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First record of *Vespa crabro* Linnaeus (Hymenoptera: Vespidae) in western North America with a review of recorded species of *Vespa* Linnaeus in Canada

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Abstract

Vespa crabro Linnaeus is newly reported as an adventive species in British Columbia, Canada which is the first record of this invasive species in western North America. The specimen of *V. crabro* was identified using morphological diagnostic keys and by comparison to authoritatively identified specimens. DNA barcoding provided support that the British Columbia specimen is conspecific with sequenced specimens of *V. crabro*. It is not possible to be certain of the origin of the specimen, but the DNA barcode was identical to sequence from specimens of *V. crabro* from South Korea. DNA barcoding was also performed on morphologically identified specimens of *Vespa simillima* and *Vespa soror* collected previously in British Columbia and the sequences were closest to *V. simillima* and *V. soror* Genbank sequences, respectively. There is no evidence that any of these species have established populations in the province. We provide diagnostic morphological characters to distinguish Canadian *Vespa* species from each other including *Vespa mandarinia* which has recently established populations in British Columbia and Washington State, USA. The potential detrimental impacts of each species are discussed.

Key words: diagnosis, DNA barcodes, hornets, invasive, morphology

Introduction

Invasive species can have detrimental impacts to human health and the economy (Wheeler & Hoebeke 2017). Mitigating these impacts requires monitoring high-risk pathways to prevent introductions, and eradicating introduced species of concern (Lodge *et al.* 2006). Species of *Vespa* Linnaeus (Hymenoptera: Vespidae) are of particular interest due to their risk of injury to humans and potential for economic loss to the apiculture industry (Matsuura & Sakagami 1973).

Vespa (Figs 1–5) can be distinguished from other genera of Vespinae by having the distance between the posterior ocellus and the posterior margin of the vertex more than twice the distance between the posterior ocellus and the eye (Fig. 5C) (Smith-Pardo *et al.* 2020). The distance between the posterior ocellus and the posterior margin of the vertex is less than or equal to the distance between the posterior ocellus and eye in other vespine genera. In addition, in *Vespa*, the prestigma of the fore wing is at least 3× the length of the pterostigma (see Fig. 4D) compared to longer than the pterostigma, but not as much as 3× its length in other vespine genera (Perrard *et al.* 2013).

Vespa species have entered Canada on multiple occasions: the west European form of *Vespa crabro* Linnaeus was first recorded in the eastern United States of America in 1854 (de Saussure 1898), and has since spread to the Canadian provinces of Ontario and Quebec (Buck *et al.* 2008). In addition to this well-established species, the following species have also been collected in Canada: *Vespa mandarinia* Smith in Nanaimo, British Columbia in 2019 (Wilson *et al.* 2020) as well as White Rock and Aldergrove, British Columbia in 2020 (British Columbia Ministry of Agriculture, Food and Fisheries, 2021); *Vespa simillima* Smith in Shawnigan Lake, British Columbia in 1977 (Cannings 1989) and *Vespa soror* du Buysson in Vancouver, British Columbia in 2019 (Kozak 2020; Kozak & Otis 2020) (see map in Fig. 6). In summary, four species of *Vespa* have been recorded in Canada, of which two: *V. crabro*

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and *V. mandarinia* have established. The purpose of this paper is to report a new adventive record of *Vespa crabro* in British Columbia, Canada and to provide molecular evidence supporting previous morphology-based identifications of *V. simillima* and *V. soror* records in the province. We provide morphological characters to distinguish the four *Vespa* species collected in Canada and discuss the potential impacts these species could have on human health and apiculture.

Materials and methods

Specimen collection and morphological identification

A resident of Mayne Island, British Columbia, Canada photographed a vespid wasp (Fig. 2) in June 2020 and submitted it to the British Columbia provincial apiculturist. This specimen was sent to the University of British Columbia, Vancouver, Canada where it was identified morphologically using keys (Bequaert 1931; Kimsey & Carpenter 2012; Smith-Pardo *et al.* 2020). DNA barcoding was also utilized so that the sequence could be compared with other sequences from *Vespa* specimens.

A specimen of *V. mandarinia* collected in Nanaimo, British Columbia (Fig. 3) in 2019 was re-examined morphologically. DNA barcoding of the Nanaimo population of *V. mandarinia* was previously done by Wilson *et al.* (2020).

The *V. simillima* specimen collected in Shawnigan Lake, British Columbia (Fig. 4) in 1977 (Cannings 1989) was re-examined morphologically and DNA barcoding was performed for comparative purposes with other sequenced specimens of *Vespa*.

Finally, the vespid specimen that was collected live in a building near the Port of Vancouver in May 2019 (Fig. 5) was photographed and these photos were sent to Professor J. Kojima (Ibaraki University, Japan) for identification. DNA barcoding of this specimen was also performed so that the specimen's sequence could be compared with previously sequenced specimens.

Examined specimens are deposited at the following collections: Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, Ontario, Canada (CNC); Royal British Columbia Museum, Victoria, British Columbia, Canada (RBCM); Spencer Entomological Museum, University of British Columbia, Vancouver, British Columbia, Canada (SEM). The collecting localities of all examined British Columbia specimens are shown in Figure 6 (red symbols).

Molecular methods

DNA was extracted destructively from a midleg with the DNeasy® Blood and Tissue Kit (Qiagen, Valencia, CA, USA), with modifications from Moreau (2014) and Cruaud *et al.* (2019). Amplification was performed in 25 µl reactions containing 15.3 µl ddH_2O, 2 µl 2.5 mM dNTPs, 2.5 µl 10X Taq Buffer, 2 µl 25 mM $MgCl_2$, 1 µl of each 10 µM universal primer LCO1490 and HCO2198 (Folmer *et al.* 1994), 0.2 µl of ExTaq HS DNA polymerase (Takara Bio USA, Madison, WI), and 1 µl of DNA template. DNA was amplified on an Eppendorf MasterCycler Pro S (Eppendorf, Hamburg, Germany) under the following conditions: 95°C for 1 minute, 35 cycles of denaturation (95°C for 15 s), annealing (49°C for 15 s), and extension (72°C for 45 s), followed by 72°C for 4 minutes. Amplification failed for *V. simillima*, so two new primer pairs targeting smaller fragments of the COI barcoding region (mini-barcodes) were created in Primer3 (Rozen & Skaletsky 2000) (Table 1). The amplification protocol was the same as listed above with the exception of a different annealing temperature for one of the primer pairs (Table 1). Amplified DNA was visualized on a gel and cleaned with ExoSAP-IT (PE Applied Biosystems, Foster City, CA, USA). Cycle sequencing was performed in 10 µl reactions with the BigDye Terminator v3.1 Cycle Sequencing kit (PE Applied Biosystems, Foster City, CA, USA). Sequencing reactions were purified and sequenced on a 3500xl DNA Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA) at the Agriculture & Agri-Food Canada Ottawa Research and Development Centre Core Sequencing Facility (Ottawa, ON, Canada). Chromatograms were trimmed and assembled with Geneious Prime v2020.0.4. Assembled sequences were assessed for sequence similarity in BLAST queries of GenBank (Altschul *et al.* 1990). Sequences were submitted to BOLD and GenBank (GenBank accession numbers OL702713–OL702715).

TABLE 1. Primers created in Primer3 to amplify regions of the COI barcode of *Vespa simillima*. The primer names, sequences, size of the targeted regions, and annealing temperatures are provided.

Forward Primer	Reverse Primer	Targeted Region (bp)	Annealing Temperature (°C)
Vsim_55F (5'-ATCAGGAACTTTAGGTGCATC-3')	Vsim_406R (5'-GGAAGGTGAATTATGTCCAGT-3')	352	50
Vsim_386F (5'- ACTGGACATAATTCACCTTCC-3')	Vsim_678R (5'-TGTTGGTAGAGAATAGGGTCT-3')	293	49

Photography and image editing

Specimen photographs taken at the Canadian National Collection of Insects were captured with a Canon EOS 7D Mark II (Canon USA, Melville, NY) mounted on an automated Stackshot macro rail (Cognysis Inc., Traverse City, MI). One of two lenses (65 mm or 100 mm) was used depending on the size of the specimen or character being photographed. Image stacks between 25 and 50 were montaged using Helicon Focus v7. Photographs from the Spencer Entomological Collection were taken with a Leica C205 microscope with zoom magnification between 5 and 160 power. A series of 5–25 photos at different focal planes were taken with the Leica Application Suite, and compiled into a single montage with a 1 mm scale bar generated by the system. Image editing and final plate layout of Figs 1–5 were completed with GIMP v2.10.24. The map (Fig. 6) was created using Simplemppr (Shorthouse 2010).

Results

Vespa crabro Linnaeus

Figs 1–2

The specimen collected on Mayne Island (Fig. 2) was identified morphologically by the authors as the Japanese colour form of *Vespa crabro*, formerly *Vespa crabro flavofasciata* Cameron (following the taxon concepts of Archer 1992) which is native to Japan, Korea, and eastern Russia (Archer 1992). A 658 base pair (bp) sequence of the specimen (GenBank Accession OL702713) was 100% identical to 14 sequences of *V. crabro* from South Korea: GenBank Accessions MN716838 – MN716841 and MN609218 – MN609227 (see Discussion for details of these sequences), with an E-value of 0.0 and a bit score of 1214.

Material examined: **CANADA**: 1 ♀, British Columbia, Mayne Island, Horton Bay, 48°49'29.16"N, 123°14'41.46"W, 28.vi.2020, E. Roth, SEM-UBC HYM-14585, (SEM) (Fig. 2); 1 ♀, Ontario, Thwartway Island, 44°17'37.50"N, 76°9'0.45"W, 23.x.1976, R.A. Turner, CNC1754075, DNA voucher AB088 (CNC); **JAPAN**: 1 ♀, Hokkaido, Onuma, 41°58'55.45"N 140°40'13.94"E, 23–24.vii.1966, A. Mutuura, CNC1754076, DNA voucher AB163 (CNC); **SOUTH KOREA**: 1 ♀, Desong-dong, Chipom, 26.v.1952, F.C.R. Chalke, CNC1754077 (CNC); **USA**: 1 ♀, Maryland, Calvert Co., Port Republic, 38°30'3.15"N 76°31'44.44"W, 12–15.x.1991. D.M. Wood, CNC1754074, DNA voucher AB087 (CNC); 1 ♀, North Carolina, Pitt Co., Stokes vic., 17.ix.1984, R.S. Jacobson (CNC) (Fig. 1).

Distribution: *Vespa crabro* is widespread across the Palaearctic region, introduced and established in the eastern Nearctic and adventive in the northwest Nearctic (current study).

Diagnosis: *Vespa crabro* can be distinguished from other *Vespa* species collected in Canada by having a combination of the following characters: 1) length of gena less than 1.6× length of eye at midheight in lateral view (Figs 1B, 2B); 2) pretegular carina complete, extending the height of the pronotal lobe (Fig. 1D); 3) female clypeus with punctures clearly defined, contiguous or nearly so (Figs 1C, 2C); 4) male metasomal sternites 6–7 with posterior margins straight or only shallowly indented medially. The Japanese form of *V. crabro* differs from the west European form in that the posterior yellow fasciae on terga 2 to 6 are generally narrow, especially on tergum 2, and sublateral black spots, if present, are not prominently projecting into the yellow fasciae (Fig. 2B) (west European colour form with larger proportion of terga 2 to 6 yellow, and with sublateral black spots generally prominently projecting into yellow fasciae, at least on terga 3 to 5) (Fig. 1B). Females of the Japanese form are generally darker than the European form in the ocellar area, as well as the scutellum; however, darkening of these regions does occur in some North American specimens of the European form.

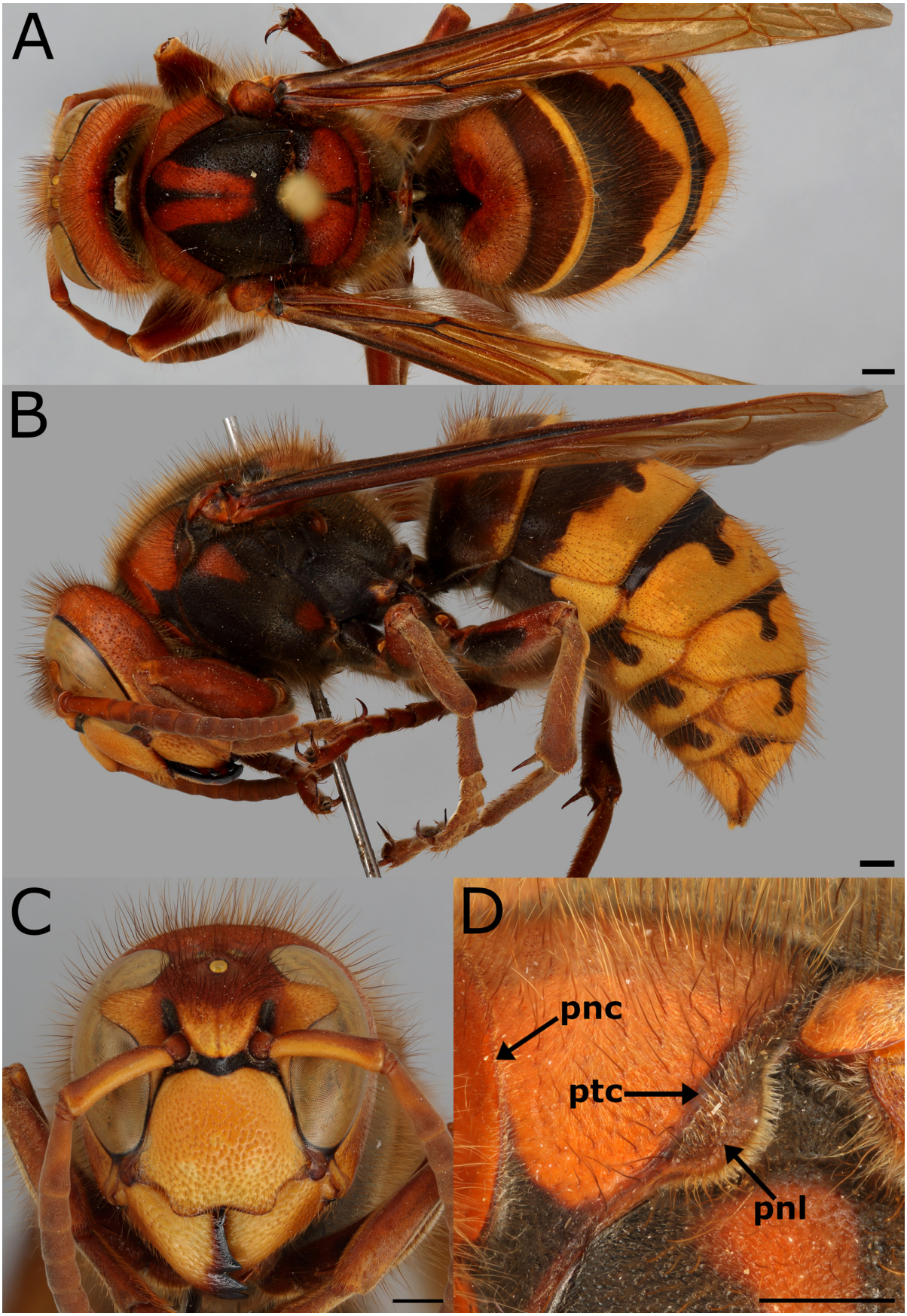

FIGURE 1. West European colour form of *Vespa crabro* (USA: NC). A. Dorsal habitus. B. Lateral habitus. C. Anterior view of head. D. Lateral view of pronotum. pnc = pronotal carina, pnl = pronotal lobe, ptc = pretegular carina. Scale bars: 1 mm.

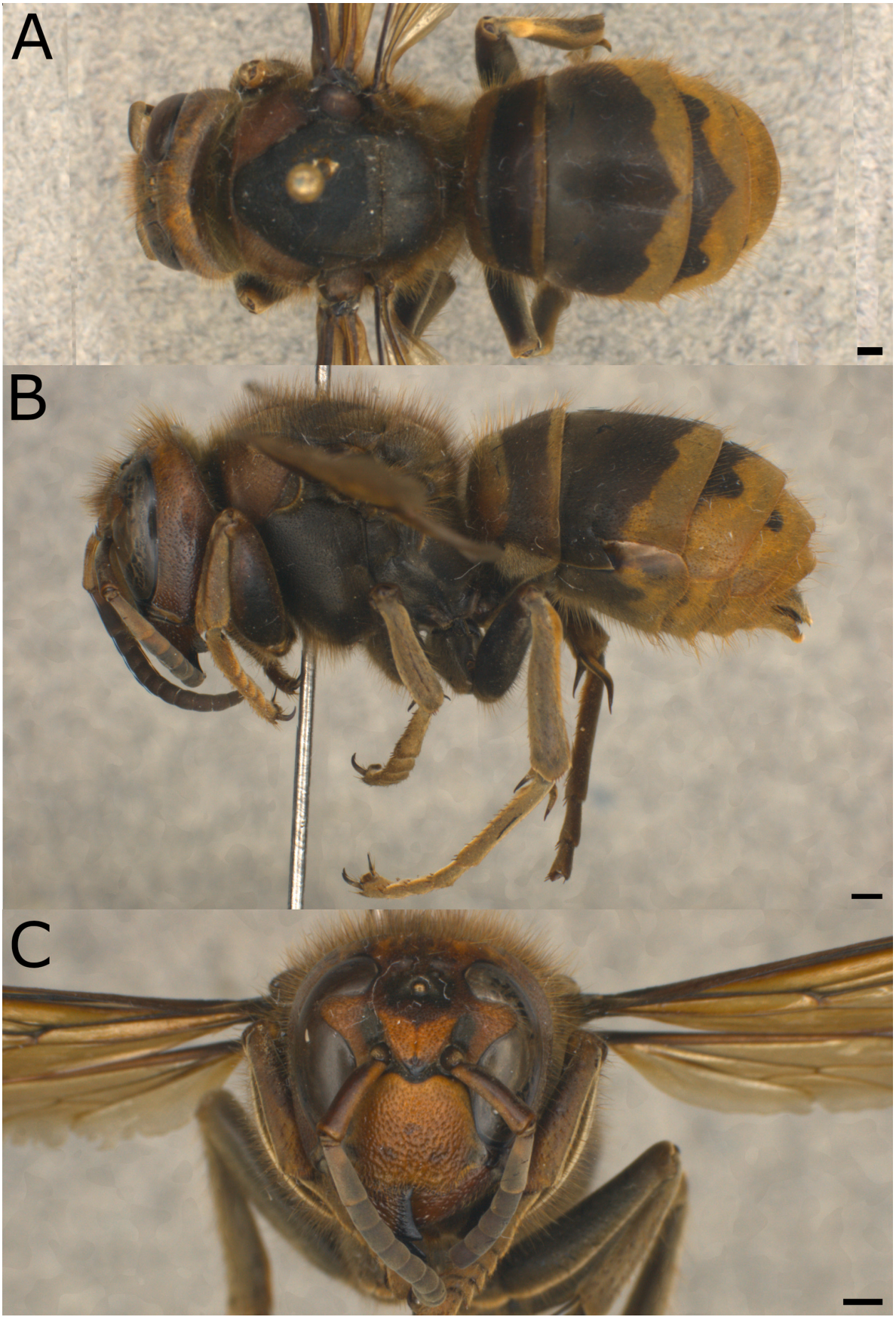

FIGURE 2. Japanese colour form of *Vespa crabro* specimen collected on Mayne Island, British Columbia, Canada. A. Dorsal habitus. B. Lateral habitus. C. Anterior view of head. Scale bars: 1 mm.

Vespa mandarinia **Smith**
Fig. 3

One specimen from the initial North American record of *Vespa mandarinia* (British Columbia Ministry of Agriculture, Food and Fisheries, 2019) was examined morphologically (Fig. 3) in comparison with specimens from Asia deposited in the CNC.

Material examined: **CANADA**: 1 ♀, British Columbia, Nanaimo, 17.viii.2019, J. Duff, SEM-UBC HYM-14395 (SEM) (Fig. 3A–C); **JAPAN**: 1 ♀, Kyushu, 32°31'25" N, 131°31'38" E, 22.IX.2006, J & R Skevington, CNC DIPTERA3231 (CNC) (Fig. 3D); 1 ♀, Kyushu, Seita, Iizuka, Fukuoka, 10–15.x.2013, A., H., & Y. Matsugama (CNC).

Distribution: India, Sri Lanka, Bhutan, Nepal, Myanmar, Thailand, Laos, Vietnam, Malaysia, China, Hong Kong, Taiwan, eastern Russia, Korea, Japan (Smith-Pardo *et al.* 2020), Canada (British Columbia) (British Columbia Ministry of Agriculture, Food and Fisheries 2021) and United States of America (Washington State) (Wilson *et al.* 2020).
Diagnosis: *Vespa mandarinia* can be distinguished from other *Vespa* species that have been recorded in Canada by a combination of the following characters: 1) length of gena at least 1.7× as long as eye at midheight as seen in lateral view (Fig. 3D), 2) metasomal terga 3–6 usually with a wide, orange posterior band, terga 3–5 sometimes completely black, but tergum 6 always predominantly orange (Fig. 3B).

Vespa simillima **Smith**
Fig. 4

The specimen identified as *Vespa simillima* (Fig. 4) was re-confirmed morphologically using the key of Smith-Pardo *et al.* (2020). Both primer pairs were successful in amplifying the COI gene, resulting in a 596 bp assembled sequence (GenBank Accession OL702714). A GenBank BLAST found this sequence was 99.83% identical (one base pair different) to a *Vespa simillima* specimen from South Korea (Accession KY172037), with an E value of 0.0 and bit-score of 1096. Including the Canadian specimen, there are 28 sequences of *V. simillima* in the BOLD database from South Korea (16), Japan (9), Russia: Primorsky Krai (1) and one of unstated country (KF933080) published in Perrard *et al.* (2013) that was collected in Japan (Perrard, pers. comm.). All sequences are grouped in a single BIN (ACB8610) with a maximum sequence divergence between species of 1.77%.

Material examined: **CANADA**: 1 ♀, British Columbia, Shawnigan Lake, viii.1977, collected live, A. Rumsby, ENT991-24452 (RBCM) (Fig. 4); **JAPAN**: 2 ♀, Hokkaido, Tomakomai, 25.viii.1983, M. Ito (CNC).

Distribution: Southeast Asia including Myanmar, parts of China, eastern Russia, Korea, and Japan, adventive in northwest Nearctic (Cannings 1989).

Diagnosis: *Vespa simillima* can be distinguished from other *Vespa* species that have been recorded in Canada by a combination of the following characters: 1) length of gena less than 1.6× length of eye at midheight in lateral view (Fig. 4B), 2) pretegular carina incomplete, not spanning the height of the pronotal lobe, 3) female clypeus with punctures medially shallow, separated by one puncture diameter or more (Fig. 4C); 4) male metasomal sterna 6–7 with posterior margin deeply emarginate medially.

Vespa soror **du Buysson**
Fig. 5

The specimen collected at the Port of Vancouver in May 2019 (Fig. 5) was initially identified morphologically as *Vespa ducalis* Smith (https://globalnews.ca/news/5326422/north-vancouver-mystery-hornet-identified) but subsequent examination changed the determination to *Vespa soror* du Buysson. We assembled a 523 bp sequence (GenBank Accession OL702715) that was 98.09% identical to a *V. soror* sequence from China on GenBank (Accession MZ191819), with an E-value of 0.0, and a bit score of 911. Including the British Columbia specimen, there are five *V. soror* sequences in the BOLD database (three from China and one with unknown provenance from Perrard *et al.* 2013) and they all cluster in the same BIN (ACQ0570) that has a maximum sequence divergence between specimens of 2.12%.

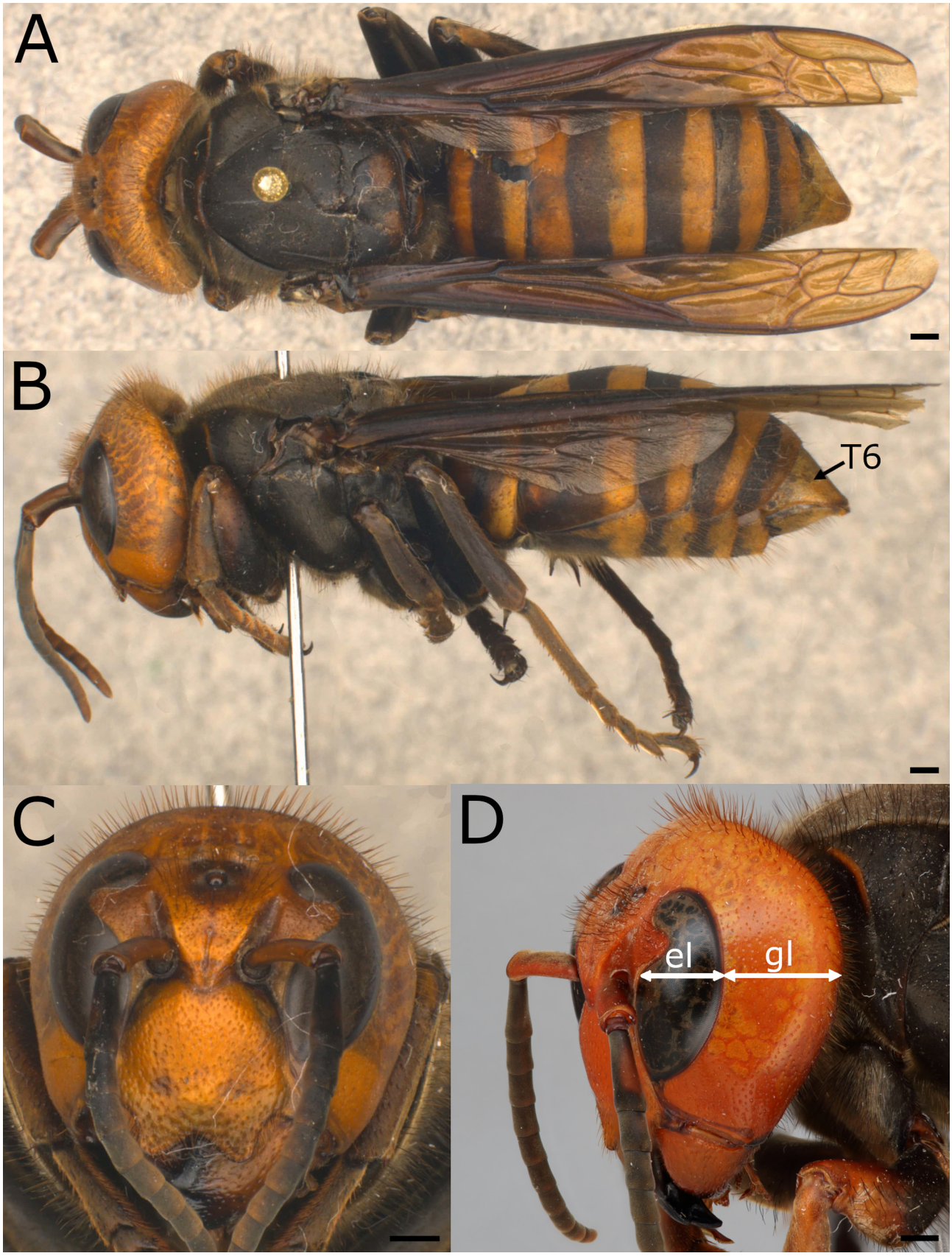

FIGURE 3. *Vespa mandarinia*. A–C. Specimen collected at Nanaimo, British Columbia, Canada. D. Specimen collected in Kyushu, Japan. A. Dorsal habitus. B. Lateral habitus. C. Anterior view of head. D. Lateral view of head. el = eye length, gl = gena length, T6 = tergum 6. Scale bars: 1 mm.

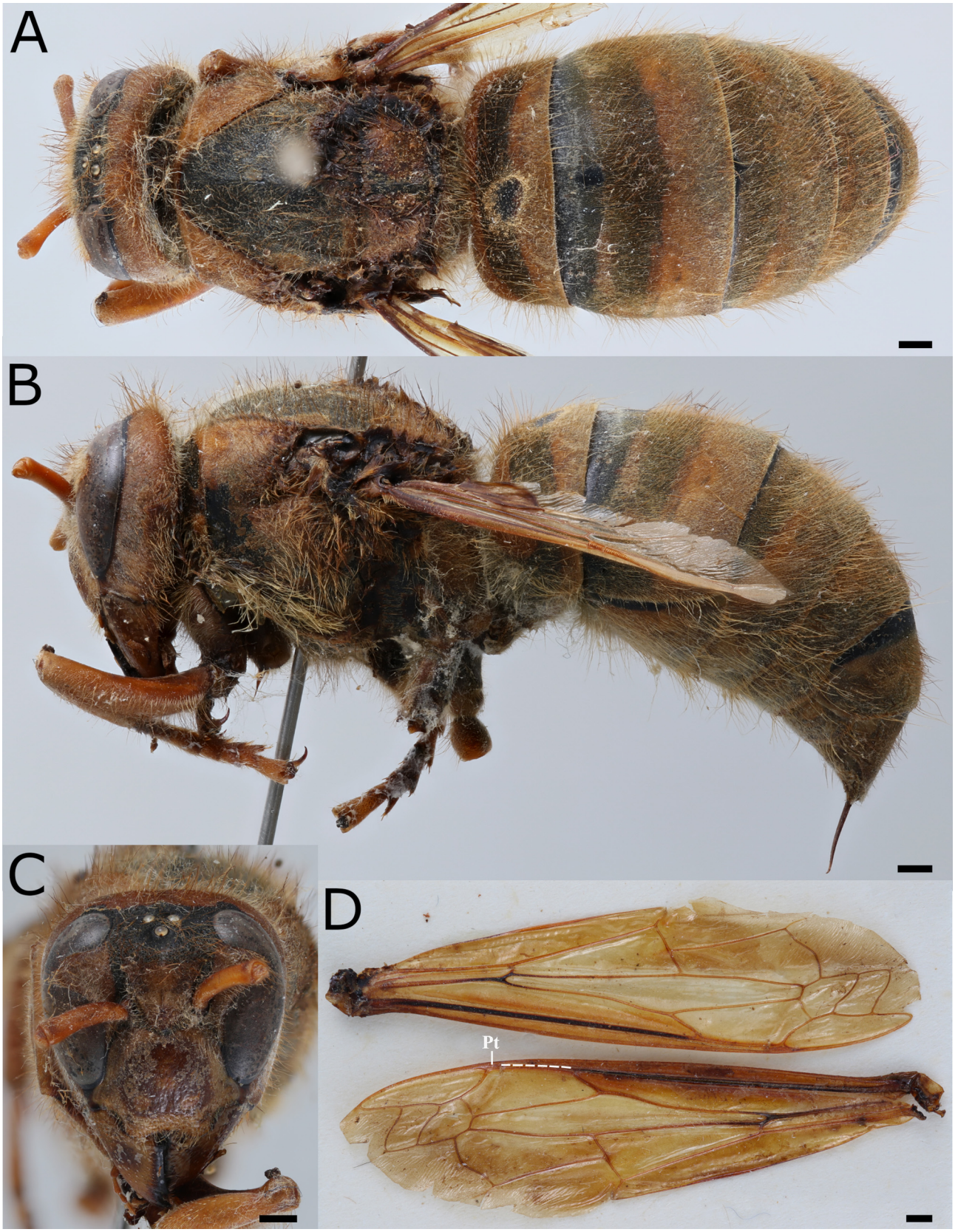

FIGURE 4. *Vespa simillima* specimen collected at Shawnigan Lake, British Columbia, Canada. A. Dorsal habitus. B. Lateral habitus. C. Anterior view of head. D. Fore wings. Scale bars: 1 mm. Pt = pterostigma. Dashed line in D is the prestigma.

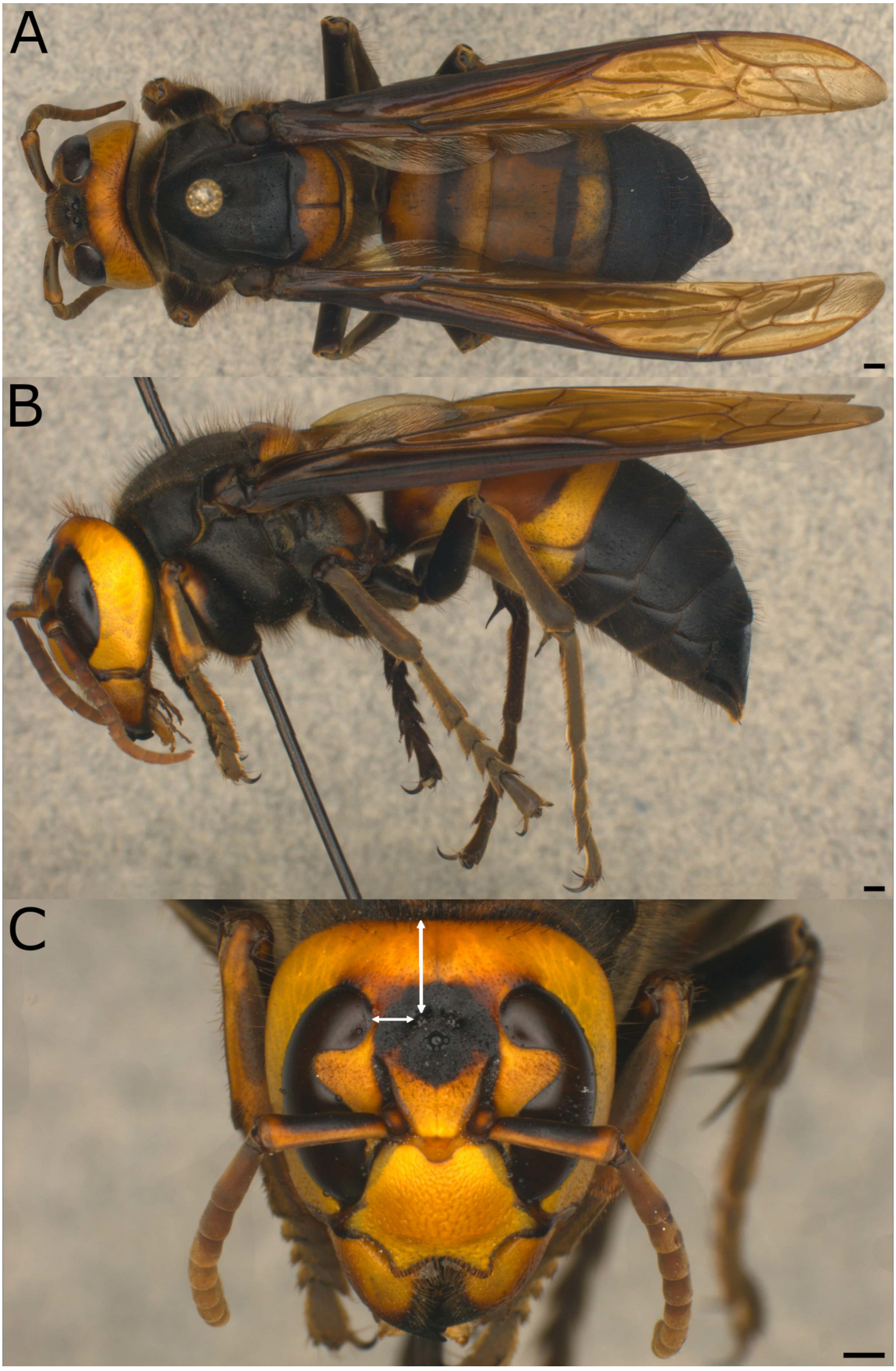

FIGURE 5. *Vespa soror* specimen collected at Vancouver, British Columbia, Canada. A. Dorsal habitus. B. Lateral habitus. C. Anterior view of head. Vertical arrow in C shows the distance between posterior ocellus and posterior edge of vertex; horizontal arrow shows distance between posterior ocellus and eye. Scale bars: 1 mm.

Material examined: **CANADA**: 1 ♀, BC, Port of Vancouver, 49°18'7.56"N, 123°6' 34.92"W, 10.v.2019, T. Hergott, SEM-UBC HYM-14359 (SEM).

Distribution: Native to south and southeast Asia including India, southwestern China, Hong Kong, Myanmar, Thailand, Laos, and Vietnam (Archer 1995; Smith-Pardo *et al.* 2020), adventive in northwest Nearctic (Kozak 2020).

Diagnosis: *Vespa soror* can be distinguished from other *Vespa* species that have been recorded in Canada by having a combination of the following characters: 1) gena length at least 1.7× the length of eye (Fig. 5B), 2) metasomal tergum 1 length at least half of posterior width, and 3) terga 3–6 black (Fig. 5B), sometimes with a narrow posterior band of orange on tergite 3.

Discussion

Morphological and molecular evidence support the hypothesis that an additional adventive *Vespa* species is recorded from British Columbia, Canada, bringing the number of *Vespa* species recorded in the province (and country) to four: *V. crabro*, *V. mandarinia, V. simillima* and *V. soror*. *Vespa basalis* Smith and *V. ducalis* have also been reported (Kozak 2020; Little 2019), but the first is attributed to a falsified record posted to iNaturalist (pers. comm. Paul Van Westendorp), and the second to a misidentification of the *V. soror* specimen noted above. In terms of the United States, a nest of *V. affinis* Linnaeus was recorded in Los Angeles Co., California, USA in 2010 (Kimsey & Carpenter 2012) and specimens of the following species were intercepted at US ports between 2010 to 2018: *V. bellicosa* de Saussure, *V. orientalis* Linnaeus and *V. tropica* (Linnaeus) (Smith-Pardo *et al.* 2020). The species diagnoses above only distinguish the four species (and two *V. crabro* colour forms) recorded from Canada. With increased global trade, it is probable that additional species of *Vespa* will be collected in Canada in the future. If the identity of a *Vespa* specimen is in doubt, refer to the key in Smith-Pardo *et al.* (2020) which includes extensive illustrations of all described species.

The west European colour form of *V. crabro* (Fig. 1) (formerly *V. crabro germana* Christ, taxon concepts from Archer 1992) has been present in North America for more than 150 years (de Saussure 1868; de Saussure 1898). The British Columbia specimen of *V. crabro* from Mayne Island (red circle in Fig. 6) shares 100% of its DNA barcode with 14 specimens from Genbank, all from South Korea. Four of these (MN716838 – MN716841) were published in Namin and Jung (2020). The other ten (MN609218 – MN609227) are unpublished sequences from a project entitled "Establishment of National Biodiversity Information Network, Integrated Database System and its Management" by the National Science Museum, Daejeon, South Korea. The British Columbia specimen and the 14 Korean specimens share three unique substitutions found in no other *Vespa crabro* specimens in Genbank or BOLD: site 230 (A instead of G); site 293 (C instead of T) and site 598 (G instead of A). The similarity of these sequences provides some support for the hypothesis that the British Columbia specimen originated from Asia. Nevertheless, the range of sequence variation between the British Columbian specimen and other Asian specimens of *V. crabro* in BOLD and Genbank is not that dissimilar to the range of variation between the BC specimen and European/ North American specimens. For example, a Chinese specimen in BOLD (GMCHK021-14) differs from the British Columbia specimen by four nucleotides (0.66 %), whereas a specimen from Canada (Ontario) (TZBCA227-06) differs by five nucleotides (0.76%). Given the relatively small sample size of specimens of *V. crabro* that have been DNA barcoded (61 in BOLD as of April 2022), it is not possible to be certain that the British Columbia specimen did not originate from eastern North America (i.e., the haplotype of the BC specimen could be present in eastern North America, but has not yet been sampled). However, based on our current molecular knowledge, the unique substitutions shared between the Korean and BC specimens and the colour pattern of the metasoma (weakly projecting dark spots on terga 3 to 6), our current hypothesis is for an Asian origin. If so, this is a new adventive record for *V. crabro* in North America rather than a range expansion from eastern North America. The Japanese colour form of *V. crabro* shares biological and behavioural traits with the west European colour form, so it is expected to have a low level of threat to human health and apiculture in North America. *Vespa crabro* is less likely to encounter and/or sting humans as it prefers forest habitats (Choi *et al.* 2012) and is docile compared to other *Vespa* species (Akre & Davis 1978; Shaw & Weidhaas 1956). This species is a generalist predator and does not target honey bee colonies (Beljavsky 1937; Matsuura & Sakagami 1973). There are no reports of *V. crabro* causing economic loss to apiculture in eastern North America (Bromley 1948), and if it were to establish in British Columbia there should be little concern that it would

impact honey bee populations in this region. Additional surveys involving both active searches and bottle trap installation were conducted on Mayne Island in August of 2020, but no further specimens have been seen or collected by the authors, or reported by the public.

Vespa mandarinia (Fig. 3) was first collected in North America in 2019 in Nanaimo, British Columbia (red star in Fig. 6) and Blaine, Washington State (black star 1 in Fig. 6), with individuals from these areas since determined to be separate maternal lines (Wilson *et al.* 2020). The *Vespa mandarinia* specimens collected in British Columbia were morphologically and genetically consistent with a colour form native to Japan (Wilson *et al.* 2020). Additional individuals were found in 2020 in British Columbia at White Rock (black star 2 in Fig. 6) and Aldergrove (black star 3 in Fig. 6) (British Columbia Ministry of Agriculture, Food and Fisheries 2021). More specimens were collected in Washington State in 2020 in the Blaine area and in 2021, a dried male specimen was collected in Marysville (black star 4 in Fig. 6) (WSDA 2021). Although this species has a painful sting, human interactions are expected to be rare due to their nesting sites in forested areas away from highly populated areas. *Vespa mandarinia* is considered one of the most destructive *Vespa* species for apiculture, frequently recorded killing all honey bees in a hive and removing the brood (Matsuura & Sakagami 1973). At present, *V. mandarinia* is not a regulated species in Canada, except in cases of intentional import (Canadian Food Inspection Agency 2020).

DNA barcoding of the *Vespa* specimen from Shawnigan Lake (red triangle in Fig. 6) placed this specimen (Fig. 4) in a cluster with specimens of *V. simillima* from South Korea, Japan and eastern Russia. *Vespa simillima* often nests in urban locations, bringing them into close proximity with humans. Due to this, *V. simillima* is responsible for most of the human sting incidents in Japan (Makino *et al.* 1981; Matsuura & Sakagami 1973; Matsuura & Yamane 1990). Workers of this species are aggressive and the nest does not have to be disturbed for stings to take place (Matsuura & Yamane 1990). *Vespa simillima* is a honey bee pest in its native range, but does not decimate colonies. *Vespa simillima* tend to kill individual bees at nest entrances, but don't attack in groups, enter the nest, or remove brood (Matsuura & Sakagami 1973). While this is not as alarming as attacks from *V. mandarinia*, it can still be detrimental to the apiculture industry and individual apiaries located close to large *V. simillima* colonies.

DNA barcoding of the specimen of *Vespa* collected in the Port of Vancouver (red square in Fig. 6) placed this specimen (Fig. 5) in a cluster with specimens of *V. soror* from China. *Vespa soror* builds subterranean nests (Smith-Pardo *et al.* 2020) in forested areas that workers aggressively protect (Lee 2009). Because of this, *V. soror* may pose a slightly higher sting risk to humans than some other species (e.g., *V. crabro*). *Vespa soror* would likely be a pest to the apiculture industry if a population becomes established in British Columbia. They are capable of decimating honey bee populations by raiding colonies in groups, killing all individuals, and removing larvae (Lee 2009). *Vespa soror* is distributed across tropical Asia, so the Canadian climate may prevent this species from establishing populations in British Columbia. However, the specimen from Vancouver was left in a home freezer (approximately -20°C) for a week and was still alive, so this species evidently has some amount of cold resistance. An additional week in a -35°C freezer killed the specimen. Based on its size, this specimen may have been a queen, but distinguishing worker from queen would require dissection of the spermathecae to look for evidence of insemination (pers. comm. Dr. J. Kojima). Given that the *V. soror* individual was collected in spring, if she is a queen, it is not likely she founded a nest before being collected.

Because of the native distribution of *V. simillima* and *V. soror*, it seems certain that these hornets arrived in Canada from Asia, and as discussed, it is hypothesized that the specimen of *V. crabro* also originated from Asia. The *Vespa soror* specimen was collected near the Vancouver harbor, so we speculate it may have entered on shipments from Asia. However, the specimens of *Vespa crabro* and *V. simillima* were both collected more than 20 kilometres from major ports (Fig. 6). As none of the specimens were directly collected from a shipment, the geographic source, vector, and pathway for these specimens cannot be confirmed.

Reporting all adventive records will alert authorities to target species for ongoing monitoring and may prevent future invasions. Prevention is usually the most cost effective solution, as it is often expensive and logistically difficult to eradicate a species once it has established a population (Beggs *et al.* 2011; Lodge *et al.* 2006). Knowledge of all adventive records and the use of comprehensive keys (Smith-Pardo *et al.* 2020) for rapid identification will be critical in quickly identifying new adventive species and/or range expansions. It is imperative that municipal, provincial, and federal agencies work together with researchers, beekeepers and the public to prevent further spread of *V. mandarinia* and monitor for future introductions of other *Vespa* species.

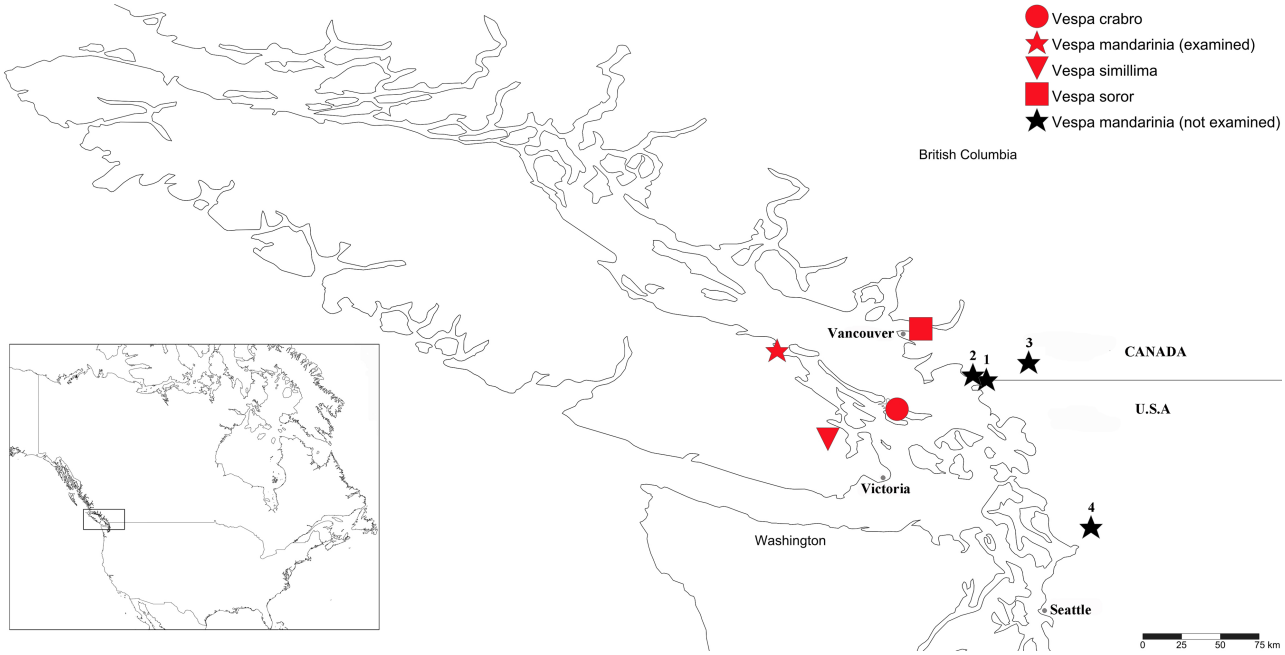

FIGURE 6. Collecting localities of *Vespa* in British Columbia, Canada and Washington State, U.S.A with inset showing location of region in North America. Numbers above black stars are collecting localities noted in Discussion for *Vespa mandarinia*.

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