



Phylogenomics and historical biogeography of the cleptoparasitic bee genus *Nomada* (Hymenoptera: Apidae) using ultraconserved elements

Katherine A. Odanaka^{a,*}, Michael G. Branstetter^b, Kerrigan B. Tobin^b, Sandra M. Rehan^a

^a Department of Biology, York University, 4700 Keele St, Toronto, ON M3J 1P3, Canada

^b U.S. Department of Agriculture, Agricultural Research Service (USDA-ARS), Pollinating Insects Research Unit, Utah State University, Logan, UT 84322, USA

ARTICLE INFO

Keywords:

Bees
Ultraconserved elements
Phylogenomics
Geodispersal
Cleptoparasitism
Brood parasitism
Divergence dating

ABSTRACT

The genus *Nomada* Scopoli (Hymenoptera: Apidae) is the largest genus of brood parasitic bees with nearly 800 species found across the globe and in nearly all biogeographic realms except Antarctica. There is no previous molecular phylogeny focused on *Nomada* despite their high species abundance nor is there an existing comprehensive biogeography for the genus. Using ultraconserved element (UCE) phylogenomic data, we constructed the first molecular phylogeny for the genus *Nomada* and tested the monophyly of 16 morphologically established species groups. We also estimated divergence dates using fossil calibration points and inferred the geographic origin of this genus. Our phylogeny recovered 14 of the 16 previously established species groups as monophyletic. The *superba* and *ruficornis* groups, however, were recovered as non-monophyletic and need to be re-evaluated using morphology. Divergence dating and historic biogeographic analyses performed on the phylogenetic reconstruction indicates that *Nomada* most likely originated in the Holarctic ~ 65 Mya. Geodispersal into the southern hemisphere occurred three times: once during the Eocene into the Afrotropics, once during the Oligocene into the Neotropics, and once during the Miocene into Australasia. Geodispersal across the Holarctic was most frequent and occurred repeatedly throughout the Cenozoic era, using the De Geer, Thulean, and the Bering Land Bridges. This is the first instance of a bee using both the Thulean and De Geer land bridges and has implications of how early bee species dispersed throughout the Palearctic in the late Cretaceous and early Paleogene.

1. Introduction

Nearly 13% of all bee species globally and around 20% of all bee species in the family Apidae exhibit the cleptoparasitic behavioral trait known as brood parasitism (Danforth, Minckley, & Neff, 2019). An ancient trait found within four different bee families (Cardinal, Straka, & Danforth, 2010), brood parasites play important ecological roles within their ecosystems. Brood parasites are known to be the main cause of brood damage (Minckley & Danforth, 2019); however, the dependency of brood parasites on their hosts make them important indicators of bee community health (Odanaka & Rehan, 2019; Sheffield, Pindar, Packer, & Kevan, 2013). Recent work indicates that the majority of brood parasitic bees (excluding the genera *Aglæ* and *Exacerbata* (Euglossini), and *Ctenoplectrina* (Ctenoplectrini)) are found within the apid subfamily Nomadinae (Bossert et al., 2019; Cardinal et al., 2010; Sless et al., 2022).

The genus *Nomada* is the largest genus of brood parasites with an estimated 795 species found globally (Alexander & Schwarz, 1994;

Litman et al., 2013). While members of the genus *Andrena* are common hosts, *Nomada* are known to parasitize species from other bee families including Apidae, Halictidae, Melittidae, and Colletidae. *Nomada* are most species diverse across the Holarctic region and use a mode of parasitism dubbed “open cell” or “larva-open strategy” (Litman et al., 2013). Adult female *Nomada* seek out and deposit eggs into host nest cells that are still being provisioned. After egg laying, a hospicial larva emerges and kills any remaining host eggs or larvae in the cell (Litman, 2019; Litman et al., 2013; Rozen, 1991).

Historically, *Nomada* taxonomy has been ambiguous; Snelling in the 1980's separated *Nomada* in the Western Hemisphere across three genera and ten subgenera based off of genus group names that had been proposed between 1880's and 1940's (Snelling, 1986). *Nomada* was last reorganized into 16 species groups by Alexander (1994) using morphological cladistic methods (App. table 1). This revision of the genus transferred some of Snelling's subgenera into species groups and created new groups for species that were formerly unplaced (=incertae

* Corresponding author at: 4700 Keele St, Toronto, ON M3J 1P3, Canada.

E-mail address: katherine.odanaka@gmail.com (K.A. Odanaka).

sedis) (Alexander, 1994). Of the currently recognized 16 species groups, the majority are found in either the Holarctic, Palearctic, or Nearctic regions; however, every ecoregion except Antarctica is represented (Alexander, 1994; Michener, 2007). Since the establishment of the 16 species groups there have been no subsequent studies, molecular or morphological, to validate these groups or examine the phylogenetic relationships among them.

There is currently no comprehensive assessment of the historical biogeography of *Nomada*. Consequently, the age and origin of the genus is rather uncertain. There are two putative hypotheses regarding the region of origin. The first hypothesis suggests that the Neotropics might be the region of origin for *Nomada* due to the diversity and abundance of species found within South America (Michener, 1979, 2007). Despite the high diversity and abundance, nearly all *Nomada* species found in the Neotropics were sorted into what eventually became the *vegana* species group (Alexander & Schwarz, 1994). From the Neotropics, it has further been suggested that *Nomada* later dispersed northward into the Holarctic and then outwards into the remaining ecoregions (Michener, 1979, 2007). The second hypothesis is based on cladistic results indicating that the “basal” clade of the genus originated in the Afrotropics, specifically South Africa (Alexander, 1989, 1991). This conclusion was drawn primarily based on the finding that the *gigas* group was the most plesiomorphic and had the least amount of shared characteristics with any of the other species groups (Alexander, 1989, 1991). However, Alexander (1991) also noted that there are not many apomorphic characters shared by all the *Nomada* groups. It was further suggested by Alexander (1991) that *Nomada* then dispersed northward from the Afrotropics into the Palearctic and then into the remaining ecoregions. Despite differences in region of origin, both hypotheses predicted a tropical southern hemisphere origin of the genus with dispersal into the northern hemisphere and then back into the remaining southern hemisphere ecoregions.

Here we provide the first global molecular phylogeny of *Nomada* representing all 16 species groups and all ecoregions where the genus occurs. We also infer the group’s historical biogeography using divergence dating and model-based biogeographic reconstruction methods. The objectives are threefold: 1) construct the first global molecular phylogeny of *Nomada* covering all known species groups, 2) determine the age and origin of the genus, and 3) map the historical biogeography of this genus including representation from all known ecoregions; in doing so, we test the two competing hypotheses regarding the origin of the genus.

2. Methods

2.1. Sample collection

A total of 144 individuals were used in the phylogenetic analyses for this study. Of these individuals, 142 represent unique species. The ingroup included 119 taxa, of which 114 were newly sequenced (App. table 2). The sampled *Nomada* represented all 16 species groups and the six biogeographic realms where the genus is found. For the outgroup, 23 taxa representing all four apid subfamilies (Anthophorinae, Apinae, Nomadinae, Xylocopinae) were included (App. table 2), 16 of which are newly sampled. Data for five ingroup and seven of the outgroup taxa were retrieved from several recent publications (Freitas et al. 2020; Grab et al. 2019; Sless et al. 2022). Samples used in this study were obtained through loans from various institutions as well as recent field collections from around the world. For rooting our phylogenetic trees we used the apid subfamily Anthophorinae.

2.2. DNA extraction

DNA was extracted from pinned specimens using non-destructive methods and Zymo Quick-DNA Miniprep Plus Kits (Zymo Research, Irvine, CA, USA). Whole body specimens were carefully removed from

the pins and placed into individual tubes with a Proteinase-K digestion buffer solution. Tubes were then incubated overnight at 55°C and extracted according to manufacturer protocols, with the modifications noted in Branstetter et al. (2021). Following extraction, specimens were washed in 95% ethanol, dried and re-mounted back on the pin. DNA concentration was checked using a Qubit 3.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA) and quality was assessed using a TapeStation instrument (Agilent, Santa Clara, CA, USA).

2.3. UCE library preparation and enrichment

We used UCE phylogenomics to generate our sequence data (Faircloth et al., 2012) and followed the protocols outlined in Branstetter et al. (2017). We used a bee-ant specific probe set (Grab et al., 2019), which is just a subset version of the principal (Branstetter et al., 2017), to enrich 2,545 UCE loci. The probes are synthesized and available through Arbor Biosciences (Ann Arbor, MI, USA).

Extracted samples were first sheared to fragment sizes of ~ 400–600 bp using a Qsonica sonicator (Q800R2; Qsonica, Newton, CT, USA). Older samples were not sheared due to their already degraded status; all other samples were sonicated for either 30, 60, or 90 s depending on their quality. Illumina libraries were generated using KAPA HyperPrep kits (Roche Sequencing, Pleasanton, CA, USA) and custom dual-indexing adapters (Glenn et al., 2019). Fragmented DNA was purified and concentrated using an in house paramagnetic-bead solution (Rohland & Reich, 2012). Once the final bead cleaning was complete, sample DNA concentration was measured using a Qubit 3 fluorometer and then pooled into 12 groups containing 9–10 samples of equimolar concentrations.

Enrichment of the samples followed protocols from both Arbor Biosciences (v4 protocol; day1) and a standard UCE protocol (Blumenstiel et al., 2010; day 2) split over two consecutive days. After enrichment, each pool was quantified using qPCR, combined into one sample and then sent off for sequencing.

2.4. Sequencing and data processing

Sequencing, data processing, and phylogenetic analysis follow similar methods outlined in (Branstetter, Muller, Griswold, Orr, & Zhu, 2021). A total of 116 samples were sent to Novogene Inc. (Sacramento, CA, USA) for single lane multiplexed sequencing using Illumina HiSeq X. Our newly generated UCE data were then combined with data from five previously sequenced *Nomada* samples and 23 outgroup samples (Freitas et al. 2020; Grab et al. 2019; Sless et al. 2022). All newly sequenced data are available on NCBI (SRR18055085 – SRR18055199; App. table 2). We extracted UCE data from already published genomes using the “Harvesting UCE Loci from Genomes” tutorial found at (<https://phyluce.readthedocs.io/en/latest/tutorial-three.html>) and described in Faircloth (2017). Raw sequence reads were demultiplexed using BBTools (Bushnell, 2014) and the reads were then cleaned, trimmed, and assembled using the package Phyluce v1.6 (Faircloth, 2016) and its associated programs. Specifically, raw reads were trimmed using Illumiprocessor v2.0 (Faircloth, 2013), a wrapper for the software Trimmomatic (Bolger, Lohse, & Usadel, 2014) and then assembled using Spades (Bankevich et al., 2012). Contigs matching UCE loci were identified and extracted using the program LastZ v1.0 (Harris, 2007) within Phyluce and then aligned using MAFFT v7.130b (Katoh & Standley, 2013). Alignments were trimmed using Gblocks (Talavera & Castresana, 2007), with reduced stringency parameters (b1 = 0.5, b2 = 0.5, b3 = 12, b4 = 7) and the publicly available program Spruceup (Borowiec, 2019), which trims poorly aligned sequence from individual samples rather than columns. For the Spruceup analysis we used Jukes-Cantor distances, a guide tree, and a lognormal cutoff of 0.88. All other settings were left at default values. We then removed empty alignment columns using a custom script and filtered alignments for 75% taxon occupancy.

2.5. Phylogenetic trees

We analyzed our data using concatenated and species tree approaches. For the supermatrix we first examined the effects of partitioning the data by testing several different partitioning schemes: no partitioning, partitioning by locus, and partitioning using the Sliding Window Site Characteristics based on Entropy method (SWSC-EN; Tagliacollo & Lanfear, 2018). The SWSC-EN partitioning scheme divides UCE loci into three separate regions, a main core and a right and left flank (Tagliacollo & Lanfear, 2018). The core region of UCEs is more conserved while the flanking regions are more variable (Faircloth et al., 2012). The initial set of partitions identified by the SWSC-EN algorithm were merged using ModelFinder2 (Kalyaanamoorthy, Minh, Wong, Von Haeseler, & Jermini, 2017), which is part of IQ-Tree v2.1.1 (Nguyen, Schmidt, Von Haeseler, & Minh, 2015). For the analysis we used rclusterf, AICc, and the GTR + G model of sequence evolution. The resulting best-fit partitioning scheme reduced the number of data subsets to 1601. Maximum likelihood analyses were then conducted on each partitioning scheme using IQ-tree v2.1.1. For each analysis we used the GTR + F + G4 model of sequence evolution and measured branch support by conducting 1000 ultrafast bootstrap replicates (UFB; Hoang et al., 2018) and 1000 replicates of the SH-like approximate likelihood ratio test (Guindon et al., 2010; Hoang et al., 2018). To test the concatenated data for potential biases, we converted the matrix to RY-coding using a Phyluce script, and then analyzed the data in IQ-Tree using the SWSC-EN partitioning scheme and the “-MFP” option for model testing. Phylogenetic inference can be influenced by the potential negative impact of data saturation and base composition bias (Phillips & Penny, 2003). RY coding has been shown to reduce these negative impacts and the approach has been used to improve phylogenetic results in bees (Praz & Packer, 2014).

For species tree inference, we estimated individual gene trees for all loci using IQ-Tree and the standard DNA alignments, i.e. not converted to RY coding. We used the “-m MFP” option for model selection and estimated branch support by conducting 1000 UFB replicates. We collapsed nodes receiving less than 10% support using Newick Utilities (Junier & Zdobnov, 2010). Using the gene trees as input, we carried out a standard species tree analysis using the summary-based method implemented in the program Astral III (Zhang, Sayyari, & Mirarab, 2017). Species tree branch supports were assessed based on local posterior probabilities.

2.6. Divergence dating and biogeography

We time dated the SWSC-EN partitioned tree from the above analyses using the program MCMCTree v4.9j found in PAML (Yang, 1997, 2007) and the R package MCMCTreeR (Puttick, 2019). The SWSC-EN tree was selected for dating because the SWSC-EN partitioning scheme had the best model fit and results among phylogenetic analyses were highly congruent. We secondarily calibrated the root node of the tree using a normal distribution prior with a mean age of $104.4 \text{ Mya} \pm 10 \text{ Mya}$ which was previously found in Cardinal, Buchmann, & Russell (2018). For all other calibrated nodes, we used fossil calibrations and applied skew normal prior distributions. Nine different outgroup fossils were used to calibrate the analysis, including one thought to be a brood parasite (App. table 3). We tested three data matrices: all loci, 1000 clocklike loci, and 500 clocklike loci. Clocklikeness was assessed using the program SortaDate (Smith et al., 2018), which measures root-to-tip variance within gene trees and then sorts them from highest to lowest variance. The 500 most clocklike loci were thus the loci with the least root-to-tip variance.

In addition to our main dating analyses described above, we tested an alternative fossil calibration scheme in which we replaced the fossil *Protomelecta brevipennis*, Cockerell (calibrating crown Nomadinae) with the fossil *Paleopeolus micheneri* Dehon et al. (calibrating stem Epeolini). Both of these fossils have somewhat uncertain placement within Apidae; however, we ultimately favored the use of *P. brevipennis* due to recent

criticism regarding the placement of *P. micheneri* within the Nomadinae (Onuferko et al., 2019). We also tested several different prior distributions with the *P. micheneri* fossil calibration to see if changing the prior produced a noticeable effect on ages. We tested uniform, skew normal, Cauchy, and modified Cauchy distributions, all based on the same minimum and maximum ages for the fossils. The results using *P. micheneri* were very similar to the results using *P. brevipennis* and can be found in the supplement (App. table 4; Supp images 1 h-k).

We carried out the approximate likelihood dating method implemented in MCMCTree and used a relaxed lognormal clock model with independent rates and a GTR + G model of sequence evolution. We did not partition the data sets. Four independent runs were conducted on each data matrix and once complete, Tracer v1.7 (Rambaut, Drummond, Xie, Baele, & Suchard, 2018) was used to investigate convergence across runs. Runs were then combined and a final summary tree with mean and 95% Highest Posterior Densities (HPD) was generated.

After dating the trees, we investigated the historical biogeography of *Nomada* using the R program BioGeoBEARS (Matzke, 2013). Three different biogeographic models were tested on each of the three main data matrices: the dispersal, extinction, cladogenesis model (DEC; (Ree, Moore, Webb, & Donoghue, 2005; Ree & Smith, 2008), a modified version of the dispersal-vicariance model that uses maximum likelihood (DIVA-like; Ronquist, 1997), and a derived version of the Bayesian Analysis of Biogeography model (BayArea-like; Landis, Matzke, Moore, & Huelsenbeck, 2013) that also uses maximum likelihood. We additionally tested the versions of each model that includes jump dispersal (+j) however, based on current criticism of the +j parameter, we did not include those results (Ree & Sanmartín, 2018). We do provide the results in the supplement (App. table 5). We considered six ecoregions in our models: Nearctic, Neotropics, Palearctic, Afrotropics, Indomalaya, and Australasia. We set the maximum range size to six so that all ecoregions were considered in analysis. Selection of the best fit model was done using AIC scores within the program.

3. Results

3.1. Molecular phylogeny

We used a data matrix filtered for 75% taxon occupancy for our downstream analysis which consisted of 2,014 loci, a total of 1,431,861 bp, and 752,356 bp informative sites. The mean locus length was 710.95 bp and the total amount of missing data was 30.1%. All five trees (no partitioning, by locus partitioning, SWSC partitioning, ry-coding, and species) tested were mostly congruent with only minor differences in placement of various species within the clades (App. Fig. 1).

All generated phylogenies support the monophyly of the genus and reveal that the sister group to all other *Nomada* is the *odontophora* species group, a small species group found in the eastern Mediterranean (Fig. 1). Trees indicated that 14 of the 16 previously established species groups are monophyletic, providing molecular confirmation for the majority of the morphological distinctions of the different groups (Alexander, 1994). Two exceptions are the *superba* and *basalis* species groups, which need to be merged into one monophyletic clade, and paraphyly of the large *ruficornis* clade. For the *superba* and *basalis* groups, the results indicate that the *basalis* species group is nested within the *superba*, thus uniting them as a single monophyletic species group. For the *ruficornis* group, analyses revealed that the group is paraphyletic, with one large main clade and one smaller clade that is sister to the *bifasciata* species group. Interestingly, while the largest main *ruficornis* clade was composed of species found throughout the Holarctic, those that clustered in the smaller clade are restricted to eastern North America and therefore should be recognized as a new species group in order to preserve monophyly.

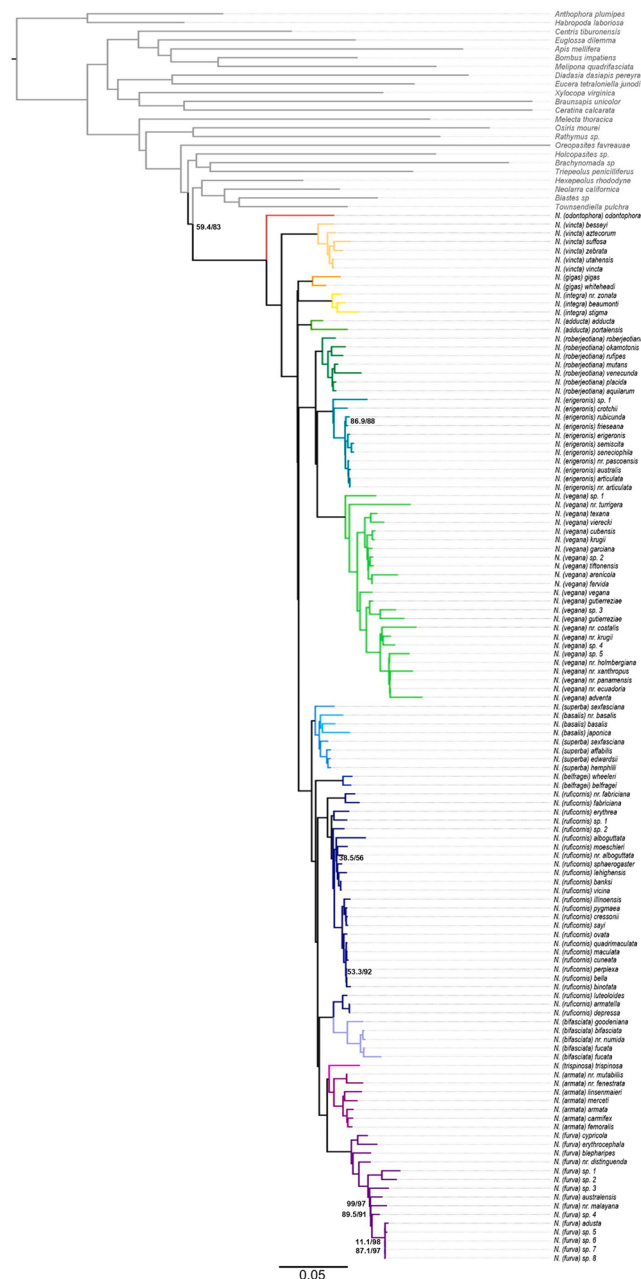


Fig. 1. Phylogram of *Nomada* with the outgroup in grey and each species group. All support values are 100/100 unless indicated.

3.2. Dating analysis and historical biogeography

Of the three data matrices tested (all loci, 1000 clocklike loci, 500 clocklike loci), we selected the all loci analysis as the main result, given that there was little variation in dates among trees, suggesting that our analyses were not biased by the inclusion of less clocklike loci. Additionally, we found the most support for the DEC biogeographic model (AIC: 339.28) performed on the all loci tree. Our dated chronogram with biogeography focusing on the ingroup (Fig. 2; App. Fig. 2) reveals that *Nomada* diverged from the rest of the Nomadinae in the late Cretaceous (stem: 79 Mya, CI: 93–63 Mya) in the Nearctic. Our divergence dating also supports a crown age for *Nomada* around 65 Mya (95% CI: 83–48 Mya) and DEC analysis places the most recent common ancestor (MRCA) for the group originating in the Holarctic during the early Paleocene (66–56 Mya). Nearly all *Nomada* species groups had crown ages between the late Oligocene to the mid Miocene (28.1–13.8 Mya) with the

exception of the younger *belfragei* group whose crown age was in the late Miocene (11.6–7.2 Mya).

The two oldest species groups (*odontophora* and *vineta*) were found to be confined to the Palearctic as early as ~ 65 Mya (CI: 83–48 Mya) and to the Nearctic ~ 57 Mya (CI: 74–37 Mya) respectively. Dispersal into the southern hemisphere was subsequently recorded in three species groups: into the Afrotropics by *gigas* ~ 21 Mya (CI: 36–5 Mya), into the Neotropics by *vegana* (CI: 36–20 Mya), and into the Indomalaya and Australasian regions by *furva* (CI: 30–14 Mya). Three species groups (*adducta*, *erigeronis*, *belfragei*) are confined to the Nearctic, however, our analyses indicates that the MRCAs for both *erigeronis* and *belfragei* reached the Nearctic independently around 33 Mya (CI: 46–25 Mya) while the MRCA for *adducta* arrived much earlier, ~46 Mya (65–33 Mya) from somewhere across the Palearctic. Three species groups, *roberjeotiana*, *superba* + *basalis* and *ruficornis*, all have Holarctic distributions; our model suggests that both *roberjeotiana* and *superba*+*basalis* only dispersed once into the Nearctic ~15 Mya (CI: 26–8 Mya) and ~16 Mya (CI: 25–6 Mya) respectively, while *ruficornis* dispersed no less than five times between ~ 19–10 Mya (CI: 27–4 Mya). The remaining four species groups (*integra*, *bifasciata*, *trispinosa*, *armata*) are confined to the Palearctic.

4. Discussion

4.1. Phylogeny

Here we provide the first comprehensive molecular phylogeny of *Nomada* to date with representation of 119 species, all currently recognized species groups, and all major biogeographic regions. We propose that the crown age for *Nomada* is between 60 and 70 Mya. This proposed age is older than previous estimates for the group which fall anywhere from ~ 61 Mya (Bossert et al., 2020) and ~ 20–23 Mya (Cardinal et al., 2010; Cardinal et al., 2018). However, recent phylogenetic analyses have pushed the crown age for all bees to between 130 and 100 Ma (Branstetter et al., 2017; Cardinal & Danforth, 2013; Sann et al., 2018). Specifically Cardinal and Danforth (2013) and Sann et al (2018) recovered an origin for bees between 123 and 128 Mya while Branstetter et al (2020), using similar methods to the ones used in this study, recovered a crown age closer to 100 Mya. The main difference between our estimate for the crown age and those previously estimated for the genus is most likely due to the depth of sampling; older analyses included at most three Holarctic species of *Nomada* (Bossert et al., 2020; Cardinal et al., 2010; Cardinal et al., 2018;) mostly found in the *ruficornis* group. We used over 100 species and included all known species groups and biogeographic realms where *Nomada* occurs. Interestingly, because Cardinal et al., (2018) only included species from the *ruficornis* group, their estimated age for *Nomada* matches the age we recovered for the same species group, indicating that our results are comparable.

Our phylogeny showed strong support for all but two of the morphologically defined species groups originally outlined by Alexander (1994) and we identified the *odontophora* species group as sister to the remaining groups in *Nomada*. Our finding that the *odontophora* group is sister to all other species groups contrasts with the predictions made by both Michener (2007) and Alexander (1994). Previously, Michener (2007) predicted that the *vegana* group would be “basal” based on their species diversity and location in the southern hemisphere; however, our molecular phylogeny indicates that this group originated later when its ancestor dispersed into the Neotropics from the Nearctic (~28 Mya). Alternatively, Alexander (1994) predicted that the *gigas* species group was sister to the remaining *Nomada* groups based primarily on male genitalia characters. Our results place the *gigas* group as more derived within the genus (Fig. 1).

In his detailed cladistic work, Alexander (1989, 1991) found little support for the monophyly of *superba* as a group as well as little support for a sister group relationship between *superba* and *basalis*, noting that observable and clearly defining characters for *superba* were difficult to

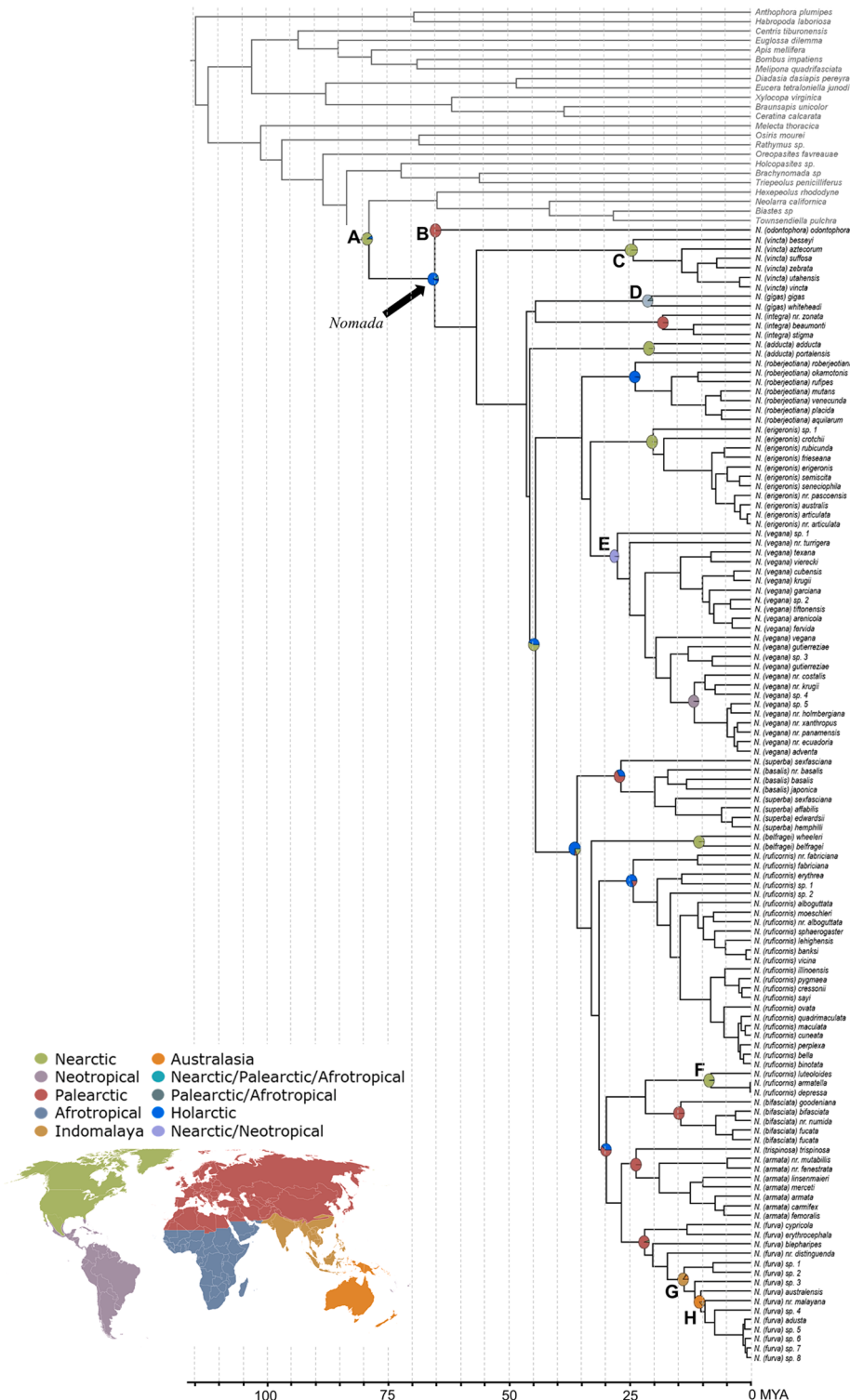


Fig. 2. Biogeography of the ingroup *Nomada* represented in our dated phylogeny. Each pie represents the probability of the ancestral region. The six included regions in our analysis are Nearctic, Neotropics, Palearctic, Afrotropics, Indomalaya, and Australasia. Large letters indicate important biogeographical events: A) most recent common ancestor of the sister stem group and *Nomada* emerges in the Nearctic; B) *odontophora* originates in the Palearctic; C) first instance of back geodispersal into the Nearctic; D) *gigas* emerges in the Afrotropics and is first dispersal into the southern hemisphere; E) movement into the Neotropics and second dispersal into southern hemisphere; F) new unnamed species group emerges in the Nearctic; G) third southern hemisphere dispersal into the Indomalaya; H) dispersal into Australasia and last new biogeographical region. Map image by Lokal Profil distributed under a CC BY-SA 3.0 license.

determine. Our results provide support for the union of the *superba* and *basalis* groups into one monophyletic clade. The *ruficornis* group is a large and historically problematic clade that was assumed to be paraphyletic, mainly due the absence of defining characters that unite the species found in the group (Alexander, 1989). In our analyses, *ruficornis* was indeed found to be paraphyletic, however, not to the extent that would be assumed for such a large clade composed of species that do not belong in any other species group. Our analyses imply that there are only two separate clades: one large main clade sister to the *armata* group, and

a smaller second smaller clade more closely related to the *bifasciata* group. Interestingly, this smaller group is composed solely of eastern North American species and it should be further investigated such that after revision, a new species group could be established.

4.2. Historical biogeography

Both Michener (2007) and Alexander (1994) proposed a southern hemisphere origin for *Nomada* with subsequent dispersal northward into

the remaining ecoregions. Specifically, Michener (2007) predicted an origin in the Neotropics while Alexander (1994) predicted an Afro-tropical origin. Our biogeographic results offer an alternative to these predictions and we found strong support that the stem ancestor for *Nomada* originally diverged from the sister clade consisting of Hexepeolini, Neolarrini, Townsendiellini and Biastini in the Nearctic ~ 79 Mya during the Late Cretaceous period. The brood parasitic bee genus *Epeolus*, was also found to have most likely originated in the Nearctic (Onuferko, Bogusch, Ferrari, & Packer, 2019) and distribution data for the tribe Hexepeolini also indicate that they are confined to the Nearctic region (Bossert et al., 2020), lending support for the origin being in the New World. Furthermore, the cleptoparasitic tribe Neolarrini, like the genus *Epeolus* most likely originated within the Nearctic as well (Sless et al., 2022). The MRCA for *Nomada* had a Holarctic origin during the early Paleocene with the ancestor dispersing into the Palearctic from the Nearctic between 79 and 65 Mya, most likely via the northern De Geer land bridge. The De Geer land bridge connected eastern North America to Europe via a northern passage through Greenland and was exposed between 71 and 63 Mya (Brikiatis, 2014). Around this same time, the Turgai Strait was open to terrestrial organisms, allowing for geodispersal (gene flow and dispersal that occurs once a physical barrier is removed) across all of Eurasia (Akhmetiev et al., 2012). Although the Bering Land Bridge (BLB) was exposed between 66 and 65.5 Mya and could have been used as an alternate route, it is unlikely for two reasons. The first reason is that around the Cretaceous-Paleogene boundary (66 Mya) the BLB was located at a higher latitude than what is observed today and therefore acted more as a filter route for organisms geodispersing across it (Brikiatis, 2014; McKenna, 2003). Secondly, movement by *Nomada* across the Holarctic region was most likely already happening prior to the estimated first opening of the BLB since *odontophora* diverged from the rest of *Nomada* ~ 65 Mya within the Palearctic.

The first instance of back geodispersal into the Nearctic occurred in the *vineta* group ~ 57 Mya, most likely via the Thulean Land Bridge (TLB) as the De Geer route closed ~ 6 Mya prior (Brikiatis, 2014) and the presence of the Turgai Strait would have hindered movement across the BLB (Brikiatis, 2014; Sanmartín, Engloff, & Ronquist, 2001). The only other recorded instance of bees using the TLB is found in the Ancylini (Praz & Packer, 2014) who are thought to have used the land bridge to reach the Palearctic from North America ~ 57–56 Mya; however, the TLB was an important connector between the Palearctic and Nearctic for many other taxa including toads (Pramuk, Robertson, Sites, & Noonan, 2007), crocodilids (Puértolas, Canudo, & Cruzado-Caballero, 2011), grasshoppers (Chintauan-Marquier et al., 2014), glass lizards (Lavin & Girman, 2019), and magnolias (Hebda & Irving, 2004) around this time.

Our analysis indicates that *Nomada* geodispersed into the southern hemisphere at least three times during the course of their evolutionary history; each dispersal event resulting in a distinct species group (*gigas*, *vegana*) or species (*furva*). The first instance of southward geodispersal occurred during the middle Eocene (stem age: 45 Mya) when the MRCA of the *gigas* species group diverged from the *integra* group and moved into the Afrotropics. By the mid Cretaceous, Africa had already been isolated from the rest of Gondwana and Laurasia, but various intermittent routes would reconnect it to the other landmasses, allowing for faunal and floral exchange (Gheerbrant & Rage, 2006). These frequent and bidirectional interchanges occurred most likely between Africa and Laurasia via two routes: the Mediterranean Tethyan Sill, whose exposure was periodic from the Jurassic to the Paleogene and closely tied to falling sea levels, and a later eastern Iranian route that emerged in the mid Eocene (Gheerbrant & Rage, 2006). Using the mid Eocene stem age of *gigas* group, we can infer that the eastern Iranian route was the most probable scenario, however, we cannot rule out use of the Mediterranean Tethyan Sill. The latter scenario would match geodispersal patterns proposed for Sericini scarab beetles (Eberle, Fabrizi, Lago, & Ahrens, 2017), the extinct predatory mammals of the family Hyenodontidae (Solé, 2013) and monitor lizards (Vidal et al., 2012).

The second instance of geodispersal by *Nomada* into the southern

hemisphere occurred between the Nearctic and the Neotropics by the diverse *vegana* species group shortly after the Eocene-Oligocene transition (~34 Mya). This corresponds to a floral shift within South America as tropical biomes gave way to more temperate and grassland habitats (Houben, van Mourik, Montanari, Coccioni, & Brinkhuis, 2012; Pound & Salzmann, 2017; Zachos, Pagani, Sloan, Thomas, & Billups, 2001). Two possible routes taken by *vegana* to reach South America include use of the Isthmus of Panama (IP) and via island hopping through the Antilles region. Movement of *vegana* through the IP is questionable as completion of the isthmus was predicted to have happened ~ 3–4 Mya (Coates & Stallard, 2013; Jackson & O'Dea, 2013), some 30 Mya after the divergence of the *vegana* group from its sister species group, *erigeronis*. However, there is some evidence that the IP may have formed much earlier than expected, possibly in the middle Miocene (Montes et al., 2015) or near the Oligocene-Miocene boundary (Bacon et al., 2015) or even existed as a chain of islands in late Eocene (Montes et al., 2012). Our analyses indicated that *Nomada* were already present in the Neotropics no later than ~ 20 Mya and therefore cannot rule out dispersal over an Oligocene-Miocene IP emergence nor an island stepping geodispersal. It is known that bee faunas in Southeast Asia and the Caribbean used scattered islands to disperse between landmasses (Michener, 1979) so it is feasible that *Nomada* island hopped between North and South America. Other organisms are found to have possibly dispersed across the IP prior to its estimated complete closure including *Ceratina* bees (Rehan et al., 2010), *Dynastes* beetles (Huang, 2016), palms (Bacon, Mora, Wagner, & Jaramillo, 2013), and *Boa* snakes (Head, Rincon, Suarez, Montes, & Jaramillo, 2012).

The third southern geodispersal happened when the *furva* group reached Australia ~ 10 Mya. During the late Miocene (~12–7 Mya), the Australian continent drifted towards southeast Asia, leading to an extension of the Sula Spur (Torsvik & Cocks, 2016) and provided a steppingstone like connection between Indonesia and Australia. Many organisms such as *Tylomelania* freshwater snails (von Rintelen, Stelbrink, Marwoto, & Glaubrecht, 2014), passerines in the Oriolidae (Jönsson et al., 2019) and *Spathius* parasitoid wasps (Zaldívar-Riverón et al., 2018) traversed this connection in order to disperse into or out of Australia.

Various *Nomada* species groups dispersed into the Nearctic between 46 and 10 Mya most likely across the Bering Land Bridge (BLB). The species groups *adductua*, *belfragei*, and *erigeronis* are all confined to North America and have MRCA's that were found across the Holarctic. Our results indicate that the ancestors for these groups geodispersed back into the Nearctic between 46 and 33 Mya, shortly after the start of the Eocene-Oligocene transition (~34 Mya). During this time period, major climatic shifts caused the vegetation of the BLB to transition from boreotropical to forests of mixed deciduous and coniferous trees (Sanmartín et al., 2001). The Holarctic *roberjeotiana*, *superba* + *basalis* and the *ruficornis* groups would have used the BLB to geodisperse to the Nearctic ~ 19–10 Mya. The BLB was a common route used by bees; genera from at least three families are known to have traversed it either to reach the Nearctic or the Palearctic: Apidae (Dorchin, López-Urbe, Praz, Griswold, & Danforth, 2018; Hines, 2008; Onuferko et al., 2019; Praz & Packer, 2014; S. Rehan & Schwarz, 2015); Colletidae (Ferrari et al., 2020); Megachilini (Branstetter et al., 2021; Trunz, Packer, Vieu, Arrigo, & Praz, 2016).

5. Conclusions

Here we constructed the first molecular phylogeny and biogeography for the *Nomada* using genome wide next-generation sequencing techniques. Overall, there was strong support for 14 of the 16 previously established species groups and additional support for one previously unknown group. *Nomada* most likely originated in the early Paleogene somewhere across the Holarctic in the latter half of the Cretaceous and began to radiate following the K-Pg extinction event. Throughout their evolutionary history, geodispersal for *Nomada* predominantly occurred

between the Nearctic and Palearctic; ten independent geodispersal events using multiple existing land bridges, including the first instance of a bee crossing the De Geer land bridge, were recorded. Three geodispersal events gave rise to the three southern hemisphere species groups. Although all species groups were sampled, future studies should include more representation within the smaller clades such as *trispinosa*, *odontophora*, and *belfragei*. With nearly 800 species worldwide, *Nomada* are the most diverse and widespread of the brood parasitic apid bees. This well resolved phylogeny and historical biogeography provide an important first step towards a deeper understanding of both *Nomada* diversification and cleptoparasitic bee evolution more broadly.

Data Availability

Unprocessed Illumina reads are deposited in BioProject # PRJNA705977, Accession # SRR18055085 – SRR18055199 on the Sequence Read Archive on NCBI. Associated DNA matrices, tree files, and analysis files are deposited on Dryad (doi: [10.5061/dryad.j9kd51cfk](https://doi.org/10.5061/dryad.j9kd51cfk)).

Credit authorship contribution statement

Katherine A. Odanaka: Conceptualization, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Michael G. Branstetter:** Methodology, Resources, Formal analysis, Investigation, Data curation, Writing – review & editing. **Kerrigan B. Tobin:** Investigation. **Sandra M. Rehan:** Conceptualization, Resources, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Nicole Gerjets, Terry Griswold, Antonia Guidotti, Gordon Hutchings, Rufus Isaacs, Sarah Mackelle, Robert Minckley, Michael Minnick, Laurence Packer, Steven Paiero, Nigel Raine, Maximilian Schwarz, Michael Veit, Karen Wright, Weiping Xie, and Doug Yanega for bee specimens. This work was funded by a National Science and Engineering Research Council Discovery Grant and Foundation for Food and Agriculture Research Pollinator Health grant 549038 to SMR, a Mitacs Research and Training award and Carswell Scholarship to KAO, and the U.S. Department of Agriculture, Agricultural Research Service (MGB and KBT), and a U.S. National Science Foundation award (DEB#2127744 to MGB). USDA is an equal opportunity provider and employer.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ympev.2022.107453>.

References

- Akhmetiev, M.A., Zaporozhets, N.I., Benyamovskiy, V.N., Aleksandrova, G.N., Iakovleva, A.I., Oreshkina, T.V., 2012. The paleogene history of the western Siberian seaway - a connection of the Peri-Tethys to the Arctic Ocean. *Austrian J. Earth Sci.* 105 (1), 50–67. Retrieved from www.geol-ges.at/.
- Alexander, B.A., 1989. A species-group revision and phylogenetic analysis of the cleptoparasitic bee genus *Nomada* Scopoli (Hymenoptera: Anthophoridae). Cornell University.
- Alexander, B.A., 1991. *Nomada* phylogeny reconsidered (Hymenoptera: Anthophoridae). *Nomada* phylogeny reconsidered (Hymenoptera: Anthophoridae). *J. Nat. Hist.* 25 (2), 315–330. <https://doi.org/10.1080/00222939100770231>.
- Alexander, B.A., 1994. Species-groups and cladistic analysis of the cleptoparasitic bee genus *Nomada* (Hymenoptera: Apoidea). *The University of Kansas Science Bulletin* 55 (6), 175–238.
- Alexander, B.A., Schwarz, M., 1994. A catalog of the species of *Nomada* (Hymenoptera: Apoidea) of the world. *The University of Kansas Science Bulletin* 55 (7), 239–270.
- Bacon, C.D., Mora, A., Wagner, W.L., Jaramillo, C.A., 2013. Testing geological models of evolution of the Isthmus of Panama in a phylogenetic framework. *Bot. J. Linn. Soc.* 171 (1), 287–300. <https://doi.org/10.1111/j.1095-8339.2012.01281.x>.
- Bankevich, A., Nurk, S., Antipov, D., Gurevich, A.A., Dvorkin, M., Kulikov, A.S., Pevzner, P.A., 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* 19 (5), 455–477.
- Blumenstiel, B., Cibulskis, K., Fisher, S., DeFelice, M., Barry, A., Fennell, T., Gabriel, S., 2010. Targeted exon sequencing by in-solution hybrid selection. *Current Protocols in Human Genetics* 66 (1), 18–24.
- Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinformatics* 30 (15), 2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>.
- Borowiec, M.L., 2019. Spruceup: fast and flexible identification, visualization, and removal of outliers from large multiple sequence alignments. *J. Open Source Software* 4 (42), 1635.
- Bossert, S., Copeland, R.S., Sless, T.J., Branstetter, M.G., Gillung, J.P., Brady, S.G., Straka, J., 2020. Phylogenomic and Morphological Reevaluation of the Bee Tribes Biastini, Neolarrini, and Townsendiellini (Hymenoptera: Apidae) With Description of Three New Species of Schwarzia. *Insect Systematics Diversity* 4, 1.
- Bossert, S., Murray, E.A., Almeida, E.A., Brady, S.G., Blaimer, B.B., Danforth, B.N., 2019. Combining transcriptomes and ultraconserved elements to illuminate the phylogeny of Apidae. *Mol. Phylogenet. Evol.* 130, 121–131.
- Branstetter, M.G., Longino, J.T., Ward, P.S., Faircloth, B.C., 2017a. Enriching the ant tree of life: Enhanced UCE bait set for genome-scale phylogenetics of ants and other Hymenoptera. *Methods Ecol. Evol.* 8 (6), 768–776.
- Branstetter, M.G., Danforth, B.N., Pitts, J.P., Faircloth, B.C., Ward, P.S., Buffington, M.L., Brady, S.G., 2017b. Phylogenomic insights into the evolution of stinging wasps and the origins of ants and bees. *Curr. Biol.* 27 (7), 1019–1025.
- Branstetter, M.G., Müller, A., Griswold, T.L., Orr, M.C., Zhu, C.D., 2021. Ultraconserved element phylogenomics and biogeography of the agriculturally important mason bee subgenus *Osmia* (Osmia). *Syst. Entomol.* 46 (2), 453–472.
- Bushnell, B., 2014. BBTools software package. URL <http://sourceforge.net/projects/bbmap> 578, 579.
- Brikatis, L., 2014. The de geer, thulean and beringia routes: Key concepts for understanding early cenozoic biogeography. *J. Biogeogr.* 41 (6), 1036–1054. <https://doi.org/10.1111/jbi.12310>.
- Cardinal, S., Buchmann, S.L., Russell, A.L., 2018. The evolution of floral sonication, a pollen foraging behavior used by bees (Anthophila). *Evolution* 72 (3), 590–600. <https://doi.org/10.1111/evo.13446>.
- Cardinal, S., Danforth, B.N., 2013. Bees diversified in the age of eudicots. *Proceedings of the Royal Society B: Biological Sciences* 280 (1755), 1–9. <https://doi.org/10.1098/rspb.2012.2686>.
- Cardinal, S., Straka, J., Danforth, B.N., 2010. Comprehensive phylogeny of apid bees reveals the evolutionary origins and antiquity of cleptoparasitism. *Proc. Natl. Acad. Sci.* 107 (37), 16207–16211.
- Chintauan-Marquier, I.C., Amédégnato, C., Nichols, R.A., Pompanon, F., Grandcolas, P., Desutter-Grandcolas, L., 2014. Inside the Melanoplinae: New molecular evidence for the evolutionary history of the Eurasian Podismini (Orthoptera: Acrididae). *Mol. Phylogenet. Evol.* 71 (1), 224–233. <https://doi.org/10.1016/j.ympev.2013.09.009>.
- Coates, A.G., Stallard, R.F., 2013. How old is the Isthmus of Panama? *Bull. Mar. Sci.* 89 (4), 801–813. <https://doi.org/10.5343/bms.2012.1076>.
- Danforth, B., Minckley, R., Neff, J., 2019. *The Solitary Bees: Biology, Evolution*. Princeton University Press, Conservation.
- Dehon, Manuel, Perrard, Adrien, Engel, S., Michael, Nel, Andre, Michez, Denis, et al., 2014. Antiquity of cleptoparasitism among bees revealed by morphometric and phylogenetic analysis of a Paleocene fossil nomadine (Hymenoptera: Apidae). *PLoS One* 9 (10), e108865.
- Dorchin, A., López-Urbe, M.M., Praz, C.J., Griswold, T., Danforth, B.N., 2018. Phylogeny, new generic-level classification, and historical biogeography of the *Eucera* complex (Hymenoptera: Apidae). *Mol. Phylogenet. Evol.* 119, 81–92. <https://doi.org/10.1016/j.ympev.2017.10.007>.
- Eberle, J., Fabrizi, S., Lago, P., Ahrens, D., 2017. A historical biogeography of megadiverse Sericini-another story “out of Africa”? *Cladistics* 33 (2), 183–197. <https://doi.org/10.1111/cla.12162>.
- Faircloth, B.C., 2013. Illumiprocessor: a trimmomatic wrapper for parallel adapter and quality trimming. Retrieved from. <https://doi.org/10.6079/J9ILL>.
- Faircloth, B.C., 2016. PHYLUCE is a software package for the analysis of conserved genomic loci. *Bioinformatics* 32 (5), 786–788. <https://doi.org/10.1093/bioinformatics/btv646>.
- Faircloth, B.C., 2017. Identifying conserved genomic elements and designing universal bait sets to enrich them. *Methods Ecol. Evol.* 8 (9), 1103–1112.
- Faircloth, B.C., McCormack, J.E., Crawford, N.G., Harvey, M.G., Brumfield, R.T., Glenn, T.C., 2012. Ultraconserved elements anchor thousands of genetic markers spanning multiple evolutionary timescales. *Syst. Biol.* 61 (5), 717–726. <https://doi.org/10.1093/sysbio/sys004>.
- Ferrari, R.R., Onuferko, T.M., Monckton, S.K., Packer, L., 2020. The evolutionary history of the cellophane bee genus *Colletes* Latreille (Hymenoptera: Colletidae): Molecular phylogeny, biogeography and implications for a global infrageneric classification. *Mol. Phylogenet. Evol.* 146, 106750 <https://doi.org/10.1016/j.ympev.2020.106750>.

- Freitas, F.V., Branstetter, M.G., Griswold, T., Almeida, E.A., 2020. Partitioned gene-tree analyses and gene-based topology testing help resolve incongruence in a phylogenomic study of host-specialist bees (Apidae: Eucerinae). *Mol. Biol. Evol.* *msaa277*.
- Gheerbrant, E., Rage, J.C., 2006. Paleobiogeography of Africa: How distinct from Gondwana and Laurasia? *Palaeogeogr. Palaeoclimatol. Palaeoecol.* *241* (2), 224–246. <https://doi.org/10.1016/j.palaeo.2006.03.016>.
- Glenn, T.C., Nilsen, R.A., Kieran, T.J., Sanders, J.G., Bayona-Vásquez, N.J., Finger, J.W., Faircloth, B.C., 2019. Adapterama I: Universal stubs and primers for 384 unique dual-indexed or 147,456 combinatorially-indexed Illumina libraries (iTru & iNext). *PeerJ* *2019* (10), e7755. <https://doi.org/10.7717/peerj.7755>.
- Grab, H., Branstetter, M.G., Amon, N., Urban-Mead, K.R., Park, M.G., Gibbs, J., Danforth, B.N., 2019. Agriculturally dominated landscapes reduce bee phylogenetic diversity and pollination services. *Science* *363* (6424), 282–284.
- Harris, R. S. (2007). Improved pairwise Alignment of genomic DNA.
- Head, J.J., Rincon, A.F., Suarez, C., Montes, C., Jaramillo, C., 2012. Fossil evidence for earliest Neogene American faunal interchange: Boa (Serpentes, Boinae) from the early Miocene of Panama. *J. Vertebr. Paleontol.* *32* (6), 1328–1334. <https://doi.org/10.1080/02724634.2012.694387>.
- Hebda, R.J., Irving, E., 2004. On the origin and distribution of magnolias: Tectonics, DNA and climate change. Washington DC American Geophysical Union Geophysical Monograph Series *145*, 43–57.
- Hines, H.M., 2008. Historical Biogeography, Divergence Times, and Diversification Patterns of Bumble Bees (Hymenoptera: Apidae: Bombus). *Syst. Biol.* *57* (1), 58–75. <https://doi.org/10.1080/10635150801898912>.
- Hoang, D.T., Chernomor, O., Von Haeseler, A., Minh, B.Q., Vinh, L.S., 2018. UFBoot2: improving the ultrafast bootstrap approximation. *Mol. Biol. Evol.* *35* (2), 518–522.
- Houben, A.J., van Mourik, C.A., Montanari, A., Coccioni, R., Brinkhuis, H., 2012. The Eocene-Oligocene transition: Changes in sea level, temperature or both? *Palaeogeogr. Palaeoclimatol. Palaeoecol.* *335*, 75–83.
- Huang, J.P., 2016. The great American biotic interchange and diversification history in Dynastes beetles (Scarabaeidae: Dynastinae). *Zool. J. Linn. Soc.* *178* (1), 88–96.
- Jackson, J.B.C., O'Dea, A., 2013. Timing of the oceanographic and biological isolation of the Caribbean sea from the tropical eastern Pacific ocean. *Bull. Mar. Sci.* *89* (4), 779–800. <https://doi.org/10.5343/bms.2012.1096>.
- Junier, T., Zdobnov, E.M., 2010. The Newick utilities: high-throughput phylogenetic tree processing in the UNIX shell. *Bioinformatics* *26* (13), 1669–1670.
- Jønsson, K.A., Blom, M.P., Marki, P.Z., Joseph, L., Sangster, G., Ericson, P.G., Irestedt, M., 2019. Complete subspecies-level phylogeny of the Orolidae (Aves: Passeriformes): Out of Australasia and return. *Mol. Phylogenet. Evol.* *137*, 200–209.
- Kalyaanamoorthy, S., Minh, B.Q., Wong, T.K.F., Von Haeseler, A., Jermiin, L.S., 2017. ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nat. Methods* *14* (6), 587–589. <https://doi.org/10.1038/nmeth.4285>.
- Katoh, K., Standley, D.M., 2013. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Mol. Biol. Evol.* *30* (4), 772–780. <https://doi.org/10.1093/molbev/mst010>.
- Landis, M.J., Matzke, N.J., Moore, B.R., Huelsenbeck, J.P., 2013. Bayesian analysis of biogeography when the number of areas is large. *Syst. Biol.* *62* (6), 789–804. <https://doi.org/10.1093/sysbio/syt040>.
- Lavin, B.R., Girman, D.J., 2019. Phylogenetic relationships and divergence dating in the Glass Lizards (Anguinae). *Mol. Phylogenet. Evol.* *133*, 128–140. <https://doi.org/10.1016/j.ympev.2018.12.022>.
- Litman, J.R., 2019. Under the radar: detection avoidance in brood parasitic bees. *Philosophical Transactions of the Royal Society B* *374* (1769), 20180196.
- Litman, J.R., Praz, C.J., Danforth, B.N., Griswold, T.L., Cardinal, S., 2013. Origins, Evolution, and Diversification of Cleptoparasitic Lineages in Long-Tongued Bees. *Evolution* *67* (10), 2982–2998. <https://doi.org/10.1111/evo.12161>.
- Matzke, N.J., 2013. Probabilistic historical biogeography: new models for founder-event speciation, imperfect detection, and fossils allow improved accuracy and model-testing. *Frontiers of Biogeography* *5* (4), 242–248. <https://doi.org/10.21425/f5fbg19694>.
- McKenna, M., 2003. Semi-isolation and lowered salinity of the Arctic Ocean in late Paleocene to earliest Eocene time. *J. Vertebr. Paleontol.* *23* (3), 77A.
- Michener, C.D., 1979. Biogeography of the Bees. *Ann. Mo. Bot. Gard.* *66* (3), 277. <https://doi.org/10.2307/2398833>.
- Michener, C.D., 2007. The bees of the world, 2nd ed. The Johns Hopkins University Press, Baltimore.
- Minckley, R.L., Danforth, B.N., 2019. Sources and frequency of brood loss in solitary bees. *Apidologie* *50* (4), 515–525. <https://doi.org/10.1007/s13592-019-00663-2>.
- Nguyen, L.T., Schmidt, H.A., Von Haeseler, A., Minh, B.Q., 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol. Biol. Evol.* *32* (1), 268–274. <https://doi.org/10.1093/molbev/msu300>.
- Odanaka, K.A., Rehan, S.M., 2019. Impact indicators: Effects of land use management on functional trait and phylogenetic diversity of wild bees. *Agric. Ecosyst. Environ.* *286*, 106663.
- Onuferko, T.M., Bogusch, P., Ferrari, R.R., Packer, L., 2019. Phylogeny and biogeography of the cleptoparasitic bee genus *Epeolus* (Hymenoptera: Apidae) and cophylogenetic analysis with its host bee genus *Colletes* (Hymenoptera: Colletidae). *Mol. Phylogenet. Evol.* *141*, 1–14. <https://doi.org/10.1016/j.ympev.2019.106603>.
- Pound, M.J., Salzmann, U., 2017. Heterogeneity in global vegetation and terrestrial climate change during the late Eocene to early Oligocene transition. *Sci. Rep.* *7* (1), 1–12. <https://doi.org/10.1038/srep43386>.
- Pramuk, J.B., Robertson, T., Sites Jr, J.W., Noonan, B.P., 2008. Around the world in 10 million years: biogeography of the nearly cosmopolitan true toads (Anura: Bufonidae). *Glob. Ecol. Biogeogr.* *17* (1), 72–83.
- Praz, C.J., Packer, L., 2014. Phylogenetic position of the bee genera *Ancyla* and *Tarsalia* (Hymenoptera: Apidae): A remarkable base compositional bias and an early Paleogene geodispersal from North America to the Old World. *Mol. Phylogenet. Evol.* *81*, 258–270. <https://doi.org/10.1016/j.ympev.2014.09.003>.
- Puértolas, E., Canudo, J.I., Cruzado-Caballero, P., 2011. A new crocodylian from the Late Mastrichtian of Spain: implications for the initial radiation of crocodyliids. *PLoS ONE* *6* (6), e20011.
- Puttick, M.N., 2019. MCMCTreeR: Functions to prepare MCMCTree analyses and visualize posterior ages on trees. *Bioinformatics* *35* (24), 5321–5322. <https://doi.org/10.1093/bioinformatics/btz554>.
- Rambaut, A., Drummond, A.J., Xie, D., Baele, G., Suchard, M.A., 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Syst. Biol.* *67* (5), 901–904. <https://doi.org/10.1093/sysbio/syy032>.
- Ree, R.H., Moore, B.R., Webb, C.O., Donoghue, M.J., 2005. A likelihood framework for inferring the evolution of geographic range on phylogenetic trees. *Evolution* *59* (11), 2299–2311.
- Ree, R.H., Sanmartín, I., 2018. Conceptual and statistical problems with the DEC+J model of founder-event speciation and its comparison with DEC via model selection. *J. Biogeogr.* *45* (4), 741–749. <https://doi.org/10.1111/jbi.13173>.
- Ree, R.H., Smith, S.A., 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Syst. Biol.* *57* (1), 4–14. <https://doi.org/10.1080/10635150701883881>.
- Rehan, S.M., Chapman, T.W., Craigie, A.L., Richards, M.H., Cooper, S.J.B., Schwarz, M.P., 2010. Molecular phylogeny of the small carpenter bees (Hymenoptera: Apidae: Ceratini) indicates early and rapid global dispersal. *Mol. Phylogenet. Evol.* *55* (3), 1042–1054. <https://doi.org/10.1016/j.ympev.2010.01.011>.
- Rehan, S., Schwarz, M., 2015. A few steps forward and no steps back: long-distance dispersal patterns in small carpenter bees suggest major barriers to back-dispersal. *J. Biogeogr.* *42* (3), 485–494. <https://doi.org/10.1111/jbi.12439>.
- Rohland, N., Reich, D., 2012. Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture. *Genome Res.* *22* (5), 939–946. <https://doi.org/10.1101/gr.128124.111>.
- Ronquist, F., 1997. Dispersal-vicariance analysis: A new approach to the quantification of historical biogeography. *Syst. Biol.* *46* (1), 195–203. <https://doi.org/10.1093/sysbio/46.1.195>.
- Rozen, J.G., 1991. Evolution of cleptoparasitism in anthophorid bees as revealed by their mode of parasitism and first instars (Hymenoptera, Apoidea). *Am. Mus. Novit.* *3029*, 36.
- Sanmartín, I., Enghoff, H., Ronquist, F., 2001. Patterns of animal dispersal, vicariance and diversification in the Holarctic. *Biol. J. Linn. Soc.* *73* (4), 345–390. <https://doi.org/10.1111/j.1095-8312.2001.tb01368.x>.
- Sann, M., et al., 2018. Phylogenomic analysis of Apoidea sheds new light on the sister group of bees. *BMC Evol. Biol.* *18* (1), 1–15.
- Sheffield, C.S., Pindar, A., Packer, L., Kevan, P.G., 2013. The potential of cleptoparasitic bees as indicator taxa for assessing bee communities. *Apidologie* *44* (5), 501–510. <https://doi.org/10.1007/s13592-013-0200-2>.
- Sless, T.J.L., et al., 2022. Phylogenetic relationships and the evolution of host preferences in the largest clade of brood parasitic bees (Apidae: Nomadinae). *Molecular Phylogenetics and Ecology* *166*.
- Smith, S.A., Brown, J.W., Walker, J.F., 2018. So many genes, so little time: A practical approach to divergence-time estimation in the genomic era. *PLoS ONE* *13* (5), e0197433.
- Snelling, R. R. (1986). Contributions toward a revision of the New World nomadine bees. A partitioning of the genus *Nomada* (Hymenoptera: Anthophoridae). *Contributions in science. Natural history museum of Los Angeles County*, (376).
- Solé, F., 2013. New proviverrine genus from the early eocene of Europe and the first phylogeny of late paleocene–middle eocene hyaenodontidans (Mammalia). *J. Syst. Paleontol.* *11* (4), 375–398. <https://doi.org/10.1080/14772019.2012.686927>.
- Tagliacollo, V.A., Lanfear, R., 2018. Estimating improved partitioning schemes for ultraconserved elements. *Mol. Biol. Evol.* *35* (7), 1798–1811. <https://doi.org/10.1093/molbev/msy069>.
- Talavera, G., Castresana, J., 2007. Improvement of phylogenies after removing divergent and ambiguously aligned blocks from protein sequence alignments. *Syst. Biol.* *56* (4), 564–577. <https://doi.org/10.1080/10635150701472164>.
- Torsvik, T.H., Cocks, L.R.M., 2016. Earth history and palaeogeography. Cambridge University Press.
- Trunz, V., Packer, L., Vieu, J., Arrigo, N., Praz, C.J., 2016. Comprehensive phylogeny, biogeography and new classification of the diverse bee tribe Megachilini: Can we use DNA barcodes in phylogenies of large genera? *Mol. Phylogenet. Evol.* *103*, 245–259. <https://doi.org/10.1016/j.ympev.2016.07.004>.
- Vidal, N., Marin, J., Sassi, J., Battistuzzi, F.U., Donnellan, S., Fitch, A.J., Hedges, S.B., 2012. Molecular evidence for an Asian origin of monitor lizards followed by Tertiary dispersals to Africa and Australasia. *Biol. Lett.* *8* (5), 853–855. <https://doi.org/10.1098/rsbl.2012.0460>.
- von Rintelen, T., Stelbrink, B., Marwoto, R.M., Glaubrecht, M., 2014. A Snail Perspective on the Biogeography of Sulawesi, Indonesia: Origin and Intra-Island Dispersal of the Viviparous Freshwater Gastropod Tydomelania. *PLoS ONE* *9* (6), e98917. <https://doi.org/10.1371/journal.pone.0098917>.
- Yang, Z., 1997. Paml: A program package for phylogenetic analysis by maximum likelihood. *Bioinformatics* *13* (5), 555–556. <https://doi.org/10.1093/bioinformatics/13.5.555>.
- Yang, Z., 2007. PAML 4: Phylogenetic analysis by maximum likelihood. *Mol. Biol. Evol.* *24* (8), 1586–1591. <https://doi.org/10.1093/molbev/msm088>.

- Zachos, J., Pagani, M., Sloan, L., Thomas, E., Billups, K., 2001. Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* 292 (5517), 686–693.
- Zaldívar-Riverón, A., Belokobylskij, S.A., Meza-Lázaro, R., Pedraza-Lara, C., García-París, M., Meseguer, A.S., 2018. Species delimitation, global phylogeny and historical biogeography of the parasitoid wasp genus *Spathius* (Braconidae: Doryctinae) reveal multiple Oligocene-Miocene intercontinental dispersal events. *Zool. J. Linn. Soc.* 182 (4), 723–734.
- Zhang, C., Sayyari, E., Mirarab, S., 2017. ASTRAL-III: Increased scalability and impacts of contracting low support branches. *Lecture Notes in Computer Science (Including Subseries Lecture Notes in Artificial Intelligence and Lecture Notes in Bioinformatics)* 10562, 53–75. https://doi.org/10.1007/978-3-319-67979-2_4.