Systematic Entomology

Systematic Entomology (2017), **42**, 596–608 DOI: 10.1111/syen.12233

Integrative taxonomy improves understanding of native beneficial fauna: revision of the Nearctic *Peristenus pallipes* complex (Hymenoptera: Braconidae) and implications for release of exotic biocontrol agents

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Abstract. The Nearctic *Peristenus pallipes* complex (Hymenoptera: Braconidae) consists of two species groups that are further divided into nine species, separated largely using ecological rather than morphological differences. The species are re-examined with an integrative approach using morphometric multivariate ratios, molecular (*COI* and *CytB*), and ecological data to test the validity of the nine species. The data support only three valid species [*P. dayi* Goulet, *P. mellipes* (Cresson) and *P. howardi* Shaw] rather than nine. New synonymies include: *P. braunae* Goulet under *P. dayi* Goulet 2006 **syn.n.**; *P. carcamoi* Goulet, *P. otaniae* Goulet and *P. pseudopallipes* (Loan) under *P. mellipes* (Cresson) **syn.n.**, and finally *P. broadbenti* Goulet 2006 and *P. gillespiei* Goulet 2006 under *P. howardi* Shaw 1999 **syn.n**. In light of these taxonomic revisions, the biology and distributions of the Nearctic *P. pallipes* complex are updated, resulting in three morphologically variable, widespread, multivoltine species rather than nine largely univoltine species with patchy distributions. The integrative taxonomic approach used here allowed for a more accurate delineation of native fauna and their potential to be competitively displaced by foreign biocontrol agents.

Introduction

Foreign biocontrol agents are often introduced intentionally to attack foreign invasive pests, or when no suitable native natural enemies can be used to suppress pest populations (van Driesche, 1994). However, extensive research on the biology (e.g. phenology, host specificity) of foreign biocontrol agents is required prior to release to ensure success of the control programme. Poorly matched phenologies between the target pest and the biocontrol agent can result in poor pest control (Boettner *et al.*, 2000). Even worse, if host-specificity tests are not completed, biocontrol agents can cause unforeseen damage to nontarget species or native congeners occupying the same niche as a result of competitive displacement (DeBach & Sundby, 1963; Bennett, 1993; Schellhorn *et al.*, 2002; van Driesche, 2008). Competitive displacement is even more of a concern when foreign agents are imported to control native pests, as their release will inevitably cause competition with the suite of natural enemies that have co-evolved with the pest (DeBach & Sundby, 1963; Bennett, 1993; Schellhorn *et al.*, 2002). Displacement of native fauna may be a particularly important phenomenon in parasitoids that are specialized on the target pest (Xu *et al.*, 2013) as they may have lost the ability to utilize alternate hosts. Therefore, understanding the possibility for interspecific competition among native and introduced parasitoid species is vital for reducing negative impacts associated with releasing foreign parasitoids for biocontrol. Displacement of native parasitoids due to foreign biocontrol agents is rarely reported in the literature, probably for two major reasons: (i) limited studies, particularly long-term studies post-establishment of a biocontrol agent (Bennett, 1993); and (ii) insufficient information on the native parasitoid community prior to release of a biocontrol agent, preventing comparative studies (Bennett, 1993). The latter is especially likely given that much of the parasitoid community remains undescribed (Godfray, 1994) and taxonomists are not always consulted in biocontrol studies.

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Fig. 1. Freshly emerged adult *Peristenus mellipes*. [Colour figure can be viewed at wileyonlinelibrary.com].

Parasitoids in the genus *Peristenus* Foerster (Braconidae: Euphorinae; Fig. 1) are found in most regions of the world except for Australia and the Neotropics. Currently there are 69 described species of *Peristenus* (Yu *et al.*, 2012), all of which are endoparasitoids of plant bugs (Hemiptera: Miridae). The actual number of species is probably much higher, as only the European (Loan, 1974a), Oriental (Chen & van Achterberg, 1997; Shamim *et al.*, 2008), Eastern Palearctic (Belokobylskij & Tobias, 2000) and North American species (Loan, 1974b; Goulet & Mason, 2006) have been revised. The taxonomic status of *Peristenus* has fluctuated depending on the author, either recognized as a distinct genus (Shaw, 1987; Chen & van Achterberg, 1997; Stigenberg *et al.*, 2015) or treated as a subgenus of *Leiophron* Nees (Tobias, 1986; Papp, 1992; Belokobylskij & Tobias, 2000). The most recent phylogenetic study (Stigenberg *et al.*, 2015) using morphological and molecular data supported *Peristenus* as a distinct clade and sister to *Leiophron*; however, taxon sampling was limited to five exemplars.

While most *Peristenus* have a partially complete occipital carina, members of the *Peristenus pallipes* species complex can be easily identified by the presence of a complete occipital carina and are found in temperate to boreal Holarctic regions (Loan, 1974a,1974b; Goulet & Mason, 2006). *Peristenus pallipes* Curtis was thought to be a single, common Holarctic species (Loan, 1974a,1974b), but recent work has identified nine Nearctic and multiple, as yet to be described Palearctic species (C. van Achterberg and H. Goulet, personal communication). *P. pallipes* is now treated as exclusively found in Europe, whereas the North American specimens are treated as *Peristenus mellipes* (Cresson), the oldest name for North American endemics (Goulet & Mason, 2006).

The nine Nearctic species are further split into two species groups: the *Peristenus dayi* group including two species (*P. braunae* Goulet and *P. dayi* Goulet) and the *P. mellipes* group with seven species [*P. broadbenti* Goulet, *P. carcamoi* Goulet, *P. gillespie* Goulet, *P. howardi* Shaw, *P. mellipes* (Cresson), *P. otaniae* Goulet and *P. pseudopallipes* (Loan)]. The two species groups were separated based on the density of punctures on the head, with the *dayi* group having large and dense puncturing, and the *mellipes* group with smaller and sparse punctures (Goulet & Mason, 2006). However, due to the lack of consistent morphological differences between species within the two species groups, identification beyond the species-group level was largely based on generalized biogeographical distributions and peak flight times (Goulet & Mason, 2006). Table S1 lists the nine species along with their distributions, hosts and life cycles based on Goulet & Mason (2006). The ecological information utilized to separate the species was largely generalized and thus calls into question the validity of the species. For example, *P. dayi* and *P. braunae* were considered not to have gene flow as populations were not found within 300 km. However, extensive sampling across the intermediate area was not completed. Additionally, the peak flight times of these two species were considered diagnostic for species delimitation by Goulet & Mason (2006), with *P. dayi* in late May and *P. braunae* in late June to early July (Table S1). However, delayed host emergence in colder climates across the larger range of *P. braunae* would probably influence the average peak flight time and thus may not be indicative of true phenological differences across species, but instead could be a result of climatic differences across the species' range.

Species of *Peristenus* attack early nymphal instars of mirids and kill their hosts in the late nymphal or adult stage (Loan, 1980). Thus, they have been used as biocontrol agents for major agricultural pests, such as the native plant bugs (*Lygus* Hahn) and the introduced *Adelphocoris lineolatus* (Goeze), that cause major economic damage into multiple North America crops, such as canola and various pulses (Haye *et al.*, 2005, 2006; Mason *et al.*, 2011). Three European species were introduced to North America to control native *Lygus* spp. and *A. lineolatus* populations in alfalfa and canola, amongst other crops (Day, 1996; Day *et al.*, 1999; Mason *et al.*, 2011). *Peristenus digoneutis* Loan and *Peristenus rubricollis* (Thomson) were introduced into eastern New Jersey and Delaware, respectively. Subsequent surveys have confirmed the establishment of both species in northeastern USA and eastern Canada (Day *et al.*, 1990, 1998, 2008; Broadbent *et al.*, 1999). *Peristenus relictus* (Ruthe) (syn. *P. stygicus*) was released in California along with *P. digoneutis*, but only the former has confirmed establishment in central California in recent surveys (Pickett *et al.*, 2007, 2013; Swezey *et al.*, 2014). The main reason for the introductions of foreign biocontrol agents was that native *Peristenus* species were not considered abundant enough to control these mirid pests based on parasitism rate assessments in some localities (Day, 1987). There was an observed decline of native *Peristenus* species in eastern Québec, Canada, after the introduction of *P. digoneutis*, which prompted the taxonomic and biological research of Goulet & Mason (2006). Recently, there has been interest in releasing foreign *Peristenus* species into western Canada to control *Lygus* (Fernández, 2016). However, competitive displacement of native parasitoids by foreign biocontrol agents remains a concern, prompting us to use integrative taxonomic approaches to re-examine the Nearctic *P. pallipes* complex in the current study. Integrative taxonomy combines information from multiple sources, such as morphology, DNA, ecology and behaviour (Dayrat, 2005; Schlick-Steiner *et al.*, 2010). This provides a more holistic taxonomic approach

using multiple independent lines of evidence and has largely improved cryptic species delimitation (Boring *et al.*, 2011; Ceccarelli *et al.*, 2012; Gebiola *et al.*, 2012; Baur *et al.*, 2014; Grossi *et al.*, 2014; Namin *et al.*, 2014; Schwarzfeld & Sperling, 2014; Zhang *et al.*, 2014).

Thus, the main objective of this study was to test the species and species-group hypotheses put forth by Goulet & Mason (2006) for the Nearctic *P. pallipes* complex using a combination of morphometrics, and molecular and ecological data. We test the validity of the nine species based on a phylogenetic species concept (monophyly) (Baum, 1992) in combination with a distinct barcoding gap (greater interspecific than intraspecific genetic distances) (Meyer & Paulay, 2005). Additionally, spatial and temporal specimen data are used in combination with phylogenetic patterns to examine possible intraclade structuring associated with phenological information. Finally, we utilize a multivariate analysis of quantitative morphological characters to provide an additional independent test of species validity. We revise the two species groups, synonymize species that are not supported, provide a key to the three native North American species of *Peristenus*, and update the biology and distribution records for the three valid species. Additionally, we discuss the implications of this study for importation of foreign parasitoids, with a focus on western Canada. We also make recommendations for the inclusion of integrative taxonomic research prior to the release of biocontrol agents, which has global implications for classical biocontrol programmes. Accurate identification of the Nearctic *P. pallipes* complex will facilitate studies on their population dynamics with hosts and crops, potentially prevent extirpation and extinction of native beneficial insects, and contribute to a better understanding of the interactions between native species and foreign agents in classical biocontrol.

Materials and methods

Sample collection

Specimens of adult species of *Peristenus* were borrowed from the following institutions and curators: the Canadian National Collections of Insects (CNCI, J. Fernández-Triana) and University of Guelph Insect Collections (DEBU, S. Paiero). Paratypes were borrowed when available and DNA was extracted for inclusion in the analyses (Table S2). Additional specimens were collected as adults using sweep nets, or reared from parasitized nymphs sampled in Manitoba, Ontario and Alberta during May–August of 2013–2015 in various agricultural fields where the hosts can be found and preserved in 95% ethanol (EtOH). Species were initially identified using a combination of morphological and ecological characters outlined in Goulet & Mason (2006). Outgroups included *Euphoriella* sp. and *Leiophron* spp., the latter being sister to *Peristenus* (Stigenberg *et al.*, 2015), and two specimens of *P. relictus*, a European species that is not in the *P. pallipes* complex. A list of the specimens used in this study is given in Table S2. For ease of interpretation of results, specimen information was added to taxon labels for the phylogenetic analyses, including province or state locality, date of collection and initial identification based on the characters outlined in Goulet & Mason (2006). Additional exemplar specimens with sequences for the barcoding region of *COI* were obtained from BOLD Systems (http://www.boldsystems.org/) to increase taxonomic sampling across a larger biogeographic range.

Morphometrics analysis

A subset of sequenced female specimens along with identified specimens that failed to generate molecular data were selected for morphometric analysis. Only females were used, as most type specimens are female, females are more abundant than males, and also to prevent any analytical issues that may be caused by sexual dimorphism. The chosen 40 female specimens were photographed using a Canon 7D Mark II with a Mitutoyo M Plan Apo 10× objective mounted onto the Canon EF Telephoto 70–200 mm zoom lens, and the Canon MT–24EX Macro Twin Lite Flash (Tokyo, Japan) with custom-made diffusers to minimize hot spots. Measurements were taken using the average of three measurements with ImageJ150 (Schneider *et al.*, 2012) and/or a Nikon SNZ18 stereomicroscope (Melville, NY, U.S.A.) with an ocular micrometer. A detailed list of measurements is presented in Table 1 and shown in Fig. 2. The multivariate ratio analysis (Baur & Leuenberger, 2011) was applied in R (R Core Team, 2016) with modified scripts (this study) as outlined in Baur *et al.* (2014). The script files can be accessed at the Dryad Digital Repository (http://datadryad.org/, doi:10.5061/dryad.vv183).

DNA protocols

Genomic DNA was extracted from mounted or EtOH-preserved specimens following the DNeasy Tissue Kit protocol (Qiagen, Valencia, CA, U.S.A.). Petioles were separated from mesosomas to ensure buffer penetration during tissue lysis, and the two body parts were mounted onto the same point post-extraction. Voucher specimens were deposited in CNCI. Two genes were amplified: mitochondrial cytochrome oxidase I (*COI*) using universal primers LCO1490 (5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′) and HCO2198 (5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′) (Folmer *et al.*, 1994); and cytochrome B (*CytB*) using CytB F (5′-TCT TTT TGA GGA GCW ACW GTW ATT AC-3′) and CytB R (5′-AAT TGA ACG TAA AAT WGT RTA AGC AA-3′) (Belshaw & Quicke, 1997). The faster rate of evolution of the mitochondrial genes compared with nuclear DNA makes mtDNA ideal for separating closely related species, and both genes are frequently used for species delimitation of Braconidae, including Euphorinae (Stigenberg & Ronquist, 2011; Ceccarelli *et al.*, 2012). All polymerase chin reactions were performed on a Bio-Rad MyCycler thermal cycler (Hercules, CA, U.S.A.), using approximately 1 μg DNA extract, 1X Standard Taq Buffer (10 mm Tris–HCl, 50 mm KCl, 1.5 mm $MgCl_2$, pH 8.3; New England Biolabs, Ipswich, Massachusetts, U.S.A.), 200 μM dNTP (Invitrogen, Carlsbad, California, U.S.A.), 4 mM $MgSO_4$,

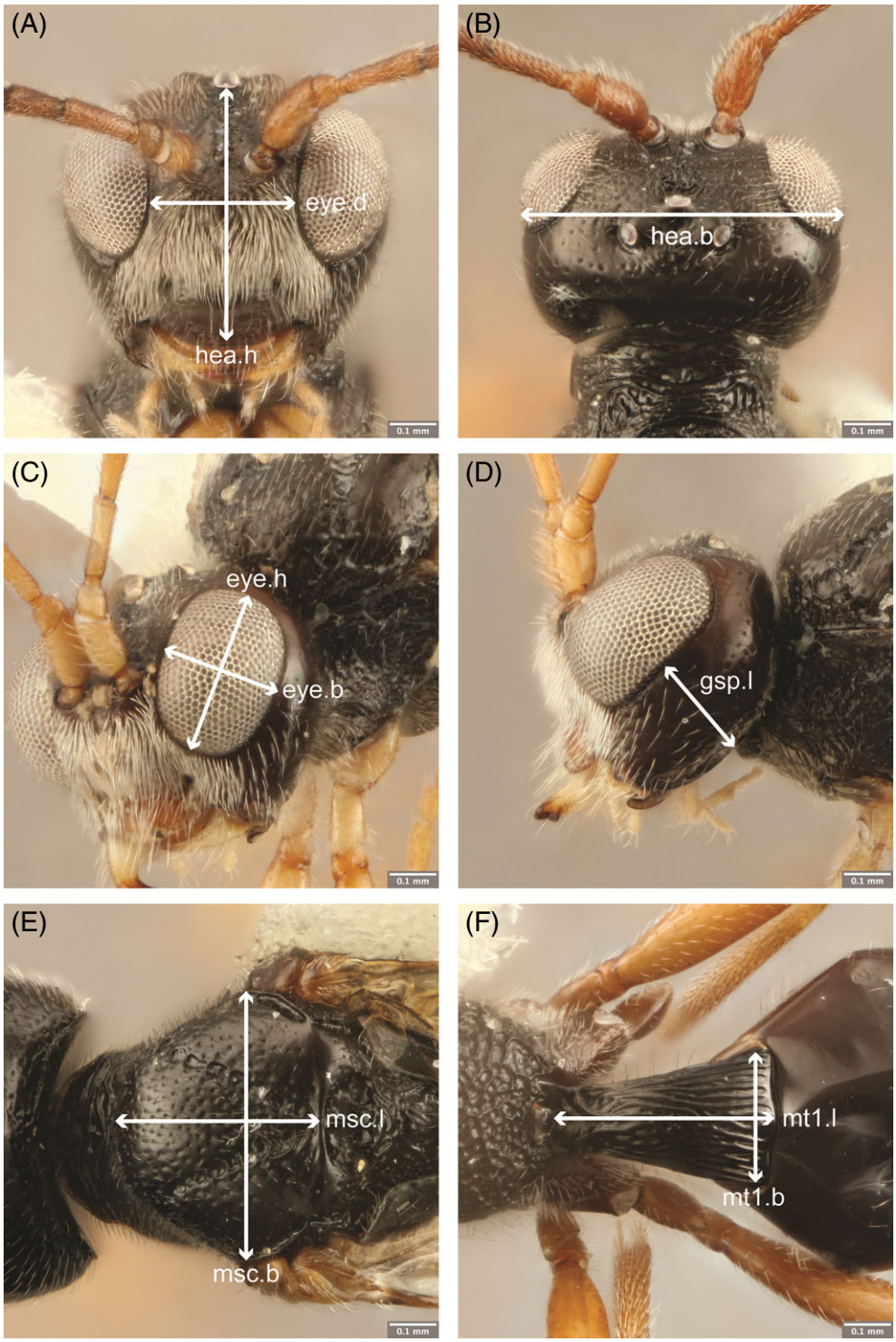

Fig. 2. (A, B, F) *Peristenus dayi* ♀; (C, D, E) *Peristenus mellipes* ♀. (A) Frontal view of the head; (B) dorsal view of the head; (C) anteriolateral view of the eye; (D) dorsal view of the mesoscutum; (E) lateral view of the head; (F) dorsal view of metasomal tergite 1. Morphometrics variables: minimum eye distance (eye.d); head height (hea.h); head breadth (hea.b); eye height (eye.h); eye breadth (eye.b); mesoscutum length (msc.l); mesoscutum breadth (msc.b); genal space length (gsp.l); metasomal tergite 1 length (mt1.l); maximum metasomal tergite 1 breadth (mt1.b). [Colour figure can be viewed at wileyonlinelibrary.com].

Table 1. Abbreviations and definitions of the 10 morphological characters used for the morphometrics analysis of *Peristenus pallipes* complex.

Abbreviation	Character name	Definition	Magnification
eye.b	Eye breadth	Greatest breadth of eye, viewed at an angle in which both anterior and posterior margins are in focus (Fig. 2C)	100×
eye.d	Eye distance	Shortest distance between eyes, frontal view (Fig. 2A)	100×
eye.h	Eye height	Greatest length of eye height, viewed at an angle in which both dorsal and ventral margins are in focus (Fig. 2C)	100×
gsp.l	Genal space	Length of the genal space taken midway between the dorsal and ventral margins of the eye from the posterior edge at 90° to the occipital carinae, lateral view (Fig. 2D)	100×
hea.b	Head breadth	Greatest breadth of head, dorsal view (Fig. 2B)	100×
hea.h	Head height	Distance between lower edge of clypeus and lower edge of anterior ocellus, frontal view (Fig. 2A)	100×
msc.b	Mesoscutum breadth	Greatest breadth of mesoscutum just in front of level of tegula, dorsal view (Fig. 2E)	100×
msc.l	Mesoscutum length	Length of mesoscutum along median line from posterior edge of pronotum to posterior edge of mesoscutum, dorsal view (Fig. 2E)	100×
mt1.b	Metasomal tergite 1 breadth	Greatest breadth of metasomal tergite 1 at the posterior margin, dorsal view (Fig. 2F)	100×
mt1.l	Metasomal tergite 1 length	Medial length from the base of metasomal tergite 1 to the posterior margin, dorsal view (Fig. 2F)	100×

400 nM of each primer, 1 unit of Taq DNA polymerase (New England Biolabs), and purified water to a final volume of 25 μL. Amplicons of COI were generated with an initial denaturation of 1 min at 95°C, followed by 35 cycles of 95°C for 15 s, 49°C for 15 s and 72°C for 45 s, and a final elongation period of 4 min at 72°C. Amplicons of *CytB* were generated with an initial denaturation of 2 min at 95°C, followed by 35 cycles of 95°C for 15 s, 45°C for 15 s and 72°C for 30 s, and a final elongation period of 4 min at 72°C. Reaction products were cleaned with Agencourt CleanSEQ magnetic beads (Beckman Coulter Life Sciences, Indianapolis, IN, U.S.A.) and sequenced in both directions using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, U.S.A.) and the Applied Biosystems 3730xl DNA Analyzer at the University of Kentucky, Advanced Genetic Technologies Center (UK-AGTC). Contigs were assembled and edited using GENEIOUS version 8.18 (Kearse *et al.*, 2012), and alignment was conducted using MUSCLE (Edgar, 2004) and then hand-corrected using reading frames as a guide in BIOEDIT (Hall, 1999). Sequences obtained from this study were deposited in GenBank (see Table S2).

Phylogenetic analyses

The two genes were concatenated for the Bayesian analysis, which was performed using MRBAYES version 3.6.11 (Ronquist *et al.*, 2012) on the CIPRES Science Gateway (Miller *et al.*, 2010). Two independent searches were carried out and four chains run for 20 000 000 generations with sampling every 1000 and 10% burn-in discarded. The dataset was not partitioned based on nucleotide position and as it would limit the amount of data needed for accurate parameter estimation. The best-fitting model of molecular evolution was tested using JMODELTEST2 (Darriba *et al.*, 2012), and the general time-reversible model, with a parameter for invariant sites and rate heterogeneity modelled under a gamma distribution (GTR+I+G) was chosen based on the Bayesian information criterion (BIC). The concatenated dataset can be accessed at the Dryad Digital Repository (http://datadryad.org/; accession # doi:10.5061/dryad.vv183). Intra- and interspecific genetic distances were calculated using MEGA version 7.0 (Kumar *et al.*, 2016) using the Kimura-2-parameter model (Kimura, 1980). The phylogenetic trees were visualized in FIGTREE v1.4.2 (Rambaut, 2014) and enhanced using INKSCAPE 0.91 (The Inkscape Team, 2016).

Results

Molecular analysis

The concatenated analysis was performed on 123 exemplars, with 122 taxa amplified for *COI* (579bp) and 31 for *CytB* (397bp). There was some difficulty amplifying *CytB* sequences, particularly for pinned type material. Of the COI sequences, 81 were downloaded from BOLD Systems (http://www.boldsystems.org/). The Nearctic *P. pallipes* complex was recovered as a monophyletic clade with strong support. The two species groups (*P. dayi* and *P. mellipes*) recognized by Goulet & Mason (2006) were also recovered as monophyletic (Fig. 3); however, only three of the nine delineated species were supported.

Within the *P. dayi* species group, *P. dayi* and *P. braunae* were recovered as paraphyletic with respect to each other, indicating only one valid species (Fig. 3). Genetic distances also supported only one species within the *dayi* species group, as the average intraspecific distance was 1.7% in *COI* and 0.8% in *CytB* (Fig. 3, Table S3A), whereas interspecific distances between other clades within the *P. pallipes* complex ranged from 9.7 to

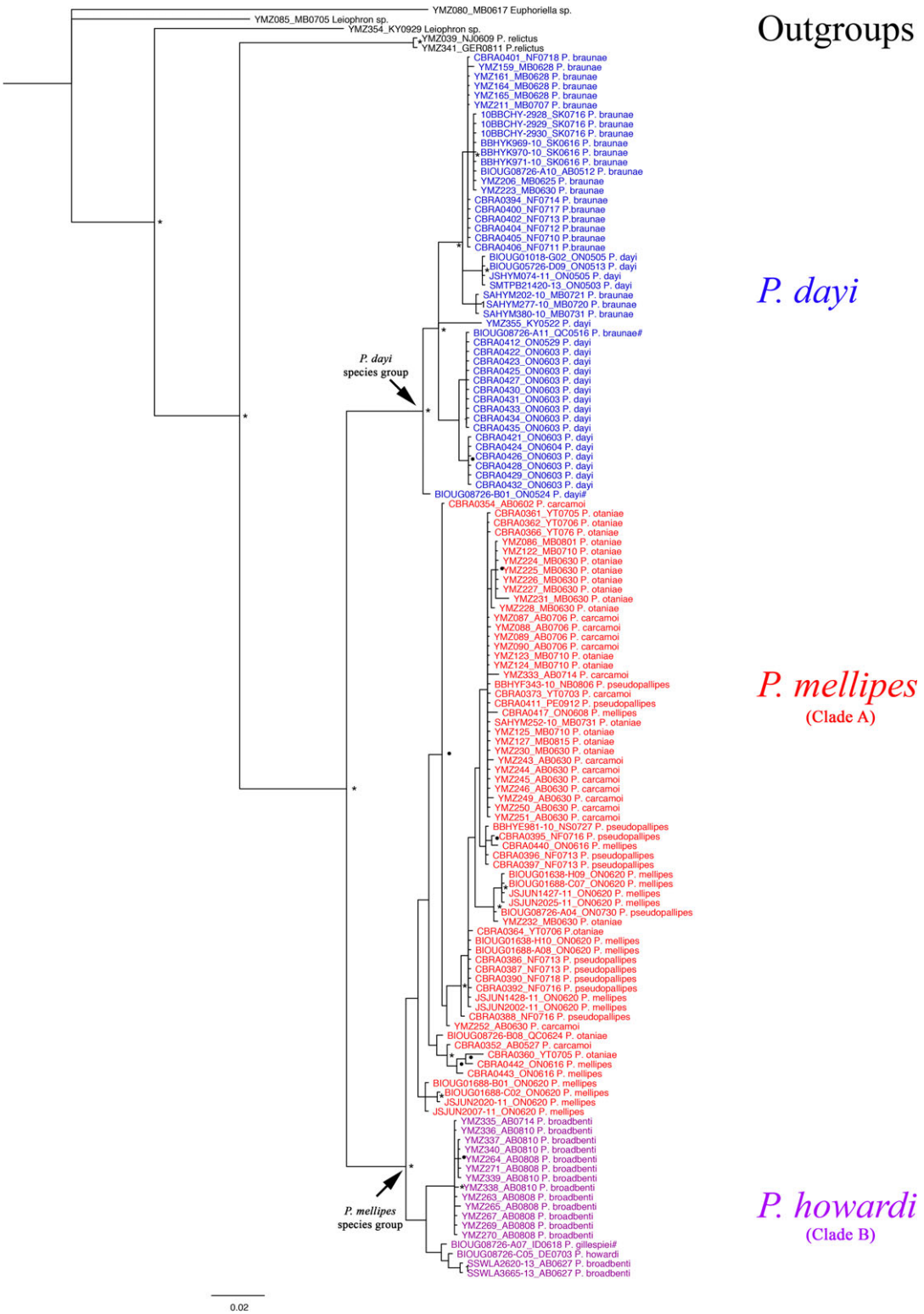

Fig. 3. Inferred concatenated topology from the Bayesian analysis of COI and CytB. Posterior probabilities ≥0.95 are indicated by an asterisk; posterior probabilities between 0.90 and 0.94 are indicated by a black dot. Arrows indicate species groups, and clades A and B represent *Peristenus mellipes* and *Peristenus howardi*, respectively. [Colour figure can be viewed at wileyonlinelibrary.com].

10.2 (Table S3B). There were no clear phylogenetic patterns based on spatial or temporal data, such that specimens from across all localities and early and late flight times were recovered in paraphyly. Thus, only one species is supported for the *P. dayi* species group, thereby invalidating the species group.

Within the *P. mellipes* species group, two distinct clades were recovered, labelled A and B (Fig. 3). Clade A included all specimens identified as *P. mellipes*, *P. pseudopallipes*, *P. otaniae* and *P. carcamoi*; however, they were all recovered as paraphyletic with respect to each other, indicating only one valid species among the four. The average intraspecific distances for the *mellipes* clade were 1.6% for *COI*, and 0.3% for *CytB* (Fig. 3, Table S3A), whereas interspecific distances between other clades within the *P. pallipes* complex ranged from 4.2 to 10.2 (Table S3B), indicating a distinct barcoding gap. Similar to *P. dayi*, there was no phylogenetic spatial or temporal patterns, indicating one widely distributed species. This clade is named as *P. mellipes*, which is the oldest synonym for the members of the Nearctic *P. pallipes* complex included in this clade. In the second clade (Fig. 3, Clade B), *P. broadbenti*, *P. gillespiei* and *P. howardi* were recovered together and paraphyletic with respect to each other, with average intraspecific distances of 0.9% in *COI* and 0.2% in *CytB* (Fig. 3, Table S3A), indicating only one valid species among the three. The smallest distance to other recovered clades was 4.2% in *COI* to *P. mellipes* (Table S3B), again signifying a distinct barcoding gap. The included exemplars ranged from Nevada to Alberta and Idaho, indicating a widely distributed species found from mid-June to early August. The clade is named *P. howardi*, as it is the senior synonym of the three included species epithets.

Morphometrics analysis

The three species supported by the molecular data (*P. dayi*, *P. mellipes*, *P. howardi*) were examined using a multivariate ratio analysis. Assignment to species was deliberately avoided and groups were assigned based on molecular operational taxonomic units (MOTUs) according to the results of the molecular analysis. A series of shape principal component analyses (PCAs) were performed to determine how well the MOTUs were supported by variation in shape. A PCA is appropriate for this study because it does not require *a priori* assignment of specimens, but instead assumes that all MOTUs belong to a single group, thus avoiding bias with respect to particular groupings (Laszlo *et al.*, 2013). Only the first and second shape principal components were informative and accounted for 56.8% of the variation (Fig. 4A). The two species groups (*P. dayi* and *P. mellipes*) were separated based on the first principal component; however, the two MOTUs within the *P. mellipes* group (*P. mellipes* and *P. howardi*) were not (Fig. 4A). The second principal component showed no separation between the two species groups. The first principal component was plotted against isometric size (Fig. 4B), which is defined as the geometric mean of all body measurements (see Baur & Leuenberger, 2011). There was no correlation between shape and size, indicating little to no allometry (Baur & Leuenberger, 2011), which indicates that differences in measured ratios across species are independent of body size.

Principle component analyses and allometry ratio spectrums are generated to show the best characters for discriminating putative species. Characters at opposite ends of the PCA spectrum show the greatest variation and therefore the best likelihood of diagnosing species, whereas characters closer together contribute very little to variation and should not be used. The allometry ratio spectrum is used in a similar manner; however, the further characters are from each other, the greater the allometry. The ratio spectrum of the first principal component showed that most of the variation was explained by ratios such as *eye.d:eye.b* or *eye.d:eye.h* (Fig. 4C). The allometric ratio spectrum showed that the ratios *mt1.b:eye.h* and *mt1.b:eye.b* contributed the most to allometry within the groups (Fig. 4D). The variables that correspond to the separation of the two species groups (Fig. 4C) were different from the variables that showed the greatest allometry (Fig. 4D), indicating that these characters are different as a result of shape and not size (Laszlo *et al.*, 2013). A linear discriminant analysis (LDA) ratio extractor was then used to determine which ratios would be the best at separating the two groups: the most discriminating ratio was *eye.d:eye.b*, with the second best being *eye.h:gsp.l* (see ranges for the LDA ratios in Table S4), which are used in the identification key to help facilitate species identification (see later).

Thus, the morphometrics data support only two species (*P. dayi* and *P. mellipes*), corresponding to the original species groups put forth by Goulet & Mason (2006). This result contrasts with the three species supported by the molecular and ecological and biogeographical data. Thus, the two species within the *P. mellipes* species group are truly cryptic, as the molecular data provide abundant evidence to support separation of the species based on a distinct barcoding gap and the phylogenetic species concept. The distributions and flight times of the three supported *Peristenus* species are expanded to reflect the current taxonomic revisions (Table 2). The ecological data further support separation between *P. mellipes* and *P. howardi* as the range of the latter species is restricted to western North America, ranging from western Canada down to California. Additionally, *P. howardi* has been reared from *Lygus hesperus*, which is only a western species (Goulet & Mason, 2006).

Discussion

Members of the *P. pallipes* complex are difficult to distinguish due to high intraspecific and low interspecific morphological variation (Goulet & Mason, 2006). Using the integrative taxonomy approach of combining morphological characters, molecular evidence and ecological data, we have re-examined the Nearctic *P. pallipes* complex to refine the species groups, and to test the validity of the species within each group. Our results did not support the species concepts put forth by Goulet & Mason (2006), in which each of the species would have resulted in a monophyletic clade with specific geographical and/or peak flight time patterns, as seen in Table S1. The morphometric, molecular and ecological data support the synonymy of *P.*

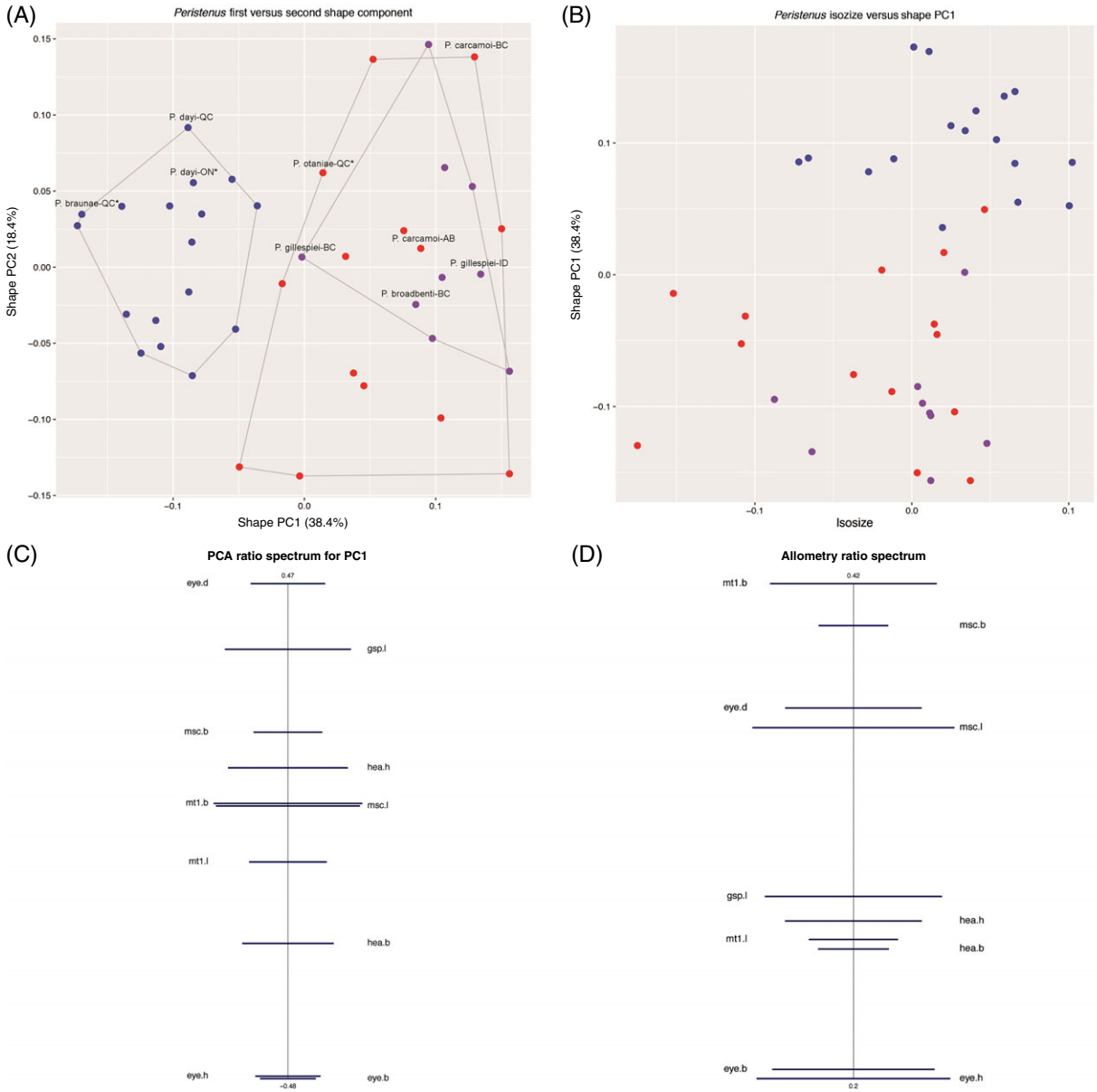

Fig. 4. Size and shape analysis of ♀ *Peristenus* using all variables. (A, B) Blue, *Peristenus dayi*; red, *Peristenus mellipes*; purple, *Peristenus howardi*: (A, B) Shape principal component analyses (PCA): (A) scatterplot of first against second shape principal component (PC); (B) scatterplot of isosize against first shape PC. The variance explained by each shape PC is shown in parentheses. (C, D) Ratio spectra: (C) PCA ratio spectrum; (D) allometry ratio spectrum. Horizontal bars in the ratio spectra represent 68% bootstrap confidence intervals based on 1000 replicates. [Colour figure can be viewed at wileyonlinelibrary.com].

braunae as a junior synonym of *P. dayi* **syn.n.** The seven members within the *P. mellipes* group (*P. broadbenti*, *P. carcamoi*, *P. gillespiei*, *P. howardi, P. mellipes*, *P. otaniae*, *P. pseudopallipes*) are split into two species, with *P. carcamoi*, *P. otaniae* and *P. pseudopallipes* synonymized under *P. mellipes* **syn. n.**; and *P. broadbenti* and *P. gillespiei* synonymized under *P. howardi* **syn. n.**. Interestingly, in regions like Lethbridge, Alberta, where multiple species occur, there seems to be evidence for niche partitioning: *P. mellipes* emerges from early May to the end of June to attack the first-generation *Lygus*, whereas *P. howardi* emerges later, from late June to early September, and attacks the second generation (Fernández, 2016). Future studies will focus on the two *Peristenus* species at the population level, rather than at the species level, to examine microevolutionary forces at a finer scale that may contribute to reproductive isolation and maintenance of these two species (Y. M. Zhang *et al.*, unpublished data).

The diversity of *Peristenus*, in particular the *P. pallipes* complex, is undoubtedly much higher than currently known and the entire group is in need of taxonomic revision. While the

Table 2. Updated list of Nearctic species of the *Peristenus pallipes* complex, their distribution, host and peak flight time incorporating the results of this study.

Species	Distribution	Flight period	Voltinism	Host(s)	Provincial/state record
P. dayi	Across North America	May–August	Bivoltine	*Adelphocoris lineolatus*, *Lygus lineolaris*	AB, AK, BC, CA, CO, DE, MB, NB, NF[a], NJ, NS, NT, NY, ON, QC, SK, UT
P. mellipes	Widespread across North America	May–September	Bivoltine	*A. lineolatus*, *L. lineolaris*, *Lygus* spp.	AB, BC, CO, CT, DE, GA, IL, KS, MA, MB, ME, MI, MO, MS, NB, NC, NF, NS, NJ, NY, OH, ON, QC, SK, VA, YT[a]
P. howardi	Western across North America	May–September	Multivoltine	*L. hesperus*, *Lygus* spp.	AB, BC, CA, ID, MT, NV, OR, WA, WY

[a]New provincial/state records.

Palearctic fauna is beyond the scope of this paper, the relationships between the Holarctic *Peristenus* fauna is integral to resolving the *P. pallipes* complex as a whole (C. van Achterberg and H. Goulet, unpublished data). A similar approach using integrative taxonomy is highly recommended as molecular evidence is vital as a screening process to avoid over-splitting based on inconsistent morphological data. Multivariate morphometrics based on characters primarily on the head was used for this study, but perhaps geometric morphometrics of the wings or interference patterns might improve species delimitation when combined with molecular and ecological data (Villemant *et al.*, 2007; Shevtsova & Hansson, 2011).

This taxonomic revision has interesting implications regarding the impact that European *Peristenus* have had on native *Peristenus*. The distributions of the three *Peristenus* species were expanded as a result of this study, demonstrating a very wide geographical range, especially for *P. mellipes* and *P. dayi*. While these species may have been locally extirpated from parts of eastern Canada (Goulet & Mason, 2006), they remain more abundant in western Canada. Local extirpation is thus less of an issue, as reintroductions from other regions could be possible, making the two species less susceptible to extinction. However, whether or not local extirpation of native *Peristenus* has affected more regions is unknown, as only limited sampling has been done across North America (Goulet & Mason, 2006). Cases of competitive displacements as a result of biocontrol agent introductions have been reported in other groups of braconids, such as the displacement the native *Cotesia glomerata* L. by the introduced *Cotesia rubecula* L. in northeastern North America (van Driesche, 2008); or the European *Aphidius ervi* Haliday outcompeting native *Praon pequodorum* Viereck in the span of 20 years (Schellhorn *et al.*, 2002). While the decline of native *Peristenus* as a result of competitive displacement has largely been anecdotal (Goulet & Mason, 2006), it still serves as a warning to future biocontrol studies on the inadvertent effects of foreign parasitoids on native beneficial insect fauna.

The integrative taxonomic approach used here allowed for a more accurate circumscription of native fauna and their potential to be competitively displaced by foreign biocontrol agents. Thus, the renewed interest in releasing the European *P. digoneutis* to western Canada should be considered carefully, as the release may have detrimental effects on native *Peristenus* populations. In particular, *P. howardi* may be more susceptible to extirpation and possibly extinction as it has a more limited distribution. Using this study as an example, researchers in biocontrol should continue to work closely with taxonomists both pre- and post-release of foreign agents, as well as augmentation and conservation of native natural enemies. However, this is particularly important when exotic biocontrol agents are imported to control native pests. Native pests have their suite of natural enemies, and foreign agents create new competitive forces for these natural enemies. This is in direct contrast to more classical biocontrol programmes where the pest is exotic and thus benefits from enemy-free space in the new environment (Holt & Lawton, 1993). Finally, post-release monitoring of pest and native population dynamics is highly recommended to better understand the impacts of foreign agents on local fauna.

Key to Nearctic *Peristenus pallipes* complex

Notes to the key: The morphometric ratios apply to > 95% of the specimens, with only minor overlap. The coloration for *P. mellipes* and *P. howardi* are consistent, and locality can be further used to separate the species.

1. Punctures large and dense between inner eye margin and lateral ocellus (Fig. 5A). Metatibia testaceous or pale reddish brown, and metatarsomere 1 testaceous (Fig. 5E). Female minimum eye distance approximately 1.25× the breadth of the eyes (mean eye.d:eye.b = 1.24; range = 1.14–1.38). Found across Canada and northern USA from California to Nova Scotia (Table 2) . . . . . ***Peristenus dayi*** Goulet

1′. Punctures fine and scattered between inner eye margin and lateral ocellus (Fig. 5B). Metatibia dark brown to black in apical half of the dorsal surface, metatarsomere 1 darker than following tarsomeres (Fig. 5F). Female minimum eye distance approximately the same as the breadth of the eyes (eye.d:eye.b = 1.05; range = 0.90–1.24). **2**

2. Clypeus (Fig. 5C), metasoma and metacoxa light brown (Fig. 5F), found mainly east of the Rocky Mountains, extending from the Yukon Territories to Newfoundland (Table 2)..... ***Peristenus mellipes*** (Cresson)

2′. Clypeus (Fig. 4D), metasoma and metacoxa black (Fig. 5G), found mainly west of the Rocky Mountains, extending from southern Alberta to pacific coast down to Nevada (Table 2) . . . . . ***Peristenus howardi*** Shaw

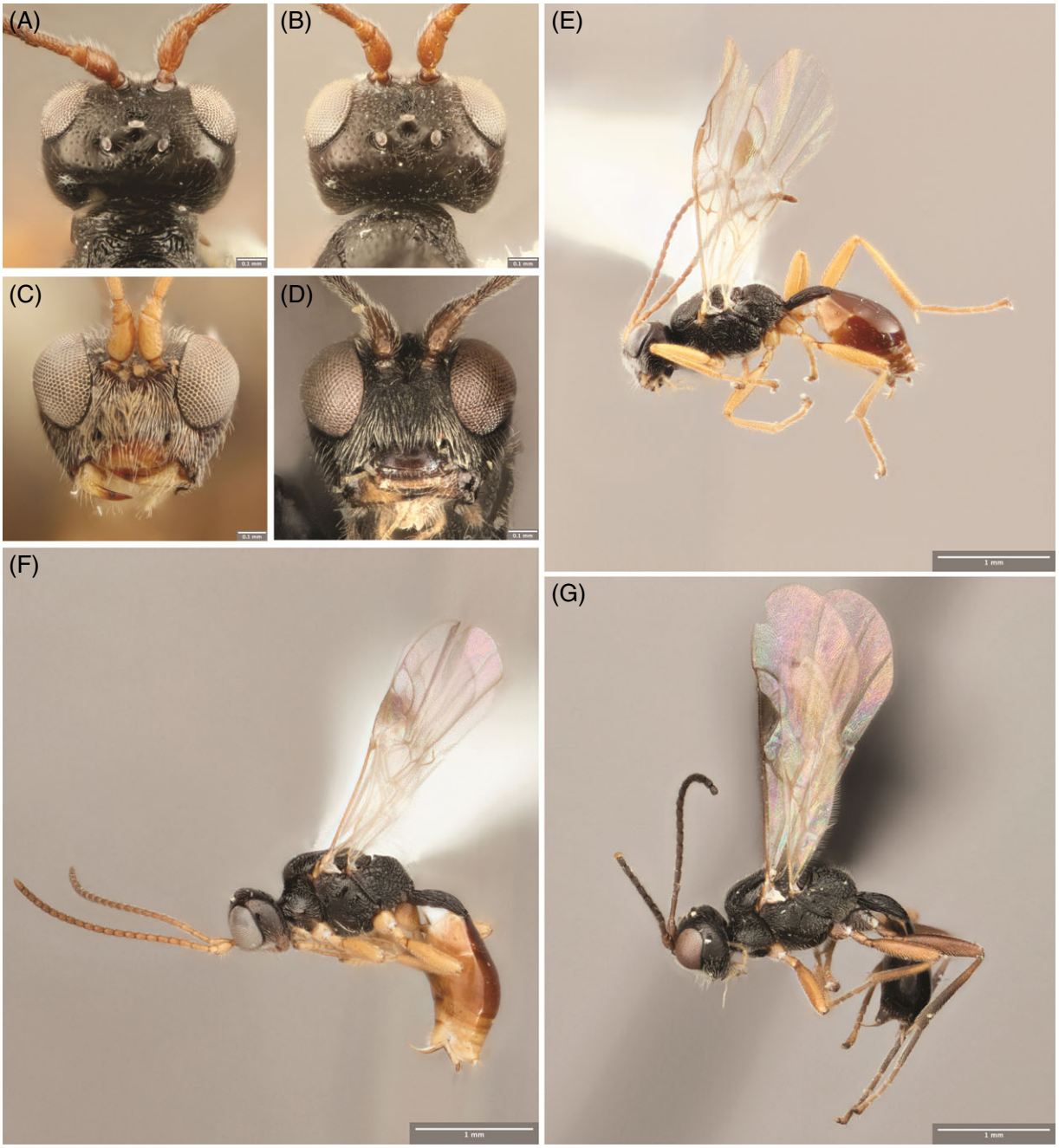

Fig. 5. (A, B) Dorsal view of the ♀ head: (A) *Peristenus dayi*, (B) *Peristenus mellipes*. (C, D) Frontal view of the ♀ head: (C) *Peristenus mellipes*, (D) *Peristenus howardi*. (D–F) Lateral habitus of ♀: (E) *Peristenus dayi*, (F) *Peristenus mellipes*, (G) *Peristenus howardi*. [Colour figure can be viewed at wileyonlinelibrary.com].

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/syen.12233

Table S1. List of Nearctic species of the *Peristenus pallipes* complex, their distribution, host and peak flight time according to Goulet and Mason (2006).

Table S2. Specimens of *Peristenus* used in this study, including the collection information and the gene that were amplified. #, type specimens; *, specimens from the Barcode of Life Database (BOLD).

Table S3. (a) Intraspecific divergence of *COI* and *CytB* for the *Peristenus* species calculated using the Kimura-2-Parameter (K2P). (b) Interspecific divergence of *COI* (1st value) and *CytB* (second value) for the *Peristenus* species calculated using the Kimura-2-Parameter (K2P).

Table S4. The best separating ratios for females of *Peristenus dayi* and *Peristenus mellipes* complex.

Acknowledgements

We thank the following people for providing specimens and for offering an insight into *Peristenus* biology needed for this study: Henri Goulet and José Fernández-Triana at Canadian National Collections of Insects (CNCI); Peter Mason at Agriculture and Agri-Food Canada; Tim Haye at Centre for Agricultural Bioscience International (CABI); Héctor Cárcamo, Carolyn Herle and Catalina Fernández at the Lethbridge Research Centre; Michael Sharkey and Eric Chapman at the University of Kentucky; Thomas Dorsey and Alexandra Villiard at the Phillip Alampi Beneficial Insect Rearing Laboratory; and Steven Paiero at the University of Guelph Insect Collection (DEBU). We also thank the Barcode of Life group at the University of Guelph for sequencing a subset of specimens as well as providing DNA extracts, and Valérie Lévesque-Beaudin for assistance with data submission. We would also like to thank Amber Bass, Leah Irwin, Pablo Fernandes, Whitney Lodge-Zaparnick, Tharshinidevy Nagalingam, Melanie Scallion and Chalsie Warren at the University of Manitoba for assistance with field work, and Jordan Bannerman, Phillip Snarr, and Leanne Peixoto at the University of Manitoba for technical support for the morphometrics and molecular work. We also extend sincere thanks to all of the organizations that have provided funding for research and travel for this study, including a Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grant (awarded to BJS) and the Western Grains Research Foundation Grant in conjunction with the Agriculture and Agri-Food Canada Growing Forward 2 Program (awarded to BJS).

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Accepted 17 March 2017
First published online 8 May 2017