.118	0.120	0.139	0.122	0.102	0.087	0.156	0.098							
13	<i>Ps.comstocki</i>	0.080	0.087	0.082	0.093	0.096	0.137	0.113	0.071	0.077	0.144	0.096	0.078						
14	<i>Ps.cryptus</i>	0.094	0.091	0.077	0.089	0.103	0.137	0.120	0.070	0.068	0.143	0.094	0.096	0.058					
15	<i>Pl.citri</i>	0.093	0.019	0.105	0.109	0.124	0.141	0.124	0.089	0.071	0.148	0.096	0.085	0.084	0.087				
16	<i>Ps.jackbeardsleyi</i>	0.087	0.091	0.100	0.096	0.114	0.111	0.096	0.093	0.087	0.139	0.112	0.109	0.089	0.093	0.093			
17	<i>De.confusus</i> _KP771952.1	0.087	0.078	0.098	0.111	0.112	0.131	0.114	0.094	0.080	0.148	0.093	0.006	0.077	0.093	0.082	0.102		
18	<i>Co.hesperidum</i>	0.319	0.346	0.348	0.343	0.331	0.326	0.353	0.348	0.333	0.328	0.374	0.366	0.343	0.363	0.348	0.331	0.356	

3.4 Name and taxonomic status

Scientific name: *Delottococcus confusus* (De Lotto, 1977).

Synonyms: *Allococcus confusus* De Lotto 1977, *Delottococcus confusus* Cox & Ben-Dov 1986.

Taxonomic status: Hemiptera, Coccoidea, Pseudococcidae, *Delottococcus*.

3.5 Geographical distribution and host plants

Delottococcus confusus originated in Southern Africa and has now spread to Hawaii and California in the United States. There are 14 families and 15 genus of host plants reported.

Apocynaceae: *Carissa*, *Carissa macrocarpa*;

Asteraceae: *Osteospermum moniliferum* subsp. *moniliferum*;

Bruniaceae: *Berzelia lanuginosa*;

Fabaceae: *Acacia*;

Iridaceae: *Bobartia orientalis*;

Lamiaceae: *Plectranthus*;

Meliaceae: *Trichilia*, *Trichilia emetica*;

Monimiaceae: *Xymalos monospora*;

Myrtaceae: *Psidium guajava*;

Proteaceae: *Protea*, *Protea cynaroides*, *Protea caffra*, *Leucadendron*, *Leucadendron arcuatum*, *Leucospermum nutans*;

Rubiaceae: *Canthium*;

Rutaceae: *Citrus*;

Sapotaceae: *Mimusops*, *Mimusops caffra*;

Solanaceae: *Lycium*, *Lycium tetrandrum*;

3.6 Biological characteristics

A single female *Delottococcus confusus* has a high fecundity and reproduces by parthenogenesis, with overlapping generations of populations. The adult females and nymphs of the piercing-sucking insects left on plants feed on the shoots, leaves, flower buds, and petioles, resulting in weak and slow growth of the affected plants or causing them to dry up and die. In addition, the insect secretes a lot of honeydew when feeding, which further induces sooty mold and affects the photosynthesis of the host plant. As a result, the infested host may be beset by poor growth, early defoliation and abscission of fruits, and lower production.

4. Discussions

Delottococcus spp. was established by Cox & Ben-Dov in 1986 to honor the contributions of Mr. De Lotto Givoanni, an insect taxonomist from South Africa, to the classification of scale insects (Cox J.M. and Ben-Dov. Y., 1986). The genus originated from the discovery of *Allococcus inamabilis* (Hambleton) in Brazilian cypresses. In 1956, Ezzat & McConnell classified mealybugs with oral-rim tubular ducts in

Planococcus and created *Allococcus* which took *A. inamabilis* as the type specimen. In later studies, however, *A. inamabilis* was found to be *P. vovae*, as a matter of fact. As *A. inamabilis* was the type specimen of *Allococcus*, the existence of *Allococcus* was questioned. In addition, De Lotto erroneously consider *trichiliae* in *Pseudococcus* to be the type species of *Allococcus* and to place the African taxon of mealybugs of the same origin with this species in this genus in 1997 because of the discovery of tubular ducts (similar to oral-rim tubular ducts) in *Pseudococcus*. In 1986, Cox & Ben-Dov recombed the mealybugs in the genus *Allococcus*, classified the mealybugs that originated in Africa and created the genus *Delottococcus* to distinguish the *Allococcus* species that were still kept at that time.

Delottococcus confusus is a newly discovered invasive species that spreads covertly and causes harm to plants. This study provides the morphological description and DNA barcode identification of *Delottococcus confusus* for the first time. *Proteaceae* plants have become a new favorite on the market of fresh-cut flowers. In particular, the protea is the most popular flower. In the context of growing trade between China and Africa year by year, the discovery of the invasive alien mealybug on the domestic circulation market has reminded us to strengthen the quarantine inspection of seedlings and cut flowers from African countries for fear that pests are introduced into China.

Mealybugs, as tiny insects, are very easy to enter China with imported fruits, fresh-cut flowers, posted succulents, and other plants, posing threats to the ecological security of China's agriculture and forestry. Mealybugs of different groups have highly similar morphological characteristics in different developmental stages, making it difficult to conduct taxonomic identification. DNA barcode, which is both universal and standard, can make up for the deficiencies in morphological taxonomy. Thus, it has been widely applied to the taxonomic identification of species (Ismail I. et al., 2020). According to the research findings, the result of molecular identification is consistent with that of morphological identification, indicating that DNA barcodes can be used as a basis for taxonomic identification of mealybugs. Meanwhile, the phylogenetic trees based on *COI* and *28S* gene sequences have revealed that the *Delottococcus* and the *Planococcus* belong to the same evolutionary branch, which explains why the two were confused in the early days. The *28S* gene sequence is conservative, and the *COI* gene sequence has a high proportion of A+T, with a low rate of base variation. Though the phylogenetic trees built based on the *COI* and *28S* gene sequences could help distinguish the genus of the mealybug family, they are inapplicable to the research on genetic differentiation at a species level. This suggests that it is

necessary to combine more than two DNA barcodes instead of a single one for the taxonomic identification of mealybugs in the future. In addition, this study has also found that both the phylogenetic trees built based on *COI* and *28S* genes grouped the mealybugs of *Dysmicoccus lepelleyi* and *Pseudococcus* into one evolutionary branch. This is inconsistent with the result of morphological taxonomy. Therefore, a further study on the mealybug needs to be conducted to determine its taxonomic status.

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