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ARTICLE

DNA barcoding as a useful tool in the systematic study of wild bees of the tribe Augochlorini (Hymenoptera: Halictidae)

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Abstract: Special care is needed in the delimitation and identification of halictid bee species, which are renowned for being morphologically monotonous. *Corynura* Spinola and *Halictillus* Moure (Halictidae: Augochlorini) contain species that are key elements in southern South American ecosystems. These bees are very difficult to identify due to close morphological similarity among species and high sexual dimorphism. We analyzed 170 barcode-compliant COI sequences from 19 species. DNA barcodes were useful to confirm gender associations and to detect two new cryptic species. Interspecific distances were significantly higher than those reported for other bees. Maximum intraspecific divergence was less than 1% in 14 species. Barcode index numbers (BINs) were useful to identify putative species that need further study. More than one BIN was assigned to five species. The name *Corynura patagonica* (Cockerell) probably refers to two cryptic species. The results suggest that *Corynura* and *Halictillus* species can be identified using DNA barcodes. The sequences of the species included in this study can be used as a reference to assess the identification of unknown specimens. This study provides additional support for the use of DNA barcodes in bee taxonomy and the identification of specimens, which is particularly relevant in insects of ecological importance such as pollinators.

Key words: DNA barcodes, cryptic species, Chile, Argentina.

Résumé : Un soin particulier est requis lors de la délimitation et de l'identification des espèces d'abeilles de la famille des Halicitidae, lesquelles sont reconnues pour être monotones sur le plan morphologique. Les genres *Corynura* Spinola et *Halictillus* Moure (Halictidae : Augochlorini) comprennent des espèces qui constituent des composantes clés des écosystèmes de la portion australe de l'Amérique du Sud. Ces abeilles sont très difficiles à identifier en raison de la grande similarité morphologique entre les espèces et d'un fort dimorphisme sexuel. Les auteurs ont analysé 170 séquences conformes de codes à barres de l'ADN provenant de 19 espèces. Les codes à barres ont permis de confirmer les associations entre les genres et pour déceler deux nouvelles espèces cryptiques. Les distances interspécifiques étaient significativement plus grandes que celles rapportées chez d'autres espèces d'abeilles. La divergence intraspécifique était inférieure à 1 % chez 14 espèces. Des numéros d'index de codes à barres (BIN) se sont avérés utiles pour identifier des espèces putatives nécessitant des études plus poussées. Plus d'un BIN ont été assignés à cinq espèces. Le nom *Corynura patagonica* (Cockerell) réfère vraisemblablement à deux espèces cryptiques. Les résultats suggèrent qu'il est possible d'identifier les espèces des genres *Corynura* et *Halictillus* au moyen de codes à barres. Les séquences des espèces issues de ce travail peuvent servir de référence pour identifier des spécimens inconnus. Cette étude fournit une preuve additionnelle de l'utilité des codes à barres de l'ADN pour des fins de taxonomie chez les abeilles et pour l'identification des spécimens, lesquelles sont particulièrement pertinentes chez des insectes pollinisateurs ayant une grande importance écologique. [Traduit par la Rédaction]

Mots-clés : codes à barres de l'ADN, espèces cryptiques, Chili, Argentine.

Introduction

Bees (Hymenoptera: Apoidea: Anthophila) are a diverse group of insects, with more than 20 000 species distributed worldwide (Ascher and Pickering 2015). They play a key role in most terrestrial ecosystems as the main pollinators of many wild and cultivated plants (Klein et al. 2007; Ollerton et al. 2012). The family Halictidae are often the most frequently collected bees in surveys (e.g.,

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Grixti and Packer 2006; Gravel 2010; Le Féon et al. 2016). This family is known to include several highly speciose but morphologically monotonous genera such as *Lasioglossum* Curtis (Gibbs 2009*a*, 2009*b*, 2010). This lack of distinguishing morphological characters is a common problem in bee taxonomy (Packer et al. 2009).

Molecular techniques like DNA barcoding (Hebert et al. 2003*a*) are increasingly being used by taxonomists to aid species delimitation (Smith et al. 2008; Butcher et al. 2012) and identification (Sheffield et al. 2009). In wild bees, DNA barcodes have proven to be a powerful tool for associating the sexes in dimorphic species (Gibbs 2009*a*; Sheffield et al. 2009), delimiting morphologically difficult-to-distinguish species (Gibbs 2009*a*; Rehan and Sheffield 2011; Williams et al. 2011), and identifying specimens in surveys (Murray et al. 2008; Bertsch 2009; Sheffield et al. 2009; Magnacca and Brown 2010, 2012). It has proven to be highly successful in correct identification of specimens to species in well-known faunas (Schmidt et al. 2015). Nonetheless, there remain some issues in some studies with respect to experimental design and analytical methods (Collins and Cruickshank 2013).

Corynura Spinola, the halictid genus with the most species in Chile (Montalva and Ruz 2010), is endemic to Chile and the Andean *Nothofagus*-dominated forests of north-west Patagonia in Argentina. *Corynura* is composed of two subgenera, *Corynura* (19 spp.; González-Vaquero 2015) and *Callistochlora* Michener (3 spp.; González-Vaquero and Galvani 2016), very different in appearance but similar in many morphological characters (González-Vaquero 2015). This genus is closely related to *Halictillus* Moure (6 spp.; González-Vaquero 2010), also restricted to southern South America, and the clade comprised of both genera is sister to the remaining genera of the tribe Augochlorini (Danforth and Eickwort 1997; Engel 2000; Gonçalves 2016).

The species of these genera exhibit strong sexual dimorphism: the males have very long antennae, reaching beyond the posterior of the mesosoma in most species, while those of the female are short. In both *Halictillus* as well as the subgenus *C.* (*Corynura*) the male metasoma is strongly petiolate whereas that of the female is of a normal ovoid shape. In addition, males and females are very different in surface sculpture. As it is details of surface sculpture that usually permit separation of closely related halictine species (e.g., Gibbs 2009*a*, 2009*b*, 2010), this makes gender associations even more difficult. Many species show intraspecific variation in colour, while at the same time being extremely similar in other morphological traits, making species delimitation a challenging task (González-Vaquero 2015).

Corynura and *Halictillus* are very abundant throughout their geographic ranges and are key components of southern South American ecosystems. *Corynura* (*Callistochlora*) *chloris* (Spinola) is very important for Chilean agriculture, being a major pollinator of some crops (De Ugarte Serra 1991; Viscarra Torrico 1996; Miranda Villalón 2002) and native plants (Lehnebach and Riveros 2003; Cares-Suárez et al. 2011; Espinoza et al. 2012). These genera are also frequent visitors of native wildflowers in Argentina (Gravel 2010; González-Vaquero et al. 2014).

We used DNA barcoding as a tool in the systematic study of *Corynura* and *Halictillus* to associate males with their conspecific females and to aid in species delimitation. New tools to correctly identify the species of these early-branching genera of Augochlorini should be very useful for future ecological studies.

Materials and methods

Taxon sampling and molecular protocols

We attempted to collect fresh specimens of all Argentinean species of *Corynura* and *Halictillus*, performing field trips to Neuquén and Río Negro near the Andes (National Parks Lanin and Nahuel Huapi), Buenos Aires province, and La Rioja between 2010 and 2012. These were supplemented with specimens collected by Laurence Packer and Anne-Isabelle Gravel in different regions of Chile and in Chubut (Argentina), between 2000 and 2009. The specimens were collected with entomological nets or pan-traps and kept in 96% ethanol. After pinning the bees in the laboratory, the right midleg was removed and processed for DNA extraction. Whenever possible, we analyzed multiple female and male individuals of a species to check gender associations and to detect intraspecific sequence divergences among specimens obtained from distant localities. Most tissue sampling was performed during the workshops "Extending and Enhancing DNA Barcoding Research in Argentina and Neighboring Countries: Third/Fourth Leading Labs Training Workshop, 2011/2012". The barcoded vouchers are housed at the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN), Buenos Aires, Argentina, and the Packer Collection at York University (PCYU), Toronto, Ontario, Canada. Collection information and lateral or dorsal photographs of the specimens are available in the project file "Southern South American Augochlorini" (CORYN) in the Completed Projects section of the Barcode of Life Data Systems (BOLD, www.boldsystems.org; Ratnasingham and Hebert 2007).

The specimens were identified by comparison to the type specimens, with the use of morphological keys and the original descriptions of the species (see González-Vaquero 2010, 2015). Although *C. aureoviridis* (Friese) was listed as a synonym of *C. chloris* (Moure 2007), it must be considered a valid species (González-Vaquero and Galvani 2016). The five morphospecies mentioned in Table 1 are new to science and are being described elsewhere (González-Vaquero and Roig-Alsina, and González-Vaquero et al. in preparation).

DNA extraction and amplification were performed either at the Canadian Centre for DNA Barcoding (CCDB) at the University of Guelph, Guelph, Ontario, Canada, or at the Laboratorio de Códigos de Barras Genéticos MACN-CONICET at MACN. All sequences were obtained at the

Table 1. Maximum within-species variation and distance to nearest neighbour (NN) of DNA barcodes from *Corynura* and *Halictillus*.

	Intraspecific variation (%)	Distance to NN (%)	Sample size	No. of localities	No. of BINs
Corynura (*Corynura*) *ampliata* (Alfken)	0.15	9.00	18	8 ARG	1
C. (*Callistochlora*) *aureoviridis* (Friese)	0.65	8.70	15	10 ARG	1
C. (*Co.*) *bruchiana* (Schrottky)	0	12.60	10	6 ARG	1
C. (*Co.*) *chilensis* (Spinola)	6.86, 0.71*	9.25	11	4 ARG, 1 CHI	2
C. (*Ca.*) *chloris* (Spinola)	0	8.70	3	3 CHI	1
C. (*Co.*) *corinogaster* (Spinola)	0.19	9.53	3	1 ARG, 2 CHI	1
C. (*Co.*) *herbsti* (Alfken)	0.31	5.53	10	3 CHI	1
C. (*Co.*) *lepida* Alfken	0.15	9.03	3	2 CHI	1
C. (*Co.*) *patagonica* (Cockerell)	4.15	7.27	6	2 ARG, 2 CHI	2
C. (*Co.*) *rubella* (Haliday)	2.50, 0.50†	5.53	18	8 ARG, 1 CHI	2
Corynura (*Co.*) sp1	0.93	11.51	11	5 ARG, 1 CHI	1
Corynura (*Co.*) sp3a	0.93	7.36	4	3 ARG	1
Corynura (*Co.*) sp3b	0	7.36	7	4 ARG	1
Corynura (*Co.*) sp5	0.93	9.00	10	4 ARG	1
Corynura (*Co.*) sp9	0.17	8.67	5	2 CHI	1
Halictillus amplilobus González-Vaquero	0.50	14.00	8	4 ARG	1
H. badiclypeus González-Vaquero	2.98	13.84	9	2 ARG	3
H. reticulatus González-Vaquero	3.46, 0.62‡	5.39	12	8 ARG	2
H. verissimus Gonçalves	0	5.39	7	1 CHI	1

Note: ARG, Argentina; CHI, Chile.
*Not considering specimen MACN-En 8136.
†Not considering specimens MACN-En 8208 and CCDB-10040 A11.
‡Not considering specimens MACN-En 9718 and MACN-En 9719.

CCDB. Laboratory procedures followed the standard protocols applied by the CCDB, which are available online (www.ccdb.ca/resources.php), using the PCR primers LepF1/LepR1. Sequences were examined for indels and stop codons as a check against pseudogenes. Only barcode-compliant sequences (>500 bp, <1% ambiguous bases, two trace files) were used for the analyses.

Data analyses

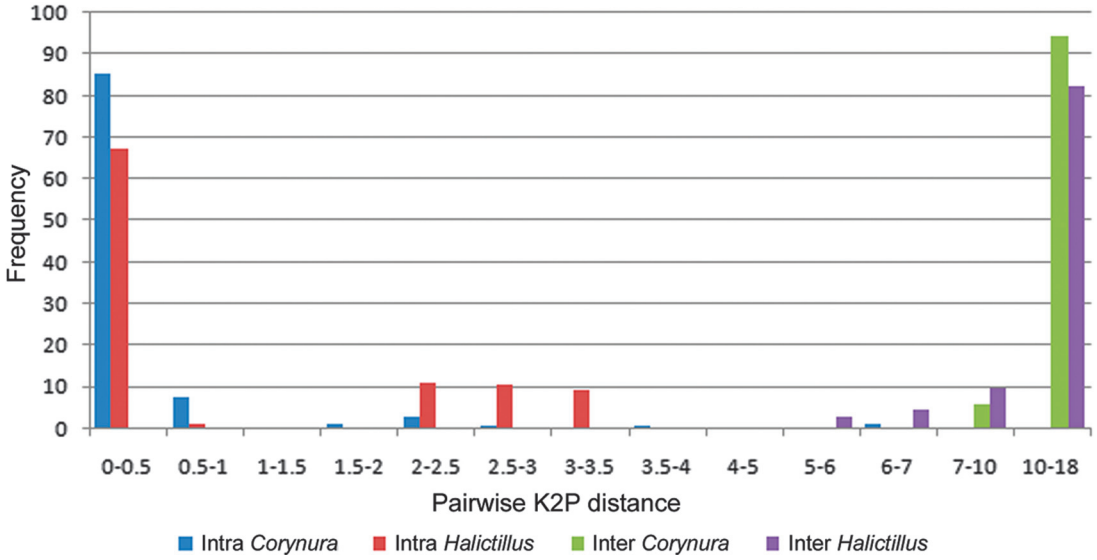
BOLD delineates molecular operational taxonomic units (MOTUs), which are recognized through sequence variation in their DNA barcodes. Since MOTUs have a close concordance to actual species (Schmidt et al. 2015), a barcode index number (BIN) is automatically assigned to each MOTU and to each new animal barcode record as it is incorporated into BOLD (Ratnasingham and Hebert 2013). All sequences obtained in this study have been deposited in GenBank (accession numbers in the supplementary data, Table S1[1]).

The program MEGA v.6 (Tamura et al. 2013) was used to align the sequences (function "Align by ClustalW (codons)", default options) and then to estimate sequence divergences among specimens, using the Kimura 2-parameter distance model (Kimura 1980), with pairwise deletion of missing data. Maximum, minimum, and mean divergences were subsequently calculated for both conspecific specimens and congeneric species. A tree was obtained using the neighbour-joining algorithm (Saitou and Nei 1987) to visualize genetic divergences. Inkscape v.0.48 (www.inkscape.org) was used to edit the tree. Nearest neighbour distances (the smallest pairwise distance to a member of a different species) were calculated using the "Barcode Gap analysis" function of BOLD, with pairwise deletion of missing data. Voucher specimens identified by morphology as a single species, which were grouped in more than one BIN, were re-examined morphologically.

Parsimony (PA) and maximum likelihood (ML) analyses were performed to assess the phylogenetic relationships among the species. A specimen of *Rhinocorynura crotonis* Ducke (BOLD number BOFTW147-08; GenBank accession number KU983470) was used to root the tree based upon earlier phylogenetic research on Augochlorini (Engel 2000). TNT v.1.1 (Goloboff et al. 2008) was used for the parsimony analysis. We performed a traditional search of 100 replications, saving 10 trees per replication, using the TBR algorithm. MEGA v.6 (Tamura et al. 2013) was used for ML, using a TN 93+G+I model of substitution as suggested by the program (function "Find best-fit substitution model", default options; model with the lowest BIC score). Branch support was assessed by bootstrapping with 1000 replicates in both analyses.

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/gen-2016-0006.

Fig. 1. Intra- and interspecific divergence values for all pair-wise comparisons of *Corynura* and *Halictillus*.



Results

We obtained 170 barcode-compliant sequences from 15 species of *Corynura* and four species of *Halictillus* (Table S1)[1]. After trimming the ends, a sequence alignment of 654 base pairs was used for analyses. Overall, 391 positions were conserved, and 263 were variable. Of the latter, 254 were phylogenetically informative. All sequences had a similar GC% (mean 29.24; min. = 26.52, max. = 32.11), with a particularly high AT% at the third codon position (mean 86.93; min. = 78.90, max. = 91.79). Mean interspecific distances were 13.72% (5.53%–17.71%) for *Corynura* and 14.20% (5.39%–17.25%) for *Halictillus* (see Tables S2–S3[1] for all species comparisons; Fig. 1). Both genera were recovered separately in the distance-based trees (Figs. 2–3). Within *Corynura*, the species with the most similar barcode sequences were *C. herbsti* (Alfken) and *C. rubella* (Haliday) (minimum pairwise interspecific divergence 5.53%), *C. rubella* and *C. patagonica* (Cockerell) (7.27%), and *Corynura* sp3a and *Corynura* sp3b (7.36%). While *C. herbsti*, *C. patagonica*, and *C. rubella* can be easily identified by morphology alone, the other two species are cryptic. In *Halictillus*, the minimum interspecific divergence was between *H. reticulatus* González-Vaquero and *H. verissimus* Gonçalves (5.39%), two partially sympatric species with very similar morphology. The distribution of these species overlaps in the Chilean regions of Araucania and Los Lagos. When we compared any other pair of specimens from different species of *Halictillus*, the divergence varied from 14.00% to 17.25%.

This analysis helped us to detect two cryptic species that could not initially be separated by morphology. The specimens first identified as *Corynura* sp3 were separated into two groups according to their DNA barcodes. The minimum sequence divergence between them was 7.36%, while the maximum intragroup divergence was 0.93%. After a detailed study of morphological structures, we found subtle differences in sculpture and pilosity between individuals from the two clusters, and we considered them as two separate species, herein termed *Corynura* sp3a and *Corynura* sp3b.

The maximum within-species variation was less than 1% in 14 out of the 19 species studied (Table 1; Fig. 1). The clusters agreed with the previously identified morphological species, and no BIN was associated with more than one species (except, initially, for the case of *Corynura* sp3 mentioned above). Three species had high intraspecific variation, which resulted in them being given more than one BIN assignment. A total of five specimens (*C. chilensis* (Spinola): MACN-En 8136; *C. rubella*: MACN-En 8208 and CCDB-10040 A11; *H. reticulatus*: MACN-En 9718 and 9719) caused these additional BINs to be allocated. Only one of these sequences were considerably shorter than the rest (511 bp, *C. rubella* CCDB-10040 A11). These specimens had not been collected in distant places and did not exhibit morphological differences when compared to the other individuals identified as being conspecific. In *Corynura* there was an overlap of the maximum intraspecific and minimum interspecific divergence values between 5.53% and 6.86%, due to the outlier specimen of *C. chilensis* (MACN-En 8136). The distance to the nearest neighbour surpassed the maximum intraspecific variation in all species (Table 1; Fig. 4).

Another species that showed a high maximum intraspecific divergence was *H. badiclypeus* González-Vaquero. Three clusters with different BINs but little intra BIN divergence were detected: BIN: BOLD:ACG2192 with 0%–0.32% divergence, BIN: BOLD:ACG2191 with 0%–0.15% divergence, and BIN: BOLD:ACG2552 with 0.46%–0.92% divergence. Each BIN was represented by three barcodes. In contrast, the divergences among any pair of specimens from different clusters were always greater than 2% (from 2.12% to 2.98%, Table 1). We have not found morphological differences among these specimens, which were collected in nearby areas of La Rioja (Argentina).

Fig. 2. Neighbor-joining tree for *Corynura* individuals, distances calculated using the Kimura-2-parameter model. The scale represents the number of substitutions per site. In orange are two clusters of *C. patagonica* that diverge up to 4.15% from each other. In red are specimens of *C. rubella* and in purple specimen of *C. chilensis* whose sequences differ considerably from the others.

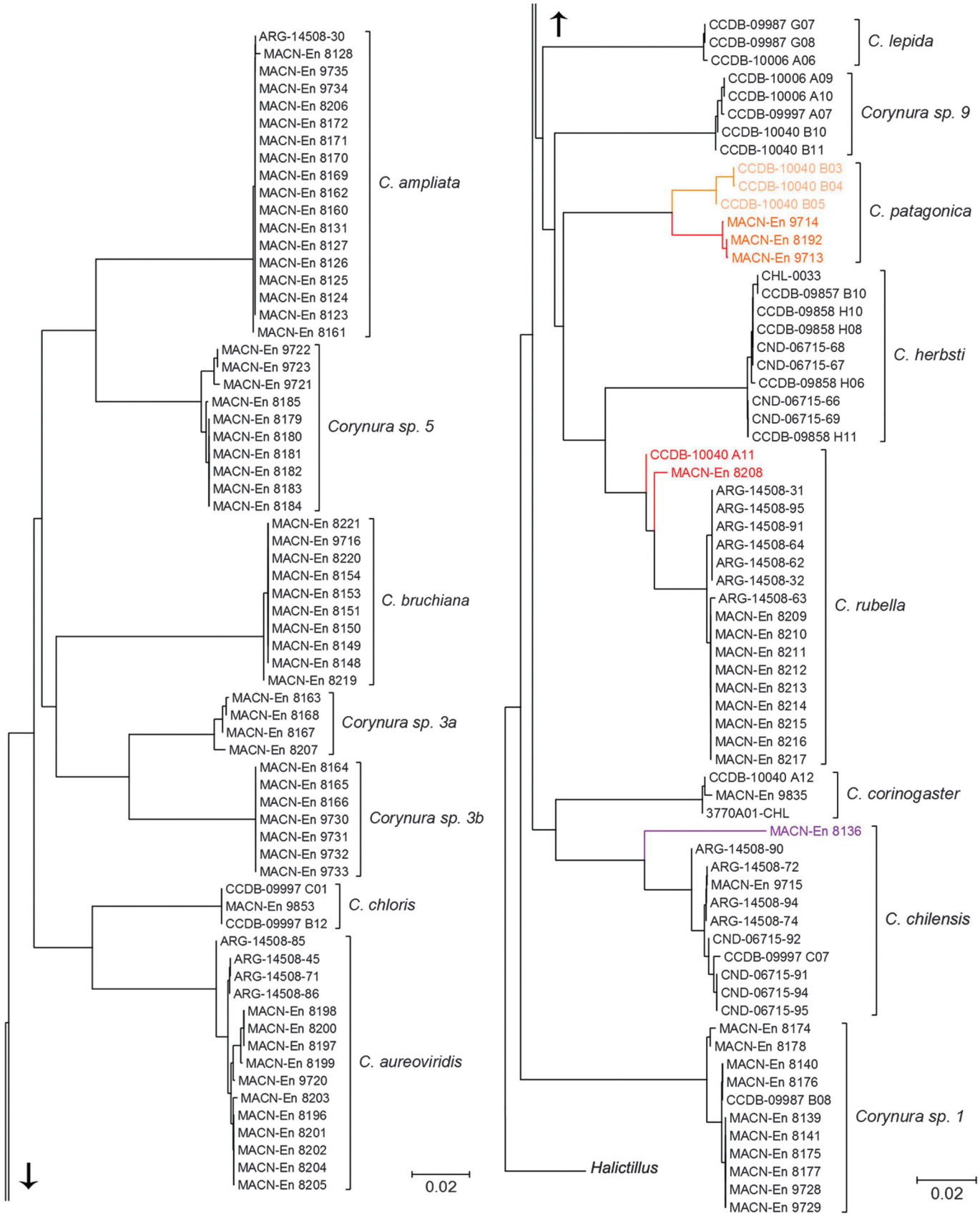
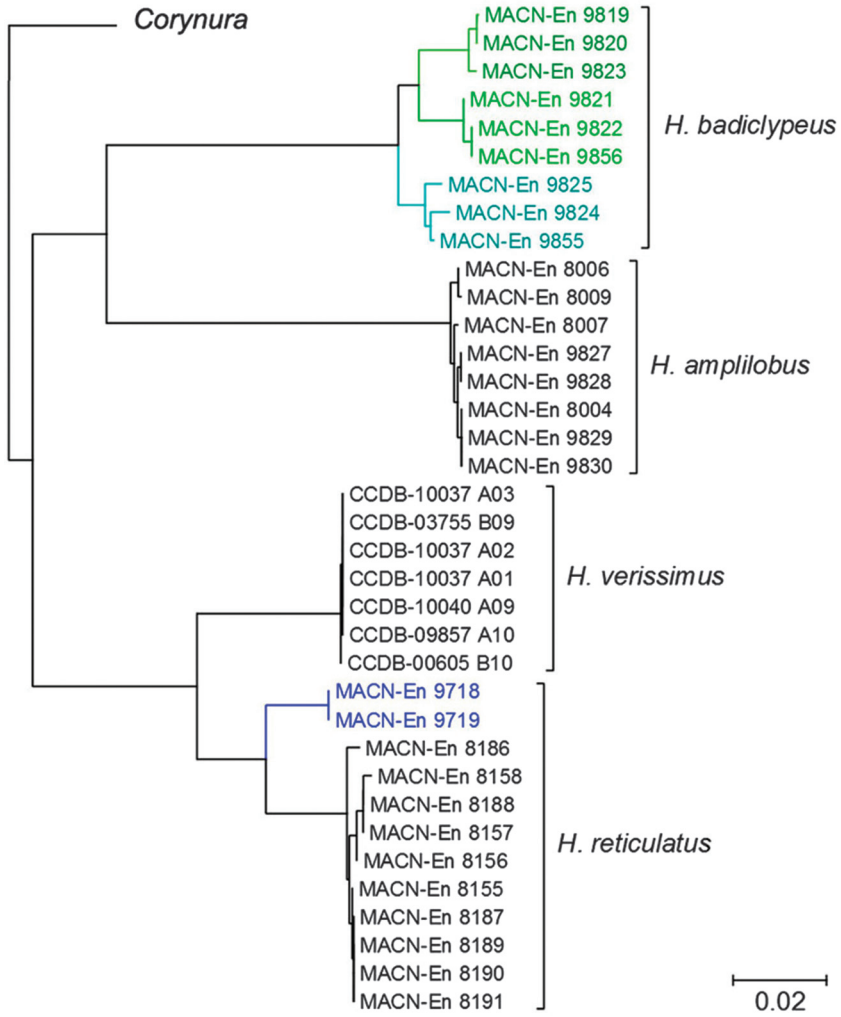


Fig. 3. Neighbor-joining tree for *Halictillus* individuals, distances calculated using the Kimura-2-parameter model. The scale represents the number of substitutions per site. In green are three clusters of *H. badiclypeus* that diverge up to 2.98% from each other. In blue are specimens of *H. reticulatus* whose sequences differ considerably from the others.



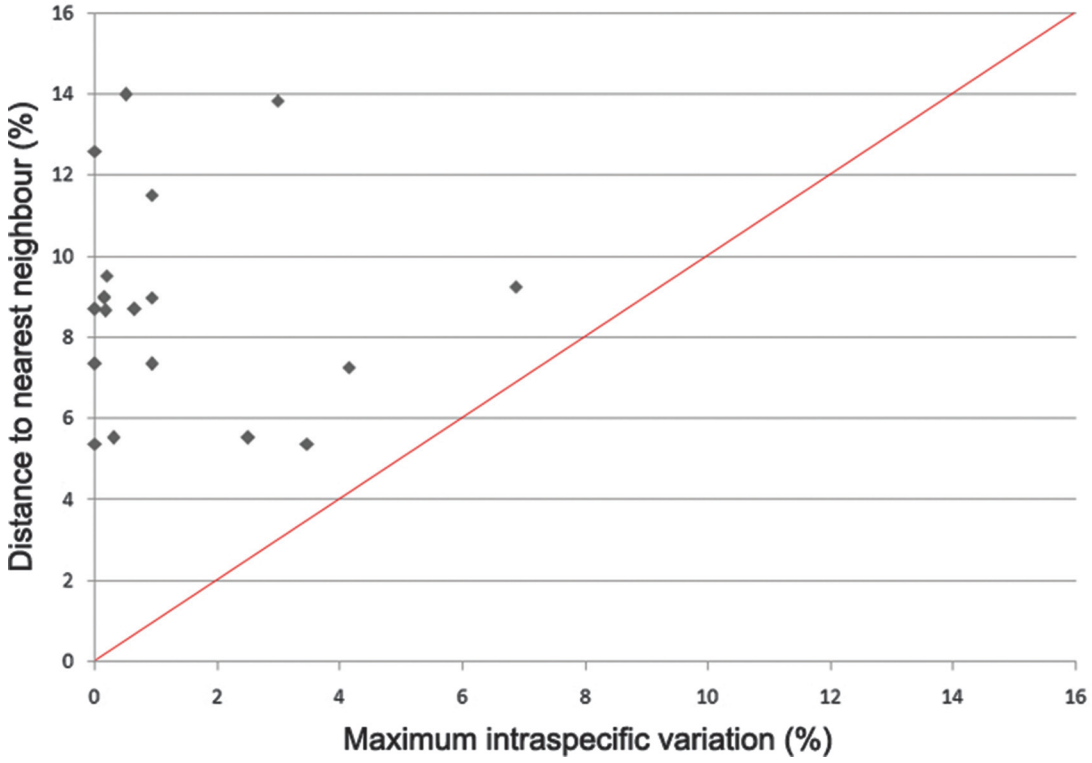
We thus consider this species to have high intraspecific variability but suggest that additional sampling and sequencing of nuclear genes, as well as additional morphological study, should take place.

Corynura patagonica showed distance ranging from 3.95% to 4.15% when specimens from Chile were compared to specimens from Argentina; each cluster was allocated to a separate BIN. The intracluster divergence was less than 0.34%. These specimens were re-examined, and we found no differences in morphology between members of different BINs. However, the specimens had been collected in different biogeographic regions. The three Chilean specimens barcoded were collected in National Park Puyehue (Los Lagos region), 500–550 m a.s.l., in the temperate and humid Subantartic subregion, Valdivian forest district (Morrone 2015), while the female and the two males from Argentina came from drier areas of National Parks Lanin and Nahuel Huapi (Neuquén and Río Negro provinces), 1000 m a.s.l., in the transition area between the Patagonian steppe and the *Nothofagus*-dominated Andean forest (Patagonian subregion; Morrone 2015).

The association of males and females of *Corynura* sp3a and *Corynura* sp3b was made by means of their DNA barcodes. This technique was useful to confirm gender associations in 15 of the 19 species analyzed, since only females were barcoded in the remaining species.

In the phylogenetic analyses the basal branches were not resolved, showing very low bootstrap values (<36% in PA, <42% in ML), failing to support the monophyly of either genus. In spite of this, some pairs of species were recovered as well supported sister species, bootstrap values in parenthesis: *H. reticulatus* and *H. verissimus* (PA 96%, ML 99%), *Corynura* sp3a and *Corynura* sp3b (PA 92%, ML 97%), *C. herbsti* and *C. rubella* (PA 87%, ML 98%), and *C. chloris* and *C. aureoviridis* (PA 79%, ML 97%). Two pairs of sister species supported by the ML analysis showed a low bootstrap value in the PA analysis but reasonably high values with ML: *C. ampliata* (Alfken) and *Corynura* sp5 (PA 36%, ML

Fig. 4. Scatterplot showing the maximum intraspecific variation versus the distance to the nearest neighbour. Each point of the scatterplot is a species.



88%) and *C. corinogaster* (Spinola) and *C. chilensis* (PA 19%, ML 80%).

Discussion

The results obtained for *Corynura* and *Halictillus* agree with previous findings in that DNA barcoding has proven to be a useful tool for bee species delimitation and specimen identification (Gibbs 2009*a*, 2009*b*; Sheffield et al. 2009; Schmidt et al. 2015). This approach relies on the finding that there is a significant difference between the degree of intra- and interspecific variation (Hebert et al. 2003*a*): the so-called "barcode gap" (Meyer and Paulay 2005). A 2%–3% sequence divergence threshold has been suggested to indicate closely related species (Hebert et al. 2003*b*), but this cutoff value is known to vary considerably among taxa (Hebert et al. 2004*a*, 2004*b*; Gibbs 2009*b*, 2010; Gibbs et al. 2013), and each case needs to be considered on its own merits.

In five species of *Lasioglossum*, previously considered as one due to close morphological resemblance, the mean interspecific divergence was 3.1%, while the minimum was 1.7% (Gibbs 2009*a*). Similar low values were found in genera of Apidae, such as in the small carpenter bees *Ceratina* Latreille (Rehan and Richards 2008; Rehan and Sheffield 2011) and in the meliponines *Liotrigona* Moure (Koch 2010) and *Scaptotrigona* Moure (Hurtado-Burillo et al. 2013). However, identical DNA barcodes have been found between some morphologically distinguishable species (e.g., *L. versatum* (Robertson) and *L. callidum* (Sandhouse); Gibbs 2010), while in some cases, deep divergences consistent with multiple species have not been related to geography (e.g., *L. cressonii* (Robertson); Gibbs 2010) or morphology (e.g., *L. ruidosense* (Cockerell); Gibbs 2010). As reported for *Lasioglossum* (Gibbs 2010; Gibbs et al. 2013) and for the genera studied herein, problematic taxa showing high intra-specific divergences apparently are not rare in halictids.

In *Corynura* and *Halictillus* we found deep divergences in both inter- and intraspecific divergences. A maximum intraspecific distance higher than 2% was found in five out of the 19 species examined. Each of these species resulted in two (*C. chilensis*, *C. patagonica*, *C. rubella*, *H. reticulatus*) or three (*H. badiclypeus*) BINs. BINs supported the view that these were cases of either high intraspecific divergence or cryptic species. Multiple BINs per species have been reported for many bee taxa from Central Europe (Schmidt et al. 2015), although in a lower proportion (11% of all species barcoded) than that found in the present study (26%). On the other hand, multiple species can share a particular BIN due to low interspecific divergence (Schmidt et al. 2015). Identification through DNA barcodes can still be possible in these cases provided either that the differences among the sequences allow the discrimination of specimens in neighbour joining trees, within a particular BIN, or there are diagnostic base substitutions or combinations of substitutions (Gibbs 2010). We found no BIN-sharing species in *Corynura*

and *Halictillus*, but in all "species" that were comprised of more than one BIN, the BINs clustered together.

Since DNA barcodes do not always distinguish closely related species (Kuhlmann et al. 2007; Gibbs 2010), morphological traits and geographical distribution were given priority in our delimitation of species. If we had considered BINs as actual species, we would have counted 25 species instead of 19. We believe that the high divergence observed in *C. patagonica* suggest either the presence of genetic lineages in different populations that are geographically separated by the Andes, or the existence of cryptic species for which we have not been able to detect morphological differences. Further study is necessary before taking a decision on the other species that occupied more than one BIN. In contrast, BINs separated what was initially considered to be one species (*Corynura* sp3) into two (*Corynura* 3a and *Corynura* 3b) for which distinguishing morphological characteristics were subsequently discovered (González-Vaquero 2015). In our research, BINs provided a useful basis for subsequent studies, highlighting the cases that need to be investigated further through integrative taxonomy, which would take into account morphological, molecular, geographical, and behavioural data, where available (Dayrat 2005). In this view, the delineation of species has to be assessed on the combined merits of all data sources.

In some cases phylogenetic relations can be inferred from DNA barcodes. For example, this technique permitted the detection of parallel changes in the diversification of *Homalictus* Cockerell species on three different Pacific Island archipelagoes (Groom et al. 2014). In the present study, both parsimony and maximum likelihood analyses resulted in poorly supported trees and a lack of monophyly for either *Corynura* (with TNT) or *Halictillus* (with ML), both of which largely resulted from groupings that had very low support. However, DNA barcode-based phylogenies did support the sister group relationships of four pairs of species, in agreement with results obtained from morphological phylogenies (González-Vaquero 2015). These findings support the view that the barcode region may be useful for phylogenetic analysis of recently diverged taxa but is of limited use for divergences that are older than a few million years (Klopfstein et al. 2010; Kjer et al. 2014).

The proper identification of wild bees is essential in all research involving these important pollinators. Because of this, DNA barcode libraries are particularly useful for studies of crop pollination because the use of such databases provide a fast and economic identification mechanism, especially in areas where the bee fauna is poorly understood and there are few taxonomists capable of accurately identifying the species. DNA barcode libraries have been published for Nova Scotia (Sheffield et al. 2009), Ireland (Magnacca and Brown 2012), and Europe (Schmidt et al. 2015), but they are badly needed for other regions. The public database presented here can be used for identification because it includes all the species of these two genera except those that are very rare, represented only by the type specimen or a few old museum specimens. These data should be very useful for ecological studies in Chile and Argentina, given the abundance of these species in the field (González-Vaquero et al. 2014).

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References

Ascher, J.S., and Pickering, J. 2015. Discover Life bee species guide and world checklist (Hymenoptera: Apoidea: Anthophila). Available from http://www.discoverlife.org/mp/20q?guide=Apoidea_species.

Bertsch, A. 2009. Barcoding cryptic bumblebee taxa: *B. lucorum*, *B. crytarum* and *B. magnus*, a case study. Beitr. Entomol. **59**(2): 287–310.

Butcher, B.A., Smith, M.A., Sharkey, M.J., and Quicke, D.L.J. 2012. A turbo-taxonomic study of Thai *Aleiodes* (*Aleidodes*) and *Aleiodes* (*Arcaleiodes*) (Hymenoptera: Braconidae: Rogadinae) based largely on COI barcoded specimens, with rapid description of 179 new species. Zootaxa, **3457**: 1–232.

Cares-Suárez, R., Poch, T., Acevedo, R., Acosta-Bravo, I., Pimentel, C., Espinoza, C., et al. 2011. Do pollinators respond in a dose-dependent manner to flower herbivory?: An experimental assessment in *Loasa tricolor* (Loasaceae). Gayana Bot. **682**: 176–181.

Collins, R.A., and Cruickshank, R.H. 2013. The seven deadly sins of DNA barcoding. Mol. Ecol. Res. **13**(6): 969–975. doi:10.1111/1755-0998.12046.

Danforth, B.N., and Eickwort, G.C. 1997. The evolution of social behavior in the augochlorine sweat bees (Hymenoptera: Halictidae) based on a phylogenetic analysis of the genera. *In* The

evolution of social behaviour in insects and arachnids. *Edited by* J.C. Choe and J.B. Crespi. Cambridge University Press, Cambridge, Mass. pp. 270–293.

Dayrat, B. 2005. Towards integrative taxonomy. Biol. J. Linn. Soc. **85**: 407–415. doi:10.1111/j.1095-8312.2005.00503.x.

De Ugarte Serra, P.M. 1991. Agentes polinizantes y rendimiento en el cultivo de alforfón (*Fagopyrum esculentum* Moench). Thesis, Facultad de Ciencias Agrarias, Universidad Austral de Chile, Valdivia.

Engel, M.S. 2000. Classification of the bee tribe Augochlorini. Bull. Am. Mus. Nat. Hist. **250**: 1–89. doi:10.1206/0003-0090(2000)250<0001:COTBTA>2.0.CO;2.

Espinoza, C.L., Murúa, M., Bustamante, R.O., Marín, V.H., and Medel, R. 2012. Reproductive consequences of flower damage in two contrasting habitats: the case of *Viola portalesia* (Violaceae) in Chile. Rev. Chil. Hist. Nat. **85**: 503–511. doi:10.4067/S0716-078X2012000400012.

Gibbs, J. 2009*a*. Integrative taxonomy identifies new (and old) species in the *Lasioglossum* (*Dialictus*) *tegulare* (Robertson) species group (Hymenoptera, Halictidae). Zootaxa, **2032**: 1–38.

Gibbs, J. 2009*b*. New species in the *Lasioglossum petrellum* species group identified through an integrative taxonomic approach. Can. Entomol. **141**: 371–396. doi:10.4039/n09-020.

Gibbs, J. 2010. Revision of the metallic species of *Lasioglossum* (*Dialictus*) in Canada (Hymenoptera, Halictidae, Halictini). Zootaxa, **2591**: 1–382.

Gibbs, J., Packer, L., Dumesh, S., and Danforth, B.N. 2013. Revision and reclassification of *Lasioglossum* (*Evylaeus*), *L.* (*Hemihalictus*) and *L.* (*Sphecodogastra*) in eastern North America (Hymenoptera: Apoidea: Halictidae). Zootaxa, **3672**: 1–117.

Goloboff, P., Farris, J., and Nixon, K. 2008. TNT, a free program for phylogenetic analysis. Cladistics, **24**: 774–786. doi:10.1111/j.1096-0031.2008.00217.x.

Gonçalves, R.B. 2016. A molecular and morphological phylogeny of the extant Augochlorini (Hymenoptera, Apoidea) with comments on implications for biogeography. Syst. Entomol. **41**(2): 430–440. doi:10.1111/syen.12166.

González-Vaquero, R.A. 2010. Revisión sistemática del género *Halictillus* (Hymenoptera: Halictidae: Augochlorini) en la Argentina. Rev. Soc. Entomol. Argent. **69**: 65–89.

González-Vaquero, R.A. 2015. Filogenia, taxonomía y biología de los géneros basales de abejas silvestres de la tribu Augochlorini (Hymenoptera: Halictidae). Ph.D. thesis, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Buenos Aires.

González-Vaquero, R.A., Gravel, A.-I., and Devoto, M. 2014. Information retrieved from specimens at Natural History Collections can improve the quality of field-based ecological networks. Community Ecol. **15**(2): 187–193. doi:10.1556/ComEc.15.2014.2.7.

González-Vaquero, R.A., and Galvani, G. 2016. Antennal sensilla analyses as useful tools in the revision of the sweat-bee subgenus *Corynura* (*Callistochlora*) Michener (Hymenoptera: Halictidae). Zool. Anz. **262**: 29–42. doi:10.1016/j.jcz.2016.03.006.

Gravel, A.-I. 2010. Bee Comunity Comparison in Northwestern Patagonia (Argentina). M.Sc. thesis, York University, Toronto, Ont.

Grixti, J.C., and Packer, L. 2006. Changes in the bee fauna (Hymenoptera: Apoidea) of an old field site in southern Ontario, revisited after 34 years. Can. Entomol. **138**(2): 147–164. doi:10.4039/n05-034.

Groom, S.V.C., Stevens, M.L., and Schwarz, M.P. 2014. Parallel responses of bees to Pleistocene climate change in three isolated archipelagos of the southwestern Pacific. Proc. R. Soc. B Biol. Sci. **281**: 20133293. doi:10.1098/rspb.2013.3293.

Hebert, P.D., Cywinska, A., Ball, S.L., and de Waard, J.R. 2003*a*. Biological identifications through DNA barcodes. Proc. R. Soc. B Biol. Sci. **270**(1512): 313–321. doi:10.1098/rspb.2002.2218. PMID:12614582.

Hebert, P.D., Ratnasingham, S., and de Waard, J.R. 2003*b*. Barcoding animal life: cytochrome *c* oxidase subunit 1 divergences among closely related species. Proc. R. Soc. B Biol. Sci. **270**: S96–S99. doi:10.1098/rsbl.2003.0025. PMID:12952648.

Hebert, P.D., Penton, E.H., Burns, J.M., Janzen, D.H., and Hallwachs, W. 2004*a*. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. Proc. Natl. Acad. Sci. U.S.A. **101**(41): 14812–14817. doi:10.1073/pnas.0406166101. PMID:15465915.

Hebert, P.D.N., Stoeckle, M.Y., Zemlak, T.S., and Francis, C.M. 2004*b*. Identification of birds through DNA barcodes. PLoS Biol. **2**(10): e312. doi:10.1371/journal.pbio.0020312. PMID:15455034.

Hurtado-Burillo, M., Ruiz, C., de Jesús May-Itzá, W., Quezada-Eúan, J.J.G., and De la Rúa, P. 2013. Barcoding stingless bees: genetic diversity of the economically important genus *Scaptotrigona* in Mesoamerica. Apidologie, **44**(1): 1–10. doi:10.1007/s13592-012-0146-9.

Kimura, M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. **16**: 111–120. doi:10.1007/BF01731581. PMID:7463489.

Kjer, K.M., Zhou, X., and Frandsen, P.B. 2014. Moving toward species-level phylogeny using ribosomal DNA and COI barcodes: an example from the diverse caddisfly genus *Chimarra* (Trichoptera: Philopotamidae). Arthropod Syst. Phylo. **72**: 345–354.

Klein, A.M., Vaissière, B.E., Cane, J.H., Steffan-Dewenter, I., Cunningham, S.A., Kremen, C., and Tscharntke, T. 2007. Importance of pollinators in changing landscapes for world crops. Proc. R. Soc. B Biol. Sci. **274**: 303–313. doi:10.1098/rspb.2006.3721.

Klopfstein, S., Kropf, C., and Quicke, D.L.J. 2010. An evaluation of phylogenetic informativeness profiles and the molecular phylogeny of Diplazontinae (Hymenoptera, Ichneumonidae). Syst. Biol. **59**: 226–241. doi:10.1093/sysbio/syp105. PMID:20525632.

Koch, H. 2010. Combining morphology and DNA barcoding resolves the taxonomy of Western Malagasy *Liotrigona* Moure, 1961 (Hymenoptera: Apidae: Meliponini). Afr. Invertebr. **51**: 413–421. doi:10.5733/afin.051.0210.

Kuhlmann, M., Else, G.R., Dawson, A., and Quicke, D.L.J. 2007. Molecular, biogeographical and phenological evidence for the existence of three western European sibling species in the *Colletes succinctus* group (Hymenoptera: Apidae). Org. Divers. Evol. **7**: 155–165. doi:10.1016/j.ode.2006.04.001.

Le Féon, V., Poggio, S.L., Torretta, J.P., Bertrand, C., Molina, G.A.R., Burel, F., et al. 2016. Diversity and life-history traits of wild bees in intensive agricultural landscapes in the Rolling Pampa, Argentina. J. Nat. Hist. **50**: 1175–1196. doi:10.1080/00222933.2015.1113315.

Lehnebach, C., and Riveros, M. 2003. Pollination biology of the Chilean endemic orchid *Chloraea lamellata*. Biodivers. Conserv. **12**: 1741–1751. doi:10.1023/A:1023666800948.

Magnacca, K.N., and Brown, M.J. 2010. Mitochondrial heteroplasmy and DNA barcoding in Hawaiian Hylaeus (Nesoprosopis) bees (Hymenoptera: Colletidae). BMC Evol. Biol. **10**: 174. doi:10.1186/1471-2148-10-174. PMID:20540728.

Magnacca, K.N., and Brown, M.J. 2012. DNA barcoding a regional fauna: Irish solitary bees. Mol. Ecol. Resour. **12**(6): 990–998. doi:10.1111/1755-0998.12001. PMID:22931682.

Meyer, C.P., and Paulay, G. 2005. DNA barcoding: error rates based on comprehensive sampling. PLoS Biol. **3**(12): e422. doi:10.1371/journal.pbio.0030422. PMID:16336051.

Miranda Villalón, J.M. 2002. Determinación de la viabilidad en la carga polínica de insectos que visitan flores de avellano chileno (*Gevuina avellana* Mol.). Thesis, Facultad de Ciencias Agrarias, Universidad Austral de Chile, Valdivia, Los Ríos.

Montalva, J., and Ruz, L. 2010. Actualización a la lista sistemática de las abejas chilenas (Hymenoptera: Apoidea). Rev. Chil. Entomol. **35**: 15–52.

Morrone, J.J. 2015. Biogeographic regionalization of the Andean region. Zootaxa, **3936**: 207–236. doi:10.11646/zootaxa.3936.2.3. PMID:25947431.

Moure, J.S. 2007. Augochlorini. *In* Catalogue of Bees (Hymenoptera, Apoidea) in the Neotropical Region. *Edited by* J.S. Moure, D. Urban, and G.A.R. Melo. Sociedade Brasileira de Entomologia, Curitiba. pp. 677–691.

Murray, T.E., Fitzpatrick, Ú., Brown, M.J.F., and Paxton, R.J. 2008. Cryptic species diversity in a widespread bumble bee complex revealed using mitochondrial DNA RFLPs. Conserv. Genet. **9**(3): 653–666. doi:10.1007/s10592-007-9394-z.

Ollerton, J., Price, V., Armbruster, W.S., Memmott, J., Watts, S., Waser, N.M., et al. 2012. Overplaying the role of honey bees as pollinators: a comment on Aebi and Neumann 2011. Trends Ecol. Evol. **27**(3): 141–142. doi:10.1016/j.tree.2011.12.001. PMID: 22209078.

Packer, L., Gibbs, J., Sheffield, C., and Hanner, R. 2009. DNA barcoding and the mediocrity of morphology. Mol. Ecol. Resour. **9**: 42–50. doi:10.1111/j.1755-0998.2009.02631.x. PMID: 21564963.

Ratnasingham, S., and Hebert, P.D.N. 2007. BOLD: the barcoding of life data system (www.barcodinglife.org). Mol. Ecol. Resour. **7**: 355–364. doi:10.1111/j.1471-8286.2007.01678.x.

Ratnasingham, S., and Hebert, P.D. 2013. A DNA-based registry for all animal species: the barcode index number (BIN) system. PLoS ONE, **8**(7): e66213. doi:10.1371/journal.pone.0066213. PMID:23861743.

Rehan, S., and Richards, M. 2008. Morphological and DNA sequence delineation of two problematic species of *Ceratina* (Hymenoptera: Apidae) from Eastern Canada. J. Entomol. Soc. Ont. **139**: 59–67.

Rehan, S.M., and Sheffield, C.S. 2011. Morphological and molecular delineation of a new species in the *Ceratina dupla* species-group (Hymenoptera: Apidae: Xylocopinae) of eastern North America. Zootaxa, **2873**: 35–50.

Saitou, N., and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol. **4**: 406–425. PMID:3447015.

Schmidt, S., Schmid-Egger, C., Morinière, J., Haszprunar, G., and Hebert, P.D.N. 2015. DNA barcoding largely supports 250 years of classical taxonomy: identifications for Central European bees (Hymenoptera, Apoidea *partim*). Mol. Ecol. Resour. **15**: 985–1000. doi:10.1111/1755-0998.12363. PMID:25588628.

Sheffield, C.S., Hebert, P.D., Kevan, P.G., and Packer, L. 2009. DNA barcoding a regional bee (Hymenoptera: Apoidea) fauna and its potential for ecological studies. Mol. Ecol. Resour. **9**: 196–207. doi:10.1111/j.1755-0998.2009.02645.x. PMID:21564979.

Smith, M.A., Rodríguez, J.J., Whitfield, J.B., Deans, A.R., Janzen, D.H., Halwachs, W., and Hebert, P.D.N. 2008. Extreme diversity of tropical parasitoid wasps exposed by iterative integration of natural history, DNA barcoding, morphology, and collections. Proc. Natl. Acad. Sci. U.S.A. **105**: 12359–12364. doi:10.1073/pnas.0805319105. PMID:18716001.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., and Kumar, S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis, version 6.0. Mol. Biol. Evol. **30**: 2725–2729. doi:10.1093/molbev/mst197. PMID:24132122.

Viscarra Torrico, R.C. 1996. Comportamiento de insectos asociados a las flores de Frambueso (*Rubus idaeus* L.), en relación a su actividad como agentes bióticos de polinización. Thesis, Facultad de Ciencias Agrarias, Universidad Austral de Chile, Valdivia, Los Ríos.

Williams, P.H., An, J., and Huang, J. 2011. The bumblebees of the subgenus *Subterraneobombus*: integrating evidence from morphology and DNA barcodes (Hymenoptera, Apidae, *Bombus*). Zool. J. Linn. Soc. **163**(3): 813–862. doi:10.1111/j.1096-3642.2011.00729.x.