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Using DNA barcoding for identification of some psyllids (Hemiptera: Psylloidea) intercepted at South Korea ports-of-entry

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Abstract. Quick and accurate identification of intercepted psyllids (Hemiptera: Psylloidea) is an essential requirement for effective pest management and phytosanitary procedures. However, due to lack of morphological characters in the immature stages that can be used to distinguish species, other molecular methods, such as DNA barcoding are proving to be useful. The current study was to designed to generate comprehensive information on the identification of all developmental stages of eight species of psyllids intercepted on consignments of infested fresh cut flowers at the ports of entry in South Korea using DNA barcoding. It is considered that DNA barcoding is a reliable technique for identification of intercepted psyllids for immature stages and will be helpful in the development of more effective pest management options for regulating pest species.

Key words. Invasive psyllids, immature stage, molecular identification, quarantine.

ZooBank registration. urn:lsid:zoobank.org:pub:2044517F-EB5B-4CE0-AEC0-712C555DBC94

Introduction

Interception of potential invasive insect pests at ports-of-entry and their identification to the species level are essential for effective biosecurity and biosurveillance programs (Tahira et al. 2018; Madden et al. 2019). However, the identification of immature insects and availability of information about them is a common and continuing problem for many taxa. The literature available to accomplish this is widely scattered, limited to certain groups, outdated, difficult to use, or non-existent (Stehr 1991). Larval or nymphal recognition of insect pests is essential in the enforcement of quarantine regulations especially at ports of entry of the importing countries.

Many foreign plants enter into South Korea every year. Recently, the amount of fresh cut flowers imported (19,888 cases of quarantine inspection in 2018) has increased due to rising demand in the quantity, quality and diversity of flowers in the country (Gim 2019). Psyllids are the most frequently intercepted pest on these commodities, 114 times between the year 2014 to 2018 (PIS 2020). They are obligate plant feeders, small in size and have an immobile nymphal stage that can easily enter countries undetected on infested plants that are imported. Psyllids therefore constitute a potential threat to crop and ornamental plants (Burckhardt 1994). Some species, such as *Blastopsylla occidentalis* Taylor (Aphalaridae) collected on imported eucalyptus cut branches during phytosanitary inspections, are recorded as important pests of ornamental plants (Halbert et al. 2001; PIS 2020). Unfortunately, approximate 46% of intercepted psyllids are females and/or immature stages that lack diagnostic morphological characters for confident identification (PIS 2020). At present, the only way to identify these psyllids involves collecting additional specimens of adult males or rearing immature nymphs until they are mature enough to permit identification. Discrimination of species that lack a morphological character is a common problem in many pests as well as psyllids.

Molecular methods are now used widely by the taxonomists to solve this problem associated with species identification based on their morphology (Navajas and Fenton 2000). Among these methods, DNA barcoding is the most frequently used technique (Nagoshi et al. 2011; Van der Bank et al. 2012). The barcode involves DNA sequence analysis of a portion of the mitochondrial gene cytochrome c oxidase subunit I (COI) (Hebert et al. 2003). Effective identification of species using short DNA fragments of DNA barcoding requires reliable sequence reference libraries of known taxa. Both taxonomically comprehensive coverage and content quality are important for sufficient accuracy. Although some studies on psyllids have been done, the comprehensive information on morphological and molecular data on psyllids is still lacking. Therefore, the main aim of this study is to generate the morphological and molecular data to identify some psyllids intercepted on imported cut flowers at Korean ports of entry. The comprehensive information on the following eight species of intercepted psyllids is provided: *Blastopsylla occidentalis* Taylor, *Ctenarytaina eucalypti* (Maskell) (Aphalaridae), *Calophya rhois* (Low) (Calophyidae), *Acizzia acaciaebaileyanae* (Froggatt), *Acizzia hakeae* (Tuthill), *Acizzia uncatoides* (Ferris and Klyver), *Cacopsylla nigriantennata* (Kuwayama) and *Livilla retamae* (Puton) (Psyllidae). A barcode reference library for intercepted psyllids as a rapid tool to be used for identification, their plant hosts and origin is necessary to alert inspectors to the possible presence of non-indigenous species and the importance of careful examination of imported cut flowers.

Materials and Methods

Morphological methods. Data on imported cut flowers were collected from the Pest Information System (PIS) database developed by the Animal Plant Quarantine Agency (2020). In total, 301 specimens of psyllids were intercepted during phytosanitary inspections on imported cut flowers and branches from 2014 to 2018. A list presented below contains the identification of specimens to the level of species or genus depending on the specimen quality, the life stage that was intercepted and the state of the current taxonomic knowledge of the taxon. Captured psyllids were examined morphologically using the keys and descriptions of Hodkinson and Hollis (1987), Tuthill (1952), Taylor (1985), Kuwayama (1908) and Hodkinson (2007). Herein the authors provide a brief description and images of major morphological characters of adults. Specimens were curated on microscope slides or triangular points mounted on pins; these were deposited in the Collection of Plant Quarantine Technology Center (CPQTC), Gimcheon, South Korea. Psyl'list was used for taxonomic classification and nomenclature and morphological identifications were made using terminology for morphological structures by Ouvrard (2020) and Hodkinson and White (1979). Photographs were taken using an AxioCam MRc5 camera mounted on a ZEISS Axio Imager M2 Microscope and a Leica M165C microscope with a Spot Flex camera.

Molecular methods. The collection information of psyllid species used in the molecular analyses is as follows (Table 1). The PCR primer C1J1709 (fwd_seq: AATTGGWGGWTTYGGAAAYTG) was paired with HCO2198 (rev_seq: TAAACTTCAGGGTGACCAAAAAATCA) to generate an amplicon of 463 bp of the mitochondrial cytochrome c oxidase subunit I gene (COI) (Folmer et al. 1994; Simon et al. 1994; Martoni et al. 2018). All DNA was extracted from either dried or ethanol-fixed samples using the Qiagen DNeasy Blood and Tissue Kit. PCR

Table 1. Collection details of psyllid species used in the molecular analyses.

Family	Species	Country (cosignment origin), host and date
Aphalaridae	*Blastopsylla occidentalis* Taylor	Italy, on *Eucalyptus cinerea* F. Muell. ex Benth. (Myrtaceae), 19-iii-2018
	Ctenarytaina eucalypti (Maskell)	Italy, on *Eucalyptus tereticornis* Sm. (Myrtaceae), 23-iv-2018
Calophyidae	*Calophya rhois* (Low)	China, on *Cotinus coggygria* Scop. (Anacardiaceae), 9-v-2018
Psyllidae	*Acizzia acaciaebaileyanae* (Froggatt)	Italy, on *Acacia retinodes* Schltdl. (Fabaceae), 26-xi-2018
	Acizzia hakeae (Tuthill)	USA, on *Grevillea* sp. (Proteaceae), 2-iv-2018
	Acizzia uncatoides (Ferris and Klyver)	Netherlands, on *Acacia* sp. (Fabaceae), 17-xii-2018
	Cacopsylla nigriantennata (Kuwayama)	Japan, on *Enkianthus perulatus* C.K. Schneid. (Ericaceae), 17-iv-2017
	Livilla retamae (Puton)	Italy, on *Retama monosperma* (L.) Boiss. (Fabaceae), 9-ii-2018

was performed using the HotStart PCR PreMix (Bioneer, South Korea). PCR thermocycling was done under the following conditions: 5 min at 94°C; 40 cycles of 30 sec at 94°C, 30 sec at 50°C, 60 sec at 72°C; 1 min at 72°C; held at 4°C. Sequences were aligned using MEGA version 7 (Kumar et al. 2016). The barcode sequences generated in present study were compared with sequences submitted by other researchers in GenBank and BOLD System databases for confirmation of the morphometric identifications (Ratnasingham and Hebert 2007).

Results and Discussion

1. *Acizzia acaciaebaileyanae* (Froggatt) (Psyllidae)

(Fig. 1–2)

Psylla acaciaebaileyanae Froggatt 1901.

Korea port interceptions. Intercepted 37 times at Korean ports of entry from France, Italy and Netherlands on cut flowers of *Acacia* plants.

Diagnosis. Adults: General body color yellowish brown. Forewing oblong oval, with a well-developed pterostigma and costal break; transparent with small irregular pale brown diffuse spots. Male proctiger with a short and broad tubular apical projection and with a rounded posterior lobe bearing a subsidiary finger-like projection; paramere simple, broad basally, gradually tapering to rounded apex, with a small denticle on inner posterior margin; aedeagus with a bulbous apex (Hodkinson and Hollis 1987).

Hosts. Fabaceae: *Acacia baileyana* F.Muell., *Acacia dealbata* Link, *Acacia decurrens* Willd., *Acacia podalyriifolia* A.Cunn. ex G.Don, *Acacia* sp., *Samanea saman* (Jacq.) Merr. (Ouvrard 2020).

Distribution. Australia, France, Germany, Italy, New Zealand, Philippines, Slovenia, South Africa, Switzerland, UK, USA (Ouvrard 2020).

DNA barcoding data for diagnosis. 1 attagtgcct ttaataattg gagcccccga tatagctttt ccccgtctta acaacttaag / 61 attttgattg ttaattccat ctttatatct actaattata agaagtttaa ttgatcaagg / 121 ggtcggtaca ggttggacag tctatcctcc tctatcgaac gctatattcc acagaggtta / 181 ttctgtagac atagcaatct tttctttaca tcttgcaggc atttcctcaa ttttaggagc / 241 tattaatttt attaccacaa ttattaatat acgaagatgt ctacataaaa tagaaactct / 301 ccctctattt gtgtgatcag ttttaatcac agcattcctt ctgctactag cattacccgt / 361 ccttgcagga gcaatcacta tgctcttaac agatcgtaat ataaacacta ccttttttga / 421 ccctgccgga ggaggagacc ctattctata tcaacactta ttt (463 bp).

2. *Acizzia hakeae* (Tuthill) (Psyllidae)

(Fig. 3–4)

Psylla (*Acizzia*) *hakeae* Tuthill 1952.

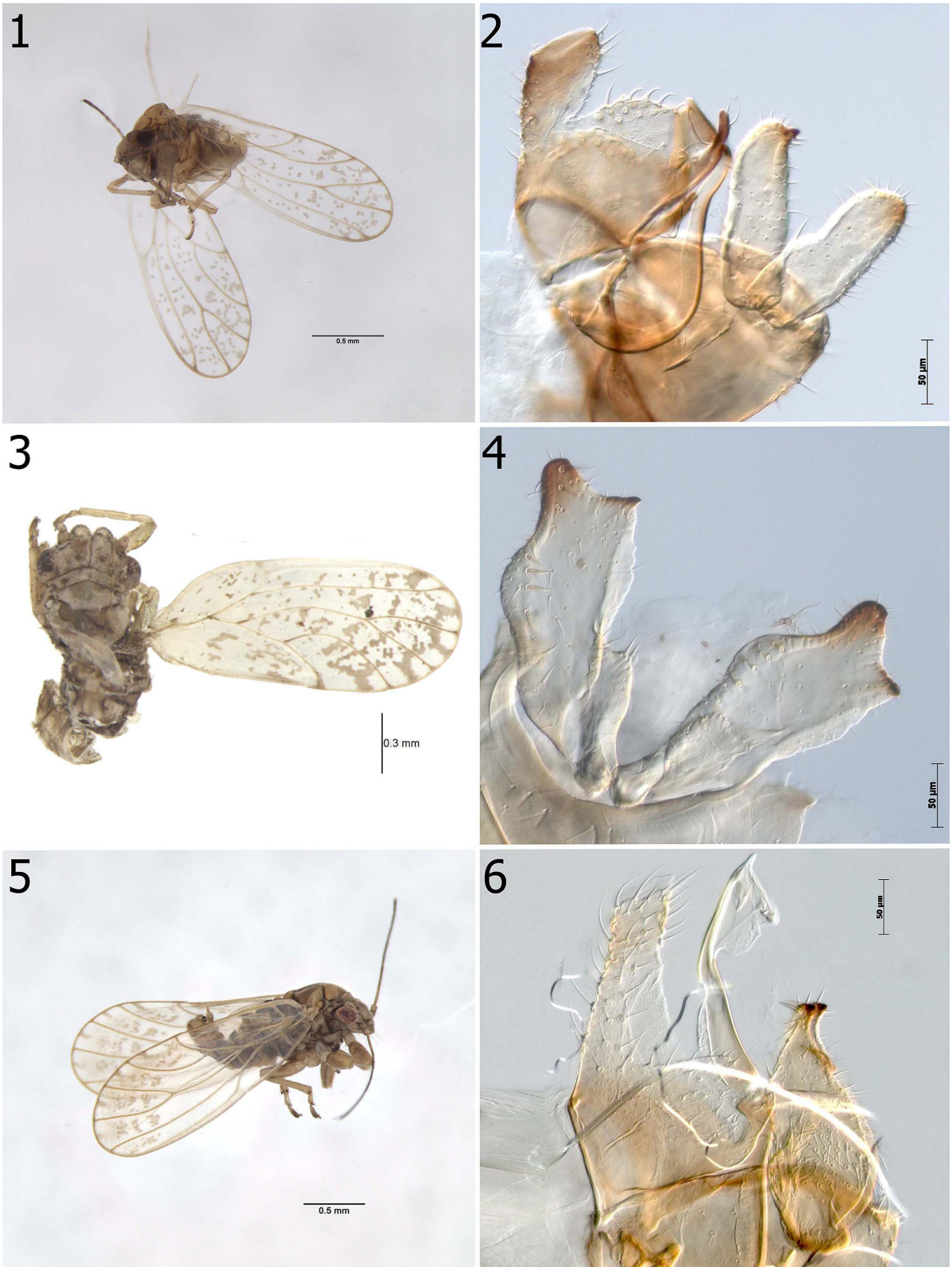
Korea port interception. Intercepted one time at Korean ports of entry from USA on cut flowers of *Grevillea* plants.

Diagnosis. Adults: General body color light reddish brown. Forewings broadly rounded, about twice as long as wide, with dark brown diffuse spots; pterostigma long and narrow, marginal cells large, about equal. Male proctiger strongly produced caudad in basal half, terminating in large, heavily sclerotized hook, apical half slender, cylindrical, apex oblique; paramere in lateral view broad, margins sinuate, apex broadly emarginate, large anteromesal lobe, anterior corner of apex small, sharp, posterior produced as blunt lobe (Tuthill 1952).

Hosts. Fabaceae: *Acacia* sp.; Proteaceae: *Grevillea banksii* R.Br., *Grevillea robusta* A.Cunn. ex R.Br., *Grevillea* sp., *Hakea acicularis* (Sm. ex Vent.) Knight, *Hakea dactyloides* (Gaertn.) Cav., *Hakea suaveolens* R.Br. (Ouvrard 2020).

Distribution. New Zealand, USA (Ouvrard 2020).

DNA barcoding data for diagnosis. 1 acttgtcccc ttgataattg gaactcctga tatagctttt ccccgactta acaatttaag / 61 attctgactt ttaattcctt ccctatattt acttatcata agaagtttaa ttgatcaggg / 121 agtaggaact ggatgaacag tatacccacc tctttcaagt tcaatattcc atagaggata / 181 ctctgttgat atagctatct tttctctcca tcttgctggg atttcctcaa tcttaggggc / 241 aattaacttt atcacaacaa ttattaacat acgaagatgc ctccacaaga tagaaaacct / 301 tcccctgttt gtctgatcgg tattgattac agctttctta ttgctcttag ccctcccagt / 361 attagcagga gctatcacaa tactattaac agaccgaaac ataaacacaa ctttctttga / 421 ccccgctggt ggaggtgacc ctattttata ccaacactta ttc (463 bp).



Figures 1–6. Three species of intercepted psyllids. **1–2)** *Acizzia acaciaebaileyanae* (Froggatt), male. **1)** Forewing. **2)** Genitalia. **3–4)** *Acizzia hakeae* (Tuthill), male. **3)** Forewing. **4)** Paramere of genitalia. **5–6)** *Acizzia uncatoides* (Ferris and Klyver), male. **5)** Forewing. **6)** Genitalia.

3. *Acizzia uncatoides* (Ferris and Klyver) (Psyllidae)

(Fig. 5–6)

Psylla uncatoides Ferris and Klyver 1932.

Korea port interceptions. Intercepted 61 times at Korean ports of entry from France, Italy and Netherlands on cut flowers of *Acacia* plants.

Diagnosis. Adults: General body color orange throughout with paler markings on dorsum of thorax. Forewing membrane pale yellow to pale amber with orange-brown diffuse spots in the apical half. Male proctiger with long tubular apical portion and a rounded posterior lobe bearing a subsidiary finger-like projection; paramere broad, with apex deflexed posteriorly; aedeagus with harpoon-shaped apex (Hodkinson and Hollis 1987).

Hosts. Fabaceae: *Acacia baileyana* F.Muell., *Acacia confusa* Merr., *Acacia dealbata* Link, *Acacia floribunda* (Vent.) Willd., *Acacia heterophylla* (Lam.) Willd., *Acacia koa* A.Gray, *Acacia koaia* W.Hillebrand, *Acacia ligulata* Benth., *Acacia longifolia* (Andrews) Willd., *Acacia melanoxylon* R.Br., *Acacia rivalis* J.M. Black, *Acacia saligna* (Labill.) H.L.Wendl., *Acacia* sp., *Acacia verniciflua* A.Cunn., *Albizia julibrissin* Durazz., *Albizia lophantha* (Willd.) Benth. (Ouvrard 2020).

Distribution. Algeria, Australia, Chile, Colombia, France, Greece, Israel, Italy, Lebanon, Malta, Mexico, Montenegro, New Zealand, Portugal, Spain, UK, USA (Ouvrard 2020).

DNA barcoding data for diagnosis. 1 acttgtacct ttaataattg gagctcctga tatagccttc ccccgtctta ataatttaag / 61 attttggctg ttaatcccct ccctgtactt actaattata agaagtctaa ttgaccaagg / 121 agtaggtaca ggttgaactg tttacccccc tctttcaaac tcaatatttc acagtgggta / 181 ctctgtagac atagcaatct tttctttaca tcttgcggga atctcctcga tcctaggagc / 241 tattaatttt atcactacaa ttatcaatat acgaagatgt ttacacaaaa tagaaaactt / 301 acctttattt gtgtgatctg tattaattac agcattttta ttactattag ctttacccgt / 361 tctagcagga gcaatcacta tactactaac tgaccgaaat ataaacacta cgttctttga / 421 ccctgctggg ggtggagacc caattctata ccaacactta ttt (463 bp).

4. *Blastopsylla occidentalis* Taylor (Aphalaridae)

(Fig. 7)

Blastopsylla occidentalis Taylor 1985.

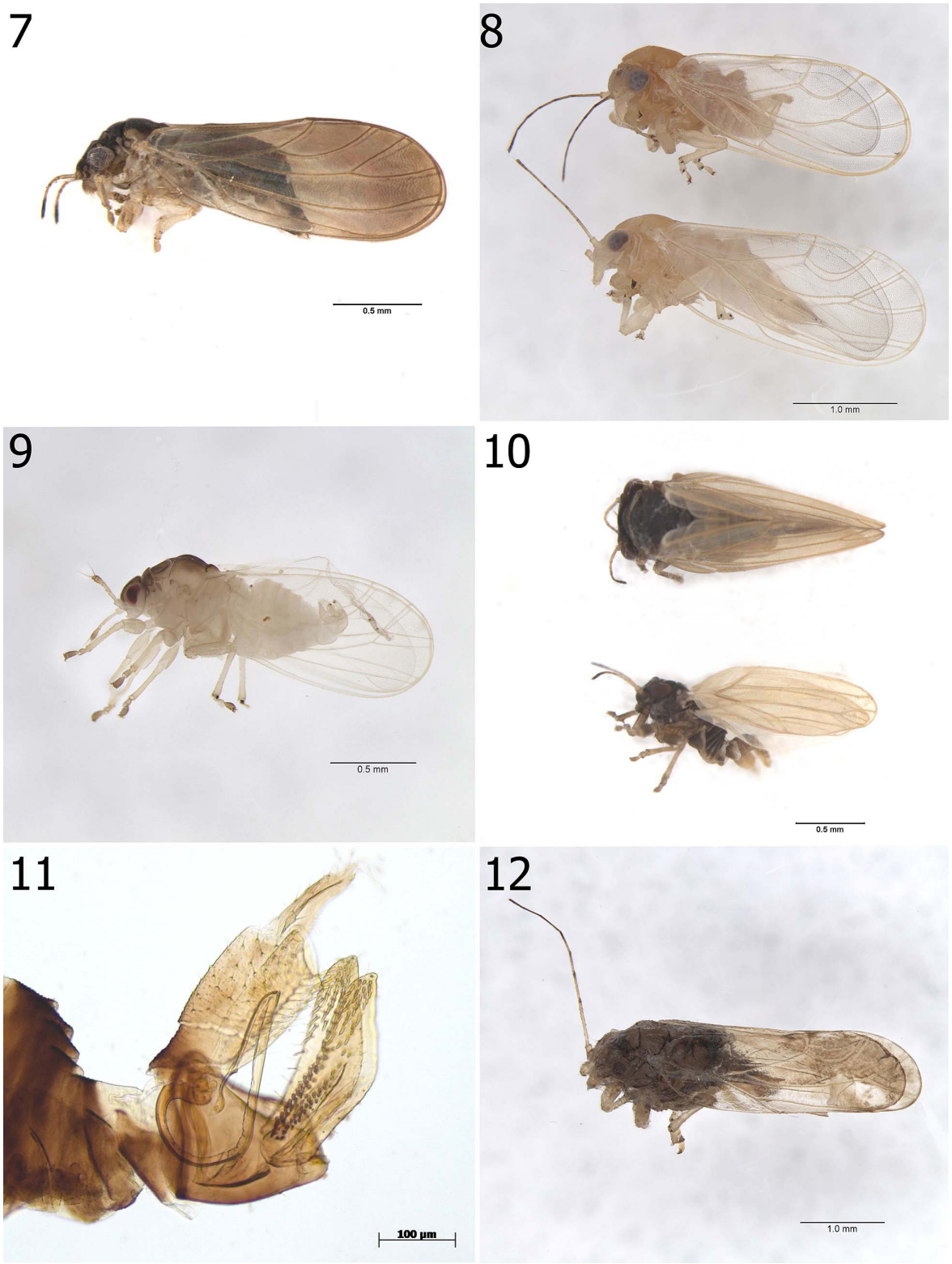
Korea port interceptions. Intercepted three times at Korean ports of entry from Israel, Italy and Netherlands on cut flowers and branches of *Eucalyptus* plants.

Diagnosis. Adults: General body color yellowish green. Forewings elongate, rounded apically; cells dark in the fore of the posterior margin. Head bearing genal cones long projections, less than 0.7× length of vertex. Metacoxae without meracanthi (large spines); proximal (basal) segment of metatarsus with one black spine. Male genitalia distal and proximal segments of proctiger more or less in straight line anteriorly; paramere with 15–18 short black setae apically, 5–7 on anterior margin in distal half, and 2–6 on posterior margin in proximal half (Taylor 1985).

Hosts. Myrtaceae: *Eucalyptus camaldulensis* Dehnh., *Eucalyptus deglupta* Blume, *Eucalyptus forrestiana* Diels, *Eucalyptus globulus* Labill., *Eucalyptus gomphocephala* DC., *Eucalyptus grandis* W. Hill, *Eucalyptus lehmannii* (Schauer) Benth., *Eucalyptus microneura* Maiden and Blakely, *Eucalyptus microtheca* F.Muell., *Eucalyptus nicholii* Maiden and Blakely, *Eucalyptus oleosa* F.Muell. ex Miq., *Eucalyptus platypus* Hook., *Eucalyptus polyanthemos* Schauer, *Eucalyptus rudis* Endl., *Eucalyptus saligna* Sm., *Eucalyptus sideroxylon* A.Cunn. ex Woolls, *Eucalyptus* sp., *Eucalyptus spathulata* Hook., *Eucalyptus tereticornis* Sm., *Eucalyptus urophylla* S.T. Blake (Ouvrard 2020).

Distribution. Argentina, Australia, Brazil, Cameroon, Chile, China, Egypt, Israel, Italy, Kenya, Mexico, New Zealand, Paraguay, Spain, Turkey, USA (Ouvrard 2020).

DNA barcoding data for diagnosis. 1 acttgttcct attataattg gagctcctga tatatctttt cctcgtttaa ataatttaag / 61 attctgatta ttaattccat ctttatattt acttattata agaagtctaa ttgatcaagg / 121 agtaggtact ggatgaacag tttaccctcc tttatctaat agagtatttc atagaggata / 181 ctctgtagat gtagctattt tttctcttca tttagcaggt atttcttcta ttttaggtgc / 241 aattaatttt attactacaa ttattaatat acgatcttct ttatatccta tagaaaaaat / 301 acctttattt gtttgatctg tttttattac tgcttttta ttattatttt ctttaccagt / 361 tttagctgga gctatcacaa tattacttac tgatcgaaat cttaatacat cattttttga / 421 tccagttggg ggaggagacc caattcttta tcaacattta ttt (463 bp).



Figures 7–12. Five species of intercepted psyllids. **7)** *Blastopsylla occidentalis* Taylor, female. **8)** *Cacopsylla nigriantennata* (Kuwayama), female (top) and male (bottom). **9)** *Calophya rhois* (Löw), male. **10–11)** *Ctenarytaina eucalypti* (Maskell), female (top) and male (bottom). **10)** Forewing. **11)** Genitalia. **12)** *Livilla retamae* (Puton), male, forewing.

5. *Cacopsylla nigriantennata* (Kuwayama) (Psyllidae)

(Fig. 8)

Psylla nigriantennata Kuwayama 1908.

Korea port interception. Intercepted one time at Korean ports of entry from Japan on cut flowers of *Enkianthus* plants.

Diagnosis. Adults: Length to tip of folded wings 2.0–2.5mm. General body color yellow to green. Antennae black with 1–2 segments dark brown. Forewing elongate, very pale yellow, transparent with dark yellow veins. Head bearing genal cones long projections, about 1× length of vertex.

Hosts. Ericaceae: *Enkianthus campanulatus* (Miq.) G.Nicholson, *Enkianthus cernuus* fo. *rubens* (Maxim.) Ohwi, *Enkianthus perulatus* C.K.Schneid., *Lyonia ovalifolia* var. *elliptica* (Siebold and Zucc.) Hand.-Mazz. (Ouvrard 2020).

Distribution. Japan (Ouvrard 2020).

DNA barcoding data for diagnosis. 1 gttagtaccc ttaataattg gagccccaga tatagccttc ccccgtttaa acaatcttag / 61 attttgactt ctaattcctt ccttatatct tcttttaatg agaagcttac tagaccaggg / 121 agtagggact gggtgaactg tatatcctcc tttatctaac tcaatatttc atagtggata / 181 ctctgtagac actgctattt tctctttaca tcttgcagga atttcatcaa tcttaggagc / 241 aattaatttt attacaacaa ttattaatat acgaagaaat ctccactcaa tagaaaaatt / 301 acctttattt gtgtgatcag tattaatcac agctttcctt ctccttttag cactccccgt / 361 tttagcagga gccatcacta tacttttaac tgatcgaaat ataaacacta ccttttttga / 421 tccagcagga ggaggagatc ctattttgta tcaacattta ttt (463 bp).

6. *Calophya rhois* (Löw) (Calophyidae)

(Fig. 9)

Psylla rhois Löw 1877.

Korea port interception. Intercepted one time at Korean ports of entry from China on cut flowers of *Cotinus* plants.

Diagnosis. Adults: General body color young specimens head and thorax reddish brown, abdomen green or yellow; older specimens, head and thorax chestnut brown, abdomen red and brown (Hodkinson and White 1979). Forewing membranous, clear; veins yellow; pterostigma long. Head bearing genal cones short. Antennae yellow, except at tip; shorter than width of head, thick always black at tip. Male anal valve broad, about 0.7× as broad as long, convex on both hind and front margins.

Hosts. Asteraceae: *Ambrosia artemisiifolia* L. Anacardiaceae: *Cotinus coggygria* Scop., *Rhus ambigua* Lav. ex Dippel, *Rhus coriaria* L., *Rhus cotinus* L. (Ouvrard 2020).

Distribution. Austria, China, Czech Republic, France, Georgia, Greece, Hungary, Inner Mongolia, Italy, Serbia, Slovakia, Slovenia, Switzerland, Turkey, UK (Ouvrard 2020).

DNA barcoding data for diagnosis. 1 attggtacct cttataattg gagctcctga tatagccttc cctcgattaa acaatttaag / 61 attttgacta ttaattccct cactttattt attattaata agaagaatta tagatcaagg / 121 agtaggaact ggttgaacag tttacccccc actatcaaat tccatatttc atagaggata / 181 ctctgtagat atagctattt tttctttaca tttagcagga atttcctcaa ttttaggagc / 241 aattaatttt atttcaacca tcttaaatat acgaagatgt ttgtataact tagaaaaaat / 301 acctttattt gtttgatctg tactaattac agctatttta ctattatttg ctttgccagt / 361 attagcagga gcaattacaa tacttttaac agaccgtaat ttaaatacat ctttctttga / 421 cccttctgga ggtggagatc ctatccttta ccaacactta ttt (463 bp).

7. *Ctenarytaina eucalypti* (Maskell) (Aphalaridae)

(Fig. 10–11)

Rhinocola eucalypti Maskell 1890.

Korea port interceptions. Intercepted seven times at Korean ports of entry from Italy on cut flowers and branches of *Eucalyptus* plants.

Diagnosis. Adults: General body color dark chocolate brown, the humeral regions of the thorax often orange. Antennae pale yellow with apical segment darkened. Forewing more oval shaped, with a broadly rounded apex;

membrane dirty whitish. Male proctiger with segment 2 broader; parameres relatively smaller and slender, at most 1.45× length of proctiger segment 1; lacking peg-like setae adjacent to posterior margin (Hodkinson 2007).

Hosts. Myrtaceae: *Eucalyptus benthamii* Maiden and Cambage, *Eucalyptus bicostata* Maiden, Blakely and Simmonds, *Eucalyptus camaldulensis* Dehnh., *Eucalyptus cinerea* F.Muell. ex Benth., *Eucalyptus cordata* Labill., *Eucalyptus dunnii* Maiden, *Eucalyptus globulus* Labill., *Eucalyptus gunii* Hook. f., *Eucalyptus leucoxylon* F.Muell., *Eucalyptus globulus* subsp. *maidenii* (F.Muell.) J.B.Kirkp., *Eucalyptus nicholii* Maiden and Blakely, *Eucalyptus nitens* Maiden, *Eucalyptus parviflora* F.Muell., *Eucalyptus perriniana* F.Muell. ex Rodway, *Eucalyptus pulverulenta* Sims (Ouvrard 2020).

Distribution. Australia, Azores, Bolivia, Brazil, Chile, Colombia, France, Germany, Hungary, Ireland, Italy, Madeira, New Zealand, Papua New Guinea, Portugal, South Africa, Spain, Sri Lanka, Switzerland, UK, USA (Ouvrard 2020).

DNA barcoding data for diagnosis. 1 gctcgtacct attataatag gagctccaga tatagcattc ccacgcataa acaacataag / 61 attttgatta ctcattcctt caatttactt actaattatt agaagtttaa ttgaccaagg / 121 agtaggaact ggatgaacag tttaccctcc tttatctaat tctatatttc atagaggata / 181 ctctgtagac acagcaattt tctctctgca tttagcagga atttcttcta tcttaggagc / 241 aattaatttc atcacaacta ttatgaatat gcgatcttct ctgtactcta tagaaaaaat / 301 acctctattt gtttgatctg ttttaattac agcaatctta cttctattat ccctacctgt / 361 attagcaggt gctattacta tacttttaac tgaccgaaat ttcaatacat cattttttga / 421 ccctagagga gggggagatc ctgttttata tcaacattta ttt (463 bp).

8. *Livilla retamae* (Puton) (Psyllidae)

(Fig. 12)

Psylla retamae Puton 1878.

Korea port interception. Intercepted one time at Korean ports of entry from Italy on cut flowers of *Retama* plants.

Diagnosis. Adults: General body color orange to brown; antennae dirty yellow, segments 3–6 darkened apically, segments 6–10 dark brown. Forewing elongate oblong-oval; costal break and rudimentary pterostigma present; spinules absent or thinly scattered in areas of brown patterning. Metatibia with 5 thick black spurs, basal metatarsus without black spurs. Male proctiger simple; paramere slightly expanded subapically, inner tooth partially hidden inside view; aedeagus with slightly hooked apex (Hodkinson and Hollis 1987).

Hosts. Fabaceae: *Retama monosperma* (L.) Boiss., *Retama raetam* (Forssk.) Webb and Berthel., *Retama sphaerocarpa* (L.) Boiss. (Ouvrard 2020).

Distribution. Algeria, Egypt, Israel, Madeira, Morocco, Portugal, Spain (Ouvrard 2020).

DNA barcoding data for diagnosis. 1 gttagtccct ctcataatcg gagctccgga tatagcattt ccacgactaa acaatcttag / 61 attttgactt cttcttccct caatctacct cttactcata agaagtttaa ttgatcaagg / 121 agtcggaaca ggatgaacag tttaccctcc cctatcgaat tctctctttc atagaggata / 181 ttctattgat atcgcaattt tttcccttca tctagcaggt atttcctcta ttttaggagc / 241 aattaatttt attacaacaa tcattaacat gcgaagaagt ctctataatc tagaaacttt / 301 acctttattt gtatgatcag ttttaattac agcttttctt ttactactag cgttaccagt / 361 gttagctgga gctatcacaa tacttctcac tgaccgaaat atgaatactt ccttttttga / 421 tccagcagga gggggagatc ctattttata tcaacattta ttt (463 bp).

Discussion

Psyllids are most abundant in tropical and subtropical areas, but are known from most geographic regions (Burckhardt 1987a, 1987b, 1994; Hollis 2004). There are 3,674 species known worldwide and among them, 107 species have been reported in Korea (Lee 2019; Ouvrard 2020). Korean psyllids make up only three percent of the total number of psyllid species that are known worldwide. However, a number of other psyllid species could enter as exotic species through trade and become established in Korea. Psyllids that were intercepted at Korean ports of entry during this study, *A. uncatoides*, *A. acaciaebaileyanae*, *B. occidentalis* and *C. eucalypti* are considered potential invasive species to South Korea. These species are frequently found on imported cut flowers and may make their way into the Korean environment in nursery and agricultural crops. Some species of herbivorous

insects are able to switch to a different host plant species found in its new geographic location that it does not utilize in the herbivore's natural range (Fox and Morrow 1981). This potential to expand its host range to different host plant species might be a reason for the current widespread and rapid movement and establishment of psyllids such as *A. uncatoides* and *B. occidentalis* (Yen et al. 2013). Therefore, rapid and accurate identification of all developmental stages of pest species such as psyllids is a fundamental requirement for effective pest management and phytosanitary procedures.

This study provides a comprehensive reference barcode library for psyllids intercepted at Korean ports of entry. With the availability of molecular tools like DNA barcodes, it is anticipated that rapid and accurate identification of psyllids could be facilitated, consequently aiding in monitoring, detection and successful management of pest species. Hence, the molecular results are not only useful for countries in which the pests have already established but also would enable countries that are at risk of invasive psyllids to strengthen their phytosanitary and quarantine measures.

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