Article

Taxonomic utility of niche models in validating species concepts: A case study in *Anthophora* (*Heliophila*) (Hymenoptera: Apidae)

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Abstract

Taxonomy has far-reaching effects throughout biology, and incorrect taxonomy can be detrimental in many ways. Polymorphic species complexes, many of which exist in the bee genus *Anthophora* Latreille, lend themselves to such difficulties. This study employs environmental niche mapping (ENM) and traditional morphological analyses to investigate the validity of the subjective synonymy of *Anthophora* (*Heliophila*) *curta* Provancher with the senior synonym *A. squammulosa* Dours. Eleven of fifty morphological characters consistently differentiate the two putative species, with an additional five characters sometimes separating them. Additionally, based on over 1000 georeferenced museum specimens, the geographic ranges of the two taxa do not overlap. The two entities also react differently to the bioclimatic variables based on correlation analysis. We further tested the two-species hypothesis by constructing ENMs with informative bioclimatic variables associated with locality records. Their modelled distributions overlapped less than 1%, suggesting discrete environmental boundaries. The variables which contributed most to each species' model also differed. These differences are explored in relation to their habitats. The combined morphological and biogeographic analysis indicates that *A. curta* and *A. squammulosa* are distinct species. Based on the accumulated evidence the synonymy is formally rejected and *A. curta* is recognized as a valid species. Five additional taxa (*A. bispinosa* Cockerell, *A. franciscana* Cockerell, *A. usticauda* Cockerell, *A. u. cinerior* Cockerell, *A. zamoranella* Cockerell) are newly synonymized with *A. squammulosa* and *Anthophora curta* var. *melanops* Cockerell is newly synonymized with *A. curta*. Implications outside of taxonomy are discussed.

Key words: Apoidea, Anthophorini, bees, taxonomy, biogeography, Nearctic, MaxEnt, niche modelling

Introduction

The Anthophorini (Hymenoptera: Apidae) are a tribe of large to small bees (6-30mm) known for their fast flight and generalist floral tendencies (Michener, 2007). A large number of synonymies were made in Brooks' (1988) review of the tribe Anthophorini without any formal argument. One such synonymy was that of *Anthophora* (*Heliophila*) *curta* Provancher, 1895 with the senior synonym *Anthophora (Heliophila) squammulosa* Dours, 1870 (Fig. 1). This synonymy came into question during the course of a revision of the New World *Anthophora* (*Heliophila*) Klug. Upon initial review of determined specimens, there appeared to be two phenotypes of *A. squammulosa*. The distribution of these two entities appeared disjunct, with a northern form in the southwestern United States that encompassed the type locality of *A. curta* and a second form limited to Mexico that included the type locality of *A. squammulosa*. Several morphological characters also seemed to distinguish the two, while others appeared to intergrade between the two entities, suggesting the need to re-examine this synonymy. The situation was further obscured by four additional, currently recognized species from Central America and three historical subspecies in the complex. Caution in analysis of morphological characters is prudent as there are numerous polymorphic species in Anthophorini (Brooks, 1983; Brooks, 1988). As morphology alone has proven inadequate for distinguishing some *Anthophora* Latreille, the incorporation of additional sources of information becomes beneficial. Molecular data is often used under these conditions but recent material for DNA extraction was

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unavailable for much of the overall distribution, including material from near type localities, preventing molecular analysis.

This study employs morphological, environmental, and biogeographic techniques to test the validity of this synonymy. Morphological comparison has a long history of use in the field of taxonomy. It is only relatively recently, with the advent of powerful computing and user-friendly technology, that environmental niche modeling (ENM) has become an additional tool for taxonomists and biogeographers of Hymenoptera (Gonzalez *et al.*, 2010; Bassin *et al.*, 2011; Wilson *et al.*, 2012; Lozier *et al.*, 2013). As the ENM approach has been successful at predicting evolutionary relationships across Hymenoptera, we predict comparable success when testing our *A. curta*/*A. squammulosa* two-species hypothesis. It is the goal of this study to determine the status of these two species names while evaluating the ability of ENMs to contribute insight into the question of species limits.
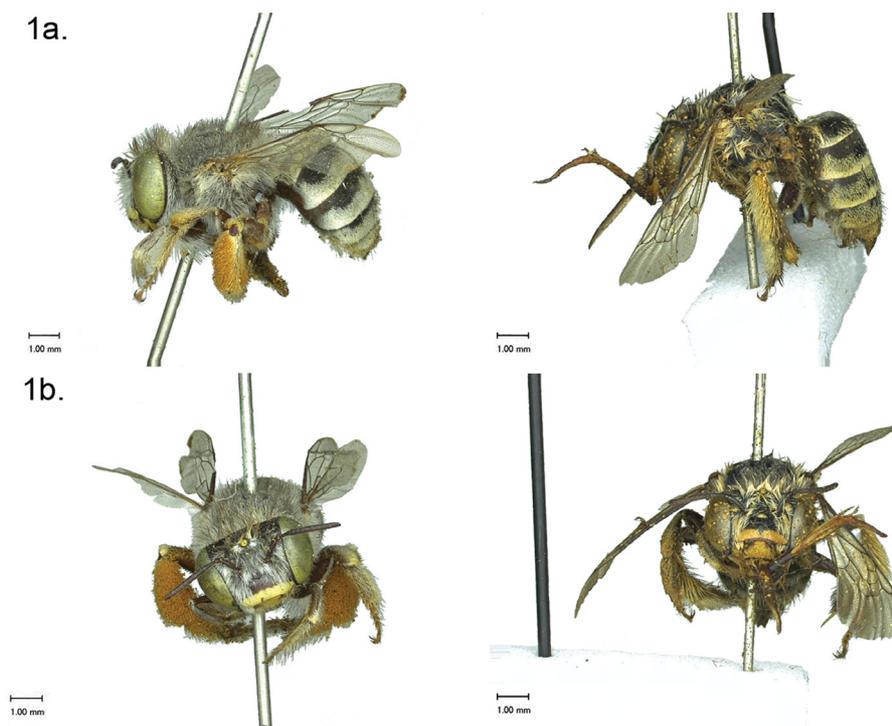

FIGURE 1. The general morphology of *Anthophora curta* and *A. squammulosa* as seen in the female lectotypes, pictured left and right respectively. **a**. Habitus view. **b.** Anterior view. Images were taken at 20x magnification.

Material and methods

A total of 1352 specimens (1099 of *A. curta* and 253 of *A. squammulosa*) from 17 collections were examined for morphological characters. The lectotype of *A. curta* and the full syntype series of *A. squammulosa* (36 specimens) were among the material examined, as were the types of all other names attributable to this complex. The morphology of a subset of the gathered specimens (100 females and 100 males for each species) from across the range of each putative species was examined for morphological differences. A total of 50 characters for this morphological analysis were selected from a larger set found useful in distinguishing New World species of the subgenus *Heliophila*. Assignment of specimens to the two putative species for the modelling was based on this morphological analysis. Locality data were captured for all specimens (426 unique locations) and all locations subsequently georeferenced. Floral records were collated for all bee specimens examined. Published floral records were not included as specimens were not directly examined by the author. All floral records were checked against the Integrated Taxonomic Information System for congruence with current taxonomy (ITIS, 2013).

The morphological terminology follows Michener (2007). All dissections were conducted with a 00 pin and a pair of Dumont forceps (#5). The dissected material was then cleared in a 10% KOH solution, for a duration commensurate with the degree of scleritization. Two 70% alcohol washes were subsequently used to end the clearing reaction and the structures stored in propylene glycol. All photographs were taken with a Keyence VHF-500x Digital Microscope. Images were then processed using Adobe Photoshop CS5 Extended Version 12.0.

ENMs were constructed for each species by aggregating informative bioclimatic variables with unique locality records. All questionable georeferences were either corrected or excluded from the analysis. The bioclimatic variables used in this study come from the WorldClim v1.4 BIOCLIM dataset (Hijmans *et al*., 2005). To reduce ENM complexity and avoid over-fitting, we calculated the Pearson product-moment correlation coefficient (r) in a pair-wise fashion for all 19 bioclimatic variables for all locality records. A single variable from a pair of highly correlated variables ($r > 0.70$) was retained for the ENM based on the expert knowledge of the species' environmental niche during field studies by the primary author (Table 2). Ultimately, the selection of a bioclimatic variable should be based on how informative it is in describing the environmental/abiotic niche of the species under investigation (Fitzpatrick *et al.,* 2013). Because there are questions regarding the specific status of the involved species, the same variables were used for both species to standardize the modeling approach of their respective distributions. In total, seven bioclimatic variables and 426 unique locality records were used in constructing the ENMs (*A. curta* = 372, *A. squammulosa* = 54).

MaxEnt v3.3.3 (Phillips *et al*., 2006) was employed to construct the ENMs as this algorithm has proven the most useful in estimating habitat suitability of various taxa, including bees (Hinojosa-Díaz *et al*., 2009; Gonzalez *et al*., 2010; Lozier *et al*., 2013). The MaxEnt algorithm, which uses the principle of maximum entropy, is discussed in great detail in both computational and ecological contexts in Phillips *et al.* (2006) and Elith *et al.* (2010), respectively. MaxEnt is an exceptionally useful algorithm as it only requires presence data (*e.g.,* museum records) along with the bioclimatic variables of interests in generating ENMs. In our study we employed MaxEnt with default parameters to generate the logistic output that was averaged across 100 replicates with each replicate being subjected to10-fold cross-validation. The performance of each ENM was assessed with the AUC-statistic with AUC values > 0.75 suggesting well-predicted ENMs. The final output of each ENM is represented as an index of habitat suitability (HS) constrained between 0–1, with 1 representing the optimal habitat as estimated by the ENM. A jackknife analysis of the seven bioclimatic variables in the MaxEnt environment was used to determine which variable contributes the most information to the ENM of each species (Elith & Leathwick, 2009). To estimate the percent overlap between *A. curta* and *A. squammulosa*, ENMs in geographic space logistic outputs were converted to shape files in ArcGIS. The range of values contributed by each bioclimatic variable was visualized using boxplots.

Maps and ENMs were processed with ArcMap10.0 (ESRI, Redland CA). Multicolinearity between bioclimatic variables was examined with the Kendall rank-sum test. We also used correlation analysis to test temperature seasonality and precipitation seasonality between *A. curta* and *A. squammulosa*. Boxplots were used to visualize the distribution of bioclimatic variables across taxa. Data visualization and statistics were implemented with R v2.15.1 (R Core Team, 2012). All ecoregion calculations were based on the World Wildlife Fund (WWF) terrestrial ecoregions of the world (Olson *et al*., 2001). Information regarding these ecoregions was taken from publications by the same group (Ricketts *et al*., 1999; World Wildlife Fund, 2002).

Results

The morphological analysis yielded numerous characters that consistently differentiated the two entities. From a total of fifty characters examined, sixteen were found to be at least circumstantially diagnostic for differentiating the two species (Table 1). An example of a character which is circumstantially viable is the extent of the light clypeal maculation, which intergrades between the two species and is only definitive at its extremes. Eleven of these sixteen characters are invariant, universally differentiating the two species. Mapping of the distributions of these species demonstrated allopatry between a northern species from the southwestern United States, Baja California, and the northern states of mainland Mexico, and a more southerly species extending from Nicaragua to the Mexican states of Sinaloa and Durango (Fig. 2). Examination of the lectotype of *A. curta* correctly aligns it with the northern species and the entire syntype series of *A. squammulosa* agrees with the southern species. The types of other synonyms, formerly subspecies, were also examined and correctly aligned with their senior synonyms. The most interesting difference is the presence of setae within the first submarginal cell of *A. squammulosa*, a condition seen otherwise only in some Old World species of the subgenus *Heliophila* (Fig. 3a). A number of additional characters, including those of the male genitalia, are given in the identification and systematics sections below.
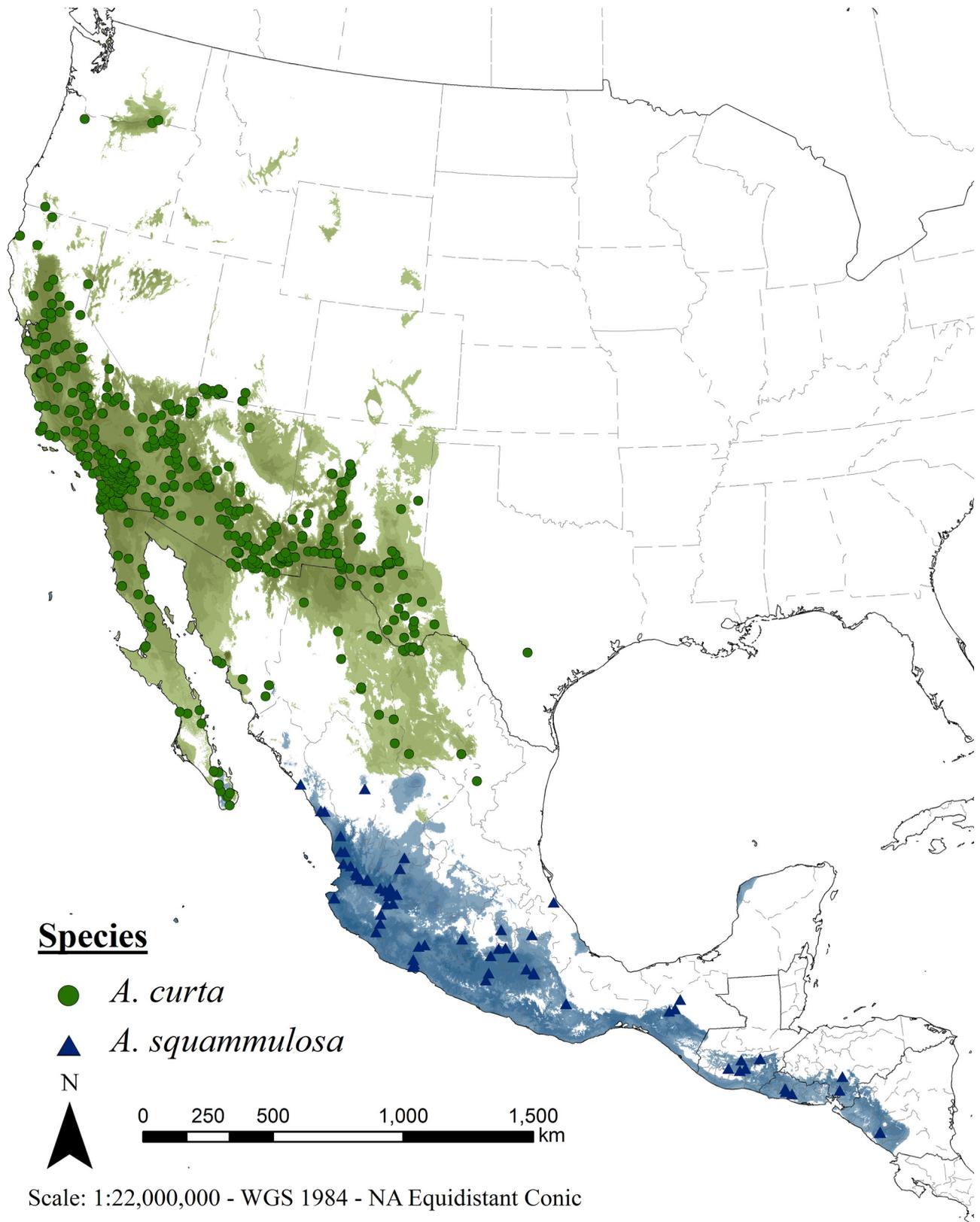
FIGURE 2. Localities and environmental niche distributions modeling of *Anthophora curta* and *A. squammulosa*. *Anthophora curta* is symbolized by dark green circles and *A. squammulosa* by dark blue triangles. The areas with >0.30 habitat suitability index are shaded green for *A. curta* and blue for *A. squammulosa*, with darker areas indicating higher suitability. The species *A. curta* and *A. squammulosa* are seen to be disjunct in Sinaloa, Mexico.

TABLE 1. The morphological characters investigated. Invariant characters are given first, followed by circumstantial (sometimes useful) and useless characters, denoted by the first letter as I, C, or U respectively. Each of these classes is then sorted according to whether it applies to both sexes, only females, or only males by a second letter, B, F, or M respectively. For characters that can be scored in only one sex, that sex is given first in the character name. Detailed descriptions of the useful characters are given in Appendix 1.

Number	Character	Usefulness
1	Scutum surface sculpting	IB
2	Wing 1st submarginal cell setae	IB
3	Propodeal enclosure surface	IB
4	Mandible form	IF
5	Legs overall setae color	IF
6	T1 erect setae color	IF
7	Female S5 setae color	IF
8	Scape integument color	IM
9	Male S7	IM
10	Male S8	IM
11	Male genital capsule	IM
12	Body length	CB
13	Supraclypeus maculation extent	CB
14	Terga appressed setae color	CF
15	Female T4 rim color	CF
16	Clypeus maculation extent	CM
17	Galea shape	UB
18	Galea length	UB
19	Galea surface sculpting	UB
20	Galea setae length	UB
21	Galea setae tip form	UB
22	Maxillary palp segment number	UB
23	Labrum rim color	UB
24	Male labrum apical dentition	UB
25	Face maculation color	UB
26	Clypeus protuberance	UB
27	Clypeus pit density	UB
28	Paraocular area integument color	UB
29	Setae color below antennae	UB
30	Eye color	UB
31	Vertex setae color	UB
32	Scutum unpitted areas	UB
33	Scutum setae color	UB
34	Mesepisternum setae color	UB
35	Basitibial plate form	UB
36	Female hindbasitarsus brush color	UB
37	Male terga rim color T5-6	UB
38	Terga apical setae bands	UB
39	Terga surface sculpting	UB

TABLE 1. (Continued)

Number	Character	Usefulness
40	T4 erect setae color	UB
41	Female T4 transparent rim width	UB
42	Female T4 apical setae band width	UB
43	Female T5 appressed setae	UB
44	Female T5 setae color	UB
45	Male T6 lateral carina	UB
46	Male T7 lateral spine	UB
47	Female pygidial plate	UB
48	Male pygidial plate	UB
49	Male S6 setae form	UB
50	Male S6 median form of rim	UB

The model performance statistics suggest that ENMs for both species fit the locality records and bioclimatic variables provided to the MaxEnt algorithm closely. The average AUC for *A. curta* and *A. squammulosa* was 0.95 (SD = 0.006) and 0.97 (SD = 0.025), respectively. The geographic transformations of the ENMs for *A. curta* and *A. squammulosa* were almost mutually exclusive (Fig. 2), mirroring the lack of overlap in existing specimen records. There is no overlap near the range boundaries of the two species and no instances of the *A. squammulosa* ENM intruding north into the *A. curta* ENM, although the tip of Baja California Sur is modeled as habitable for both. The ENM of *A. curta* is seen to reappear near the southern tip of the *A. squammulosa* model in Nicaragua. This "suitable" area is almost certainly uninhabited by *A. curta* given the distance from other suitable habitat. The total area of overlap is negligible (<1%).

The most informative bioclimatic variables for each species' ENM differ (Table 2, Appendix 2). For *A. curta*, precipitation appears to be an important variable for constructing the suitability models. All precipitation variables summed contribute about 75% of the information necessary to construct an ENM for the species. Overall, the habitat of *A. curta* seems best characterized by aridity during the driest and warmest parts of the year. In contrast, for *A. squammulosa*, precipitation in itself is a relatively weak contributor to the construction of its ENM. The sum of all precipitation variables contributed less than 10% to the ENM when precipitation seasonality was excluded, as it is a measure of change in precipitation between seasons rather than a measure of quantity of precipitation. Seasonality of both precipitation and temperature are much more important for the *A. squammulosa* ENM, contributing over 90% of the information to the final ENM. For *A. squammulosa*, relatively little difference in temperature between seasons and relatively greater disparities in amount of precipitation between seasons seem to best characterize its habitat. From these results, the distributions of temperature seasonality and precipitation seasonality were explored further between the putative species (Fig. 4). A significant positive correlation between the two variables was found in *A. squammulosa* (tau=0.49, p-value=4.767e-09), while a significant negative correlation was seen in *A. curta* (tau=-0.59, p-value<2.2e-16). Not only is there a significant correlation between the two variables found for both species, but the directionality of the relationship also differs dramatically, giving further ecological evidence for unique bioclimatic niche occupation.

Systematics

Anthophora curta and *A. squammulosa* are separated as follows:

1 First submarginal cell lacking pubescence, except sometimes a small number of setae directly on vein where the cell borders the stigma (Fig. 3a); integument of male scape dark; unworn females with light setae of terga white (Fig. 1a); United States, Baja Peninsula, and northern tier of mainland Mexican states on United States border . . . . *A. curta*
- First submarginal cell of forewing pubescent near the border with the stigma (may be absent in worn specimens, Fig. 3a); integument of male scape lightly maculated, at least in part, usually fully maculated from top to bottom of underside; fresh female specimens with the light setae of the terga golden (Fig. 1a); Nicaragua north to the Mexican states of Sinaloa and Durango . . . . *A. squammulosa*
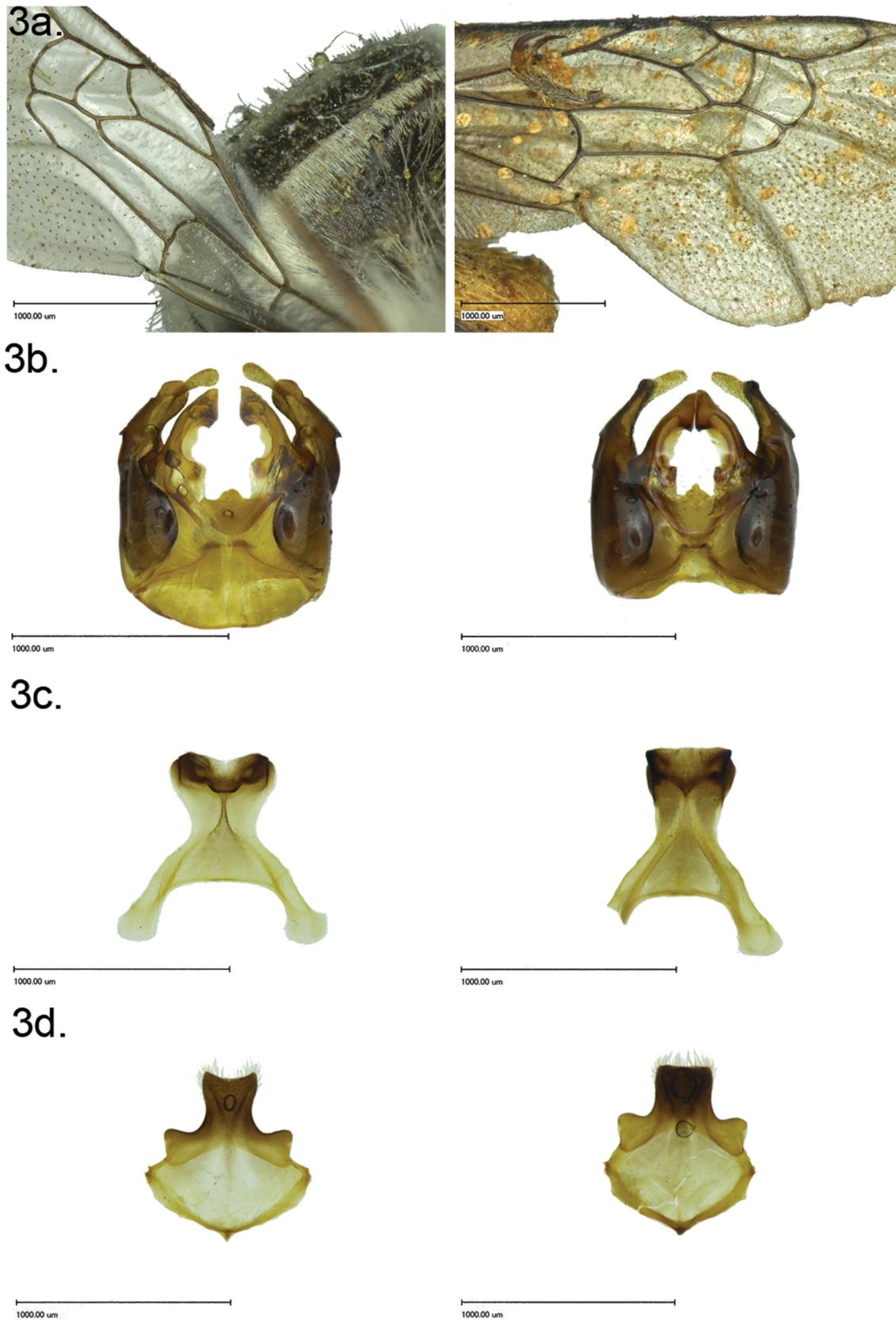
FIGURE 3. The morphology of *Anthophora curta* and *A. squammulosa*, left and right respectively. **a**. The first submarginal cell of each lectotype. **b**. Dorsal view of the genital capsule. **c**. Sternite 7. **d**. Sternite 8. Wing images were taken at 100x magnification and genitalia images were taken at 150x magnification. Note that setae were removed from figures 3b-d to improve clarity of the structures.
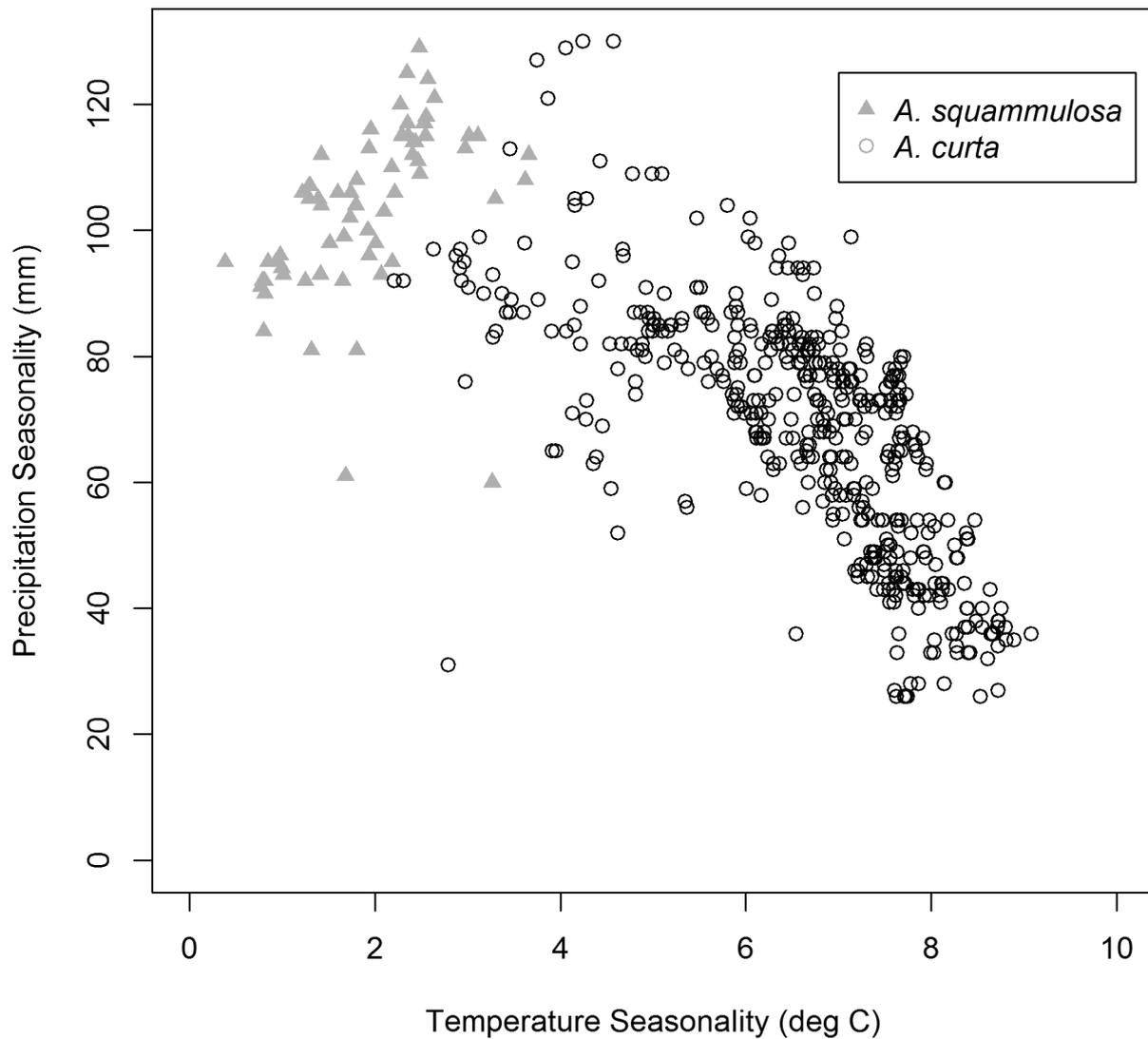
FIGURE 4. Correlation test of temperature seasonality and precipitation seasonality associated with the distribution of *A. squammulosa* and *A. curta*. A significant positive correlation was seen in *A. squammulosa*, while a significant negative correlation was seen in *A. curta*.

TABLE 2. The importance of the contributing variables to the environmental niche models for *Anthophora curta* and *A. squammulosa* based on the jackknife analysis. Variable are sorted from greatest to least importance for each species. The gain without is generated by excluding the variable and the gain with only is generated by using only that variable.

Anthophora curta

	Percent contribution	Training gain without	Training gain with only	Test gain without	Test gain with only
Precipitation of driest quarter	47.3	1.7	1.1	1.9	1.1
Precipitation of warmest quarter	22.8	1.7	1.1	1.9	1.1
Temperature seasonality	18.1	1.6	0.5	1.9	0.5
Precipitation of coldest quarter	4.2	1.7	0.1	1.9	0.1
Precipitation seasonality	3.1	1.6	0.4	1.9	0.5
Mean temp. of wettest quarter	2.6	1.7	0.6	1.9	0.6
Mean diurnal range	1.9	1.7	0.5	1.9	0.5

	Percent contribution	Training gain without	Training gain with only	Test gain without	Test gain with only
Temperature seasonality	48.4	2.4	1.9	1.3	2.0
Precipitation seasonality	44.2	2.8	1.9	1.7	1.9
Precipitation of driest quarter	3.6	2.7	1.5	1.8	0.7
Precipitation of coldest quarter	2.4	2.8	0.6	2.5	-0.2
Mean temp. of wettest quarter	1	2.8	0.5	1.8	0.5
Mean diurnal range	0.2	2.8	0.009	1.9	-0.02
Precipitation of warmest quarter	0.1	2.8	0.3	1.9	0.3

Anthophora curta Provancher, 1895

Anthophora curta Provancher, 1895: 173 (lectotype: female, Los Angeles, California, USA; Les Collections de l'Université Laval, Pavillon Louis-Jacques-Casault, Ste Foy, Québec, Canada) (Prior Lectotype Designation (Sheffield & Perron, 2013))
Anthophora curta var. *melanops* Cockerell, 1926: 84 (holotype: male, 2 miles east of Oracle, Arizona, USA; California Academy of Sciences, San Francisco, California, USA) (**New Synonym**)
Anthophora curta var. *ensenadensis* Cockerell, 1941: 349 (holotype: male, Ensenada, Baja California, México; California Academy of Sciences, San Francisco, California, USA)

Diagnosis. Males of *Anthophora curta* are easily separated from all Nearctic species, other than *A. squammulosa,* by the apically truncate medial projection on T7. In other species, there is either a sharp pygidial plate or a pair of submedial projections. In addition to the distinctions in the key, *A. curta* males differ from *A. squammulosa* in the thickness of the longitudinal clypeal maculation, which typically only equals a third or less of the height of the clypeus in *A. curta*, but usually about half that height in *A. squammulosa*. *Anthophora curta* also typically lacks a supraclypeal maculation, while it is present in most specimens of *A. squammulosa*. The male genitalia are also distinct (Fig. 3b–e). The gonostylus is more robust in *A. curta* overall, and the poorly scleritized digit near the end of the gonostylus is attached for half or less its length, while in *A. squammulosa* it is attached for more than half its length. There are also differences in overall structure and tips of S7 and S8 (Fig. 3c–d).

Females of *A. curta* are separated from other species of Nearctic *Anthophora* (*Heliophila*), save for *A. squammulosa*, by the unique combination of the following characters: basal bands of black setae on the metasomal terga; the lack of strongly curved or bent setae on the galea; the supraclypeal maculation either negligible or more often absent; the near-flat distal edge of the basitibial plate; the distinct presence of appressed, branched setae on T5; the very narrowly transparent apical rim of T4; and the relatively flat clypeus, which does not appear fully half-circular in ventral view. From *A. squammulosa*, it is distinguished using the couplet above. In addition, the supraclypeal maculation is typically absent or weak in *A. curta*, and generally strong and distinct in *A. squammulosa*, although rarely such maculations are absent.

Geographical and ecoregion distribution. The geographical range of this species is quite broad (Fig. 2). Although it is difficult to identify absolute limits of distribution, the absence of this species in Utah, except on its southern border, despite extensive collection efforts throughout the state, is likely telling of its northern boundary there. Its northern limits to the east are likely farther south, in New Mexico rather than Colorado. More collections are necessary in northern California, Nevada, and Oregon to discern the northern limits of the distribution of *A. curta* in these three states, although it at least reaches southern Washington. It is highly unlikely that *A. curta* ranges farther east than Texas, but its limits within the state are unclear. This species ranges south throughout the Baja Peninsula and much of northern Mexico. There are too few collections to be certain of its southernmost limit in Mexico, although it currently appears largely restricted to the Sonoran and Chihuahuan Deserts. Corresponding to its large geographical range, *A. curta* also inhabits a wide variety of ecoregions, based on 541 unique locations. This species' range spans a total of six WWF biomes encompassing 27 ecoregions. Two-thirds of all collection events (65%) are from 10 ecoregions in the Deserts & Xeric Shrublands biome. *Anthophora curta* is also present in the Mediterranean Forests Woodlands, & Scrub biome, with 19% of collection events spread across three

ecoregions. The remaining four biomes are primarily forest and grassland, accounting for 16% of all collection events (Temperate Conifer Forests; Temperate Grasslands, Savannas, & Shrublands; Tropical & Subtropical Coniferous Forests; and Tropical & Subtropical Dry Broadleaf Forests).

Phenology. In the Mojave Desert, 73% of collection events took place in the spring and early summer (March through June). A similar trend exists in the adjacent Sonoran Desert, with 73% of collection events during the same spring period. Contrastingly, in the Chihuahuan Desert, 62% of all collection events occurred during August through October. Additional collections during spring in the Chihuahuan Desert and fall of the Sonoran Desert are necessary to confirm the apparent phenological differences for *A. curta*. The phenology of *A. curta* in the Mojave and Sonoran Deserts appears similar to that of the Mediterranean ecoregions of California from the Mediterranean Forests, Woodlands, and Scrub biome and the Temperature Grasslands, Savannas, and Shrublands biome, with 71% of collections events in these ecoregions during the same spring period. Despite the trend, *A. curta* has been collected as late as November in the Arizona Mountains forests, California montane chaparral and woodlands, Chihuahuan Desert, and Sonoran Desert ecoregions.

Floral hosts. *Anthophora curta* appears broadly polylectic due to the long list of plant associations: 61 plant genera from 17 families. Despite this, past authors have suggested that females use only Asteraceae pollen (Moldenke & Neff, 1974). Collated floral records support this (72% from Asteraceae). The known floral hosts are as follows:

Amaranthaceae: *Salsola* sp.; Asteraceae: *Adenophyllum cooperi, Baccharis salicina, Bahia absinthifolia, Baileya multiradiata, B. pleniradiata, Bebbia juncea, B. juncea* var. *aspera, Bidens pilosa, Carduus tenuiflorus, Carthamus tinctorius, Centromadia pungens, Chaenactis glabriuscula, Chaetopappa ericoides, Chrysopsis* sp., *Coreopsis* sp., *Deinandra fasciculata, Dieteria canescens, Encelia californica, E. farinosa, E. frutescens, E. virginensis, Erigeron* sp., *Gaillardia pinnatifida, G. pulchella, Grindelia* sp., *Gutierrezia microcephala, G. sarothrae, Helianthus annuus, Hemizonia corymbosa* ssp. *macrocephala, Heterotheca subaxillaris* ssp. *latifolia, H. villosa, Hymenopappus filifolius, Hymenothrix wislizeni, Isocoma tenuisecta, Malacothrix* sp., *Palafoxia arida, P. arida* var. *gigantea, Pectis papposa, Psilostrophe* sp., *Symphyotrichum spathulatum, S. tenuifolium, Verbesina encelioides, Viguiera deltoidea*; Bignoniaceae: *Chilopsis* sp.; Boraginaceae: *Heliotropium* sp., *Phacelia coerulea, P. distans, P. robusta*; Brassicaceae: *Physaria* sp.; Cleomaceae: *Cleomella* sp.; Convulvulaceae: *Ipomoea* sp.; Fabaceae: *Dalea lanata, D. lanata* var. *terminalis, D. leporina, Medicago sativa, Melilotus* sp., *Parryella filifolia, Psoralidium lanceolatum, Psorothamnus emoryi, P. scoparius*; Loasaceae: *Cevallia sinuata, Mentzelia multiflora*; Malvaceae: *Melochia tomentosa, Sphaeralcea emoryi, S. grossulariifolia*; Nyctaginaceae: *Allionia incarnata*; Papaveraceae: *Argemone* sp.; Plantaginaceae: *Penstemon centranthifolius*; Polemoniaceae: *Eriastrum* sp., *Gilia capitata, Ipomopsis congesta* ssp. *congesta*; Polygonaceae: *Chorizanthe douglasii, Eriogonum gypsophilum, E. roseum, E. trichopes*; Rosaceae: *Adenostoma* sp., *Fallugia paradoxa*; Zygophyllaceae: *Larrea* sp.

Comments. *Anthophora curta* is more variable than most other species of the subgenus *Heliophila*, likely owing to its large distribution. This is only problematic in the females, given the ease of identification for the male of this species. In the female, the supraclypeus almost always lacks a light integumental marking. There are a few specimens in which there is a negligible dot of light integument centrally at the border with the clypeus. The extent of the apical bands of appressed setae of the metasomal terga is variable in both sexes, from narrow bands restricted to the apical rims to covering the majority of the terga. This character is more evident in females as the band variation is more constrained in males. The bands are generally narrower in California, while they are thicker eastward in eastern Arizona, New Mexico, and Texas. The synonyms *A. curta* var. *melanops* and *A. curta* var. *ensenadensis* were transferred to *A. curta* following examination of the types. Although *A. curta* var. *ensenadensis* was previously synonymized with *A. curta, A. curta* var. *melanops and A. curta* were synonymized with *A. squammulosa* at the same time (Michener, 1951; Brooks, 1988). As such, this is a new synonymy for *A. curta* var. *melanops.*

Anthophora squammulosa Dours, 1870

Anthophora squammulosa Dours, 1870: 78 (lectotype: female, "Mexique"; Muséum national d'Histoire naturelle, Paris, France) (**New Lectotype Designation**)
Anthophora usticauda Cockerell, 1912: 22 (lectotype: female, Antigua, Guatemala; National Museum of Natural History, Washington, D.C., USA) (**New Synonym**)

Anthophora usticauda cinerior Cockerell, 1949: 470 (holotype: female, Antigua, Guatemala; National Museum of Natural History, Washington, D.C., USA, USNM Type No. 58873) (**New Synonym**)

Anthophora franciscana Cockerell, 1949: 470 (holotype: female, San Francisco finca, Zamorano Valley, Honduras; National Museum of Natural History, Washington, D.C., USA, USNM Type No. 58874) (**New Synonym**)

Anthophora zamoranella Cockerell, 1949: 471 (holotype: male, Zamorano, Honduras; National Museum of Natural History, Washington, D.C., USA, USNM Type No. 58875) (**New Synonym**)

Anthophora bispinosa Cockerell, 1949: 472 (holotype: male, San Francisco finca, Zamorano Valley, Honduras; National Museum of Natural History, Washington, D.C., USA, USNM Type No. 58877) (**New Synonym**)

Diagnosis. Male *Anthophora squammulosa* and *A. curta* can be separated from other species of New World *A.* (*Heliophila*) by the apically truncated medial projection of T7. It is distinguished from *A. curta* with the above couplet and additional characters given in the *A. curta* male diagnosis.

Females of *A. squammulosa* are separated from other New World *A.* (*Heliophila*) based upon the following character combination: basal bands of black setae on the metasomal terga; the lack of strongly curved or bent setae on the galea; the supraclypeal light maculation present, although sometimes reduced to a dot; the near-flat distal edge of the basitibial plate; the clear presence of appressed, branched setae on T5; the very narrowly transparent apical rim of T4; and the relatively flat clypeus, which does not appear fully half-circular in ventral view. From *A. curta*, it is distinguished using the couplet above and additional characters given in the *A. curta* section above.

Geographical and ecoregion distribution. This species is found throughout much of central Mexico, scarcely farther north than the Trans-Mexican Volcanic Belt, and ranges south in Central America as far as Nicaragua. Its northern limit appears to be near the southern tip of the Mexican state of Sinaloa, eastward to at least the southern tip of Zacatecas. Collection records are limited (72 unique locations) and consequentially its distribution and habitat requirements are less clear. This species is known from four WWF biomes spread across 20 ecoregions. The Tropical & Subtropical Dry Broadleaf Forests biome is most represented in collection events of *A. squammulosa*, with 64% of all events made in eight ecoregions. An additional 18% of all collection events took place in the four ecoregions comprising the Tropical & Subtropical Coniferous Forests biome. The remaining 18% of collection events are in the Desert & Xeric Shrublands and Tropical & Subtropical Moist Broadleaf Forest biomes. Overall, this species appears most prevalent in forested environments, although a greater number of collection events are necessary to get a good picture of its abundance in poorly sampled ecoregions.

Phenology. Throughout its range, *A. squammulosa* is active from August to March. There does not appear to be a significant phenological trend for this species, because flight time varies widely at both the northern and southern limits of its range. At the north end, collections exist from Sinaloa and Zacatecas in both October and March. To the south, it has been collected in Nicaragua and Honduras from October and February. Although there is no overall geographical trend in phenology, *A. squammulosa* does appear to be most common from September through November, during which 73% of all collection events have taken place.

Floral hosts. There are only 17 floral records for *A. squammulosa*. It is likely a generalist, as is the closely related *A. curta*, based on recorded visitation of three plant families despite only being known from six genera. The known floral records for *A. squammulosa* are:

<u>Araceae</u>: *Zantedeschia aethiopica*; <u>Asteraceae</u>: *Anthemis* sp., *Bidens aurea*, *Cosmos sulphureus*, *Melanthera nivea*; <u>Fabaceae</u>: *Dalea foliolosa* var. *citrina*.

Comments. This species is unique among the New World bees of the subgenus *Heliophila* in several ways. It is the only species which has setae in the first submarginal cell and one of only two species in which the male has a single, apically truncated medial projection on T7. It is also the only species in the Neotropics. In Guatemala and Honduras, nearing the southernmost reaches of the distribution of *A. squammulosa*, Cockerell (1912, 1949) described five taxa: *A. bispinosa*, *A. franciscana*, *A. usticauda*, *A. usticauda cinerior*, and *A. zamoranella*. All of these entities are synonyms of *A. squammulosa* based upon examination of the types housed at the US National Museum in Washington, D.C. The lectotype of *A. squammulosa* was chosen for specimen quality (intactness) and visibility of characters. The labels of the lectotype read as follows (each label separated by a semicolon): "12. ♀ Sh; mex 63 Sich; 16 ♀; squammulosa Sich. 16 ♀ 19 ♂ 63 mex." It should be noted that Brooks (1988) examined a specimen of "*A. squammulosa*" in the Berlin Museum from Guayaquil, Ecuador and concluded that it was likely mislabeled. We have not studied this specimen but at present this is the most reasonable conclusion, given its absence in extensive collections in Costa Rica and its apparent absence from Panamá (Griswold *et al*., 1995). No *Anthophora* were included in the account of the bees of Panamá nor have any been found in more recent material (Michener, 1954).

Discussion

The morphological analysis provides strong support for the distinction of *A. curta* and *A. squammulosa*, with 11 definitive characters that unequivocally separate the pair. Previously, species and even species groups in *Anthophora* have been distinguished based on few, often variable, characters such as setal color characters (Brooks, 1999). Contrastingly, in this study, a number of non-color characters such as structures of the mandible, sterna, and genitalia indicate the distinctiveness of these two taxa. Although polymorphism is commonly present in the group, definitive characters do exist.

The mutually exclusive distributions of *A. curta* and *A. squammulosa* provide one of the strongest pieces of evidence for the two-species hypothesis. This geographic separation is reflected in the ENMs, which scarcely overlap (<1% overlap). The only points of overlap, found in Baja California Sur and Nicaragua, are far removed from the ranges of *A. squammulosa* and *A. curta* respectively. More interesting are the apparently uninhabited areas modeled as suitable *A. curta* habitat in Colorado, Wyoming, Montana, Idaho, and northwestern Nevada. It must be kept in mind that suitability of habitats does not ensure habitation. ENMs do not take into account either connectivity of suitable habitat or potential barriers, such as mountain ranges, to colonization of isolated suitable habitat.

The asymmetrical contributions of the bioclimatic variables for *A. curta* and *A. squammulosa* are informative. These variables reflect the importance of the different climatic factors to each of the species throughout their entire range. When these variables are different for two groups, and there is documented geographic separation, environmental separation is also probable. Although these models have identified several important factors for these bees, additional variables may prove useful for future investigations. Given that *Anthophora* are almost all ground nesters, it would be interesting to incorporate soil parameters into future analyses to determine if this would refine the habitat suitability models.

The correlation of temperature seasonality and precipitation seasonality with *A. curta* and *A. squammulosa* is explicable in light of bioclimatic niche space (Fig. 4). The relatively low annual temperature range experienced by *A. squammulosa* across its range explains why this temperature seasonality contributes a significant amount to the species' ENM. The more pronounced temperature seasonality of *A. curta* shows that this species experiences a greater annual temperature range than *A. squammulosa*. This is due to the colder winter seasons of the xeric and Mediterranean areas it inhabits, in comparison to the more uniform temperatures experienced year-round by *A. squammulosa*. Precipitation seasonality is another important variable for *A. squammulosa*, although it provides only a negligible contribution to the ENM of *A. curta*. The high precipitation seasonality of the *A. squammulosa* ENM corresponds with the dominance of dry forests throughout its range, where there are pronounced wet and dry seasons. In this way, a large portion of the habitat of *A. squammulosa* is similar to that of *A. curta* for the strong precipitation signals available. While the wet/dry seasons are similar throughout the range of *A. squammulosa*, they are less homogeneous across the larger range of *A. curta*, most markedly in the hot deserts which differ in the season of primary precipitation (MacMahon & Wagner, 1985). Taken altogether, these records from disparate precipitation regimes would obscure any importance of precipitation seasonality. As such, it may be that precipitation seasonality is important for A. curta, but that the different timing of precipitation events across its range obscure this in the bioclimatic analysis.

A strong precipitation signal, as is suggested by high precipitation seasonality, may ultimately provide a cue for emergence timing in bees. Several previous studies have raised this possibility in desert environments (Hurd, 1957; Danforth, 1999). This hypothesis could be tested in future studies by combining historical rainfall data with collection records spread equally throughout the year. If true, this should enhance synchrony with ephemeral floral resources. Such an opportunistic strategy would benefit *A. curta* greatly in deserts, where it is collected most often. Preliminary study of its phenology (see taxonomic section for *A. curta*) suggests that emergence may be biased toward the periods of greatest rainfall for each of the deserts it inhabits (MacMahon & Wagner, 1985). The possibility of precipitation as an emergence cue could also be tested for *A. squammulosa* in future studies, although additional collections would be necessary.

The morphological differences and the observed geographic isolation are strong indicators of species-level differences between *A. curta* and *A. squammulosa*. The correlation test shows that the two entities are associated with different climates. The environmental niche models provide novel information, elucidating climatic factors that define geographic boundaries. Based on the morphological, climatic, and geographic evidence, the synonymy of *A. curta* to *A. squammulosa* is formally reversed here, and *A. curta* is once again recognized as a valid species.

FIGURE 5. Collections of *A. curta* and *A. squammulosa* superimposed on a map of the WWF ecoregions. The ecoregions are color coded by 30-year status predictions. Green indicates "relatively stable or intact", yellow indicates "vulnerable", and red indicates "critical or endangered."

In light of the many recent studies focusing on pollinators at broad scales, it becomes all the more important to provide good taxonomy for bees. This is because different species have different preferences for floral hosts, nesting substrates, and overall habitat types. Floral specificity is most well-studied, with differences in floral host preference documented even within the same genus (Müller, 1996; Sipes & Wolf, 2001; Larkin *et al*., 2008). This

study has major implications for the conservation status of these species. With the separation of *A. curta* from *A. squammulosa*, the latter's geographic range and climatic niche are much reduced. The unique distribution of *A. squammulosa*, separate from all other *A.* (*Heliophila*), becomes especially apparent. This is of conservation importance because much of Mexico's forests, especially the dry forests, are being logged (Myers *et al.*, 2000; Olson & Dinerstein, 2002). Based on projections by the WWF, virtually all of the ecoregions in which *A. squammulosa* has been detected are soon to be in critical or endangered status (Fig. 5). In contrast, *A. curta* inhabits a number of ecoregions projected to be relatively stable or only vulnerable in the future. The incorrect synonymy of these two species obscured the potential threat to *A. squammulosa* posed by habitat loss. Only through the combination of traditional taxonomy and ENMs did this become apparent. This study demonstrates the value of ENMs in taxonomic studies where molecular data are not available and suggests its usefulness in future studies.

Acknowledgements

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APPENDIX 1. Detailed descriptions of the useful characters from the morphological analysis. Numbers here correspond to those of the in-line Table 1.

1. Scutum surface sculpting—Differentiates both sexes

The scutum in *Anthophora curta* is pitted throughout as in *Anthophora squammulosa*. Despite this, clear reflections are visible throughout the scutum due to its smooth surface. In contrast, *A. squammulosa* has a more tessellate scutum and very few, if any, reflections as a result. It should be noted that the anterior limit of the scutum is smooth and shiny in both species.

2. Wing 1st submarginal cell setae—Differentiates both sexes

There are at most setae along the veins of the first submarginal cell in *A. curta*, as is typical for the New World *A.* (*Heliophila*). *Anthophora squammulosa* is the only species of New World *A.* (*Heliophila*) with setae reaching into the first submarginal cell. There are normally at least five such setae in the cell, although rarely they may be worn off and only the point of attachment is visible. In such a case, this point of attachment will often appear similar to, although less well-defined than, the papillae seen at the edge of the wing in *Anthophora*.

3. Propodeal enclosure surface—Differentiates both sexes

The propodeal enclosures are quite similar in extent in these two species, but within them the level of tessellation is distinctly different. The propodeal enclosure of *A. curta* is only weakly tessellate, allowing for an appreciable degree of reflection. The propodeal enclosure of *A. squammulosa* is comparatively more coarsely tessellate, giving it a duller appearance overall.

4. Mandible form—Differentiates females

The female mandible of *A. curta* is a short, linear blade as is typical of the New World *A.* (*Heliophila*). The tip of the mandible in *A. squammulosa* is comparatively much closer to that of *A. peritomae*, which has an elongate, scoop-like mandible tip. Although not as greatly enlarged as that of *A. peritomae*, the scoop of *A. squammulosa* is still distinctly elongate and the subapical tooth appears slightly reduced in comparison as a result.

5. Legs overall setae color—Differentiates females

The legs of *A. squammulosa* are clothed in noticeably darker setae than those of *A. curta*. This is most obvious when looking at the long fringe of setae on the posterior of the fore leg's tibia and basitarsus, which are a smoky gray to black in *A. squammulosa* and a white to light brown color in *A. curta*. This is also seen on the inner edge of the mid and hind femur, where the white setae of *A. curta* are again opposed by the smoky black setae of *A. squammulosa*.

6. T1 erect setae color—Differentiates females

There are very long, erect setae present on T1 in addition to the appressed setae. These setae are all white in *A. curta*. In *A. squammulosa*, an appreciable number of these erect setae are instead black, intermixed with the dominant white setae. This may not be the case in specimens whose setae have been worn off.

7. Female S5 setae color—Differentiates females

In the apical half of S5, there are relatively longer setae in both species. These setae are typically a mix of white to light brown in *A. curta*, while in *A. squammulosa* they are largely dark brown to black, with few if any light setae.

8. Scape integument color—Differentiates males

The integument of the scape is quite commonly maculated in the New World *A.* (*Heliophila*), this maculation found on the underside such that it is visible when the antennae are raised. The species *A. squammulosa* follows this trend, with strong maculations typically present from the base to tip of the scape, although in some specimens it is somewhat reduced. There is no such maculation present in any observed specimens of *A. curta*.

9. Male S7—Differentiates males

The male S7 of these two species is highly diagnostic. The strongest characters are found near the apical tip. The medial portion of the broad tip is emarginated in *A. curta*, while in *A. squammulosa* this area is nearly straight, at most weakly undulate. There are also characters near the base of the tip. The midline of the venter is seen to be strongly constricted in *A. curta* such that it appears like a strongly produced, thin ridge. The midline of the venter is much more gradually constricted in *A. squammulosa*, making it appear to be more of a broad mound than a thin ridge. The apical tip of this ventral midline is also diagnostic. In *A. curta*, this area takes the form of a trapezoid. This area is triangular in *A. squammulosa*, although the sides are slightly concave.

10. Male S8—Differentiates males

As was the case with the male S7, S8 is most distinctive at its medial tip. The tip is roundedly concave in *A. curta*, with each side of this concavity coming to a point. The tip is nearly straight or weakly undulate in *A. squammulosa*, at most with a very slight shallowing medially. The sides leading to this relatively straight medial portion of the tip are also distinctive, with that of *A. curta* clearly concave while those of *A. squammulosa* are nearly parallel.

11. Male genital capsule—Differentiates males

The gonostyli are significantly thicker in *A. curta* when viewed from. For *A. curta* the gonostylus is about twice the width of the unscleritized digit attached near its tip. The gonostyli is much thinner in *A. squammulosa*, its width barely greater than that of the unscleritized digit if at all. The unscleritized digits are also different, that of *A. curta* widened greatly at the tip and that of *A. squammulosa* with roughly parallel sides. The attachments of the digits may also be used for diagnosis. The digit of *A. curta* is relatively free from the gonostylus and attached for less of its length. In contrast, the digit of *A. squammulosa* is secured to the gonostylus almost to the tip of the gonostylus.

12. Body length—Differentiates both sexes circumstantially

Both species exhibit a considerable amount of variation in size, although there is a slight trend for greater size in *A. squammulosa*. *Anthophora curta* averages about 8.5mm in total body length, while *A. squammulosa* has a slightly larger average total body length of 9mm.

13. Supraclypeus maculation extent—Differentiates both sexes circumstantially

There is almost never a supraclypeal maculation in *A. curta*, and when present it is usually a very minute dot of light integument. In contrast, *A. squammulosa* almost always has a distinct, slightly rectangular maculation just above the clypeus. It should be noted that a small number of *A. squammulosa* have been seen with no supraclypeal maculation, some also with very minute maculations.

14. Terga appressed setae color—Differentiates females circumstantially

The appressed setae on the terga of both species are relatively dense, mostly obscuring the integument below where they occur. The color of these setae is typically diagnostic in these species, with those of *A. curta* being white to off-white and those of *A. squammulosa* being gold to orange in color. This color difference is also usually visible on the scutum, although it is much less distinctive there. This color difference is prone to fading if overexposed to sun, making it only circumstantially useful.
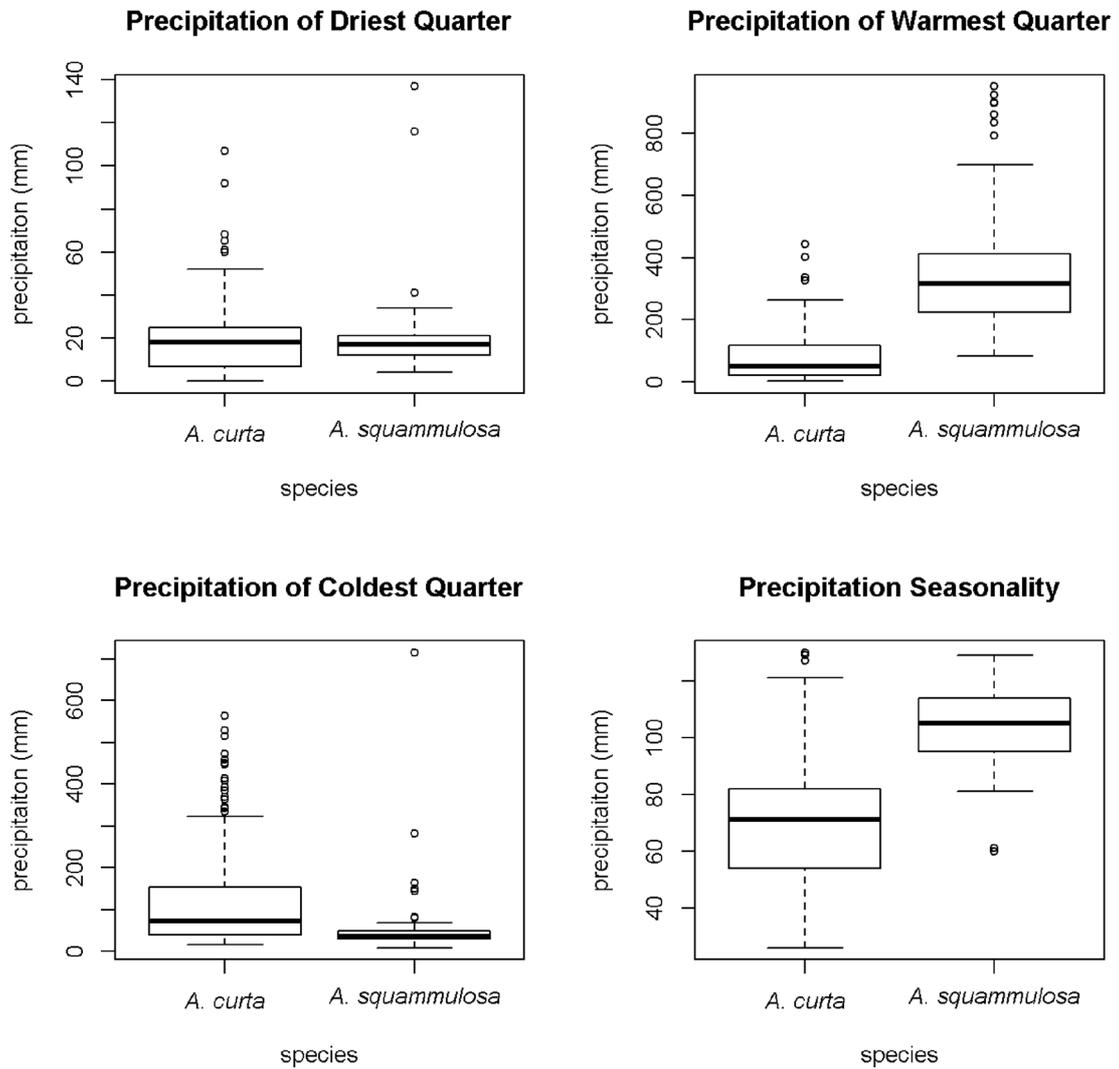
15. Female T4 rim color—Differentiates females circumstantially

The rim of T4 in females is bare of setae apicomedially, leaving this area visible. The integument here is relatively transparent, allowing one to see through to T5 below. In *A. squammulosa*, the integument is a darker brown color, leaving it only weakly transparent, closer to translucent. In contrast, *A. curta* has slightly tanned integument there such that it is nearly completely transparent.
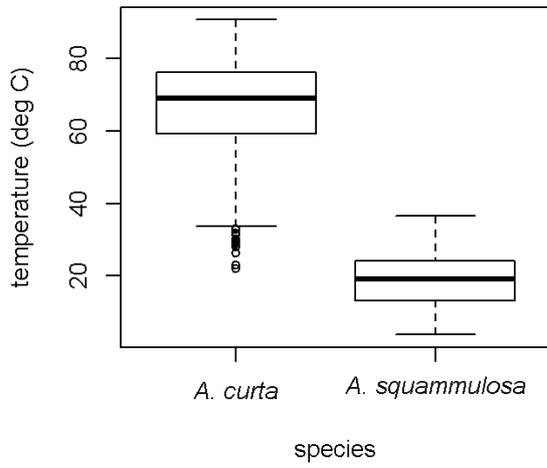
16. Clypeus maculation extent—Differentiates males circumstantially

In both species, the clypeus is typically maculated in the form of a light yellow to off-white band along the rim. This band is usually slightly thickened medially in both species and the total medial height of this light maculation is usually diagnostic. The maculation usually extends upward from the clypeal rim about one fourth to one third of its height in *A. curta*. The maculation is typically larger in *A. squammulosa*, extending upward half or more the height of the clypeus. Exceptions are seen in specimens of *A. squammulosa* uncommonly having a maculation height less than half the clypeal height and specimens of *A. curta* with nearly half the clypeal height maculated.
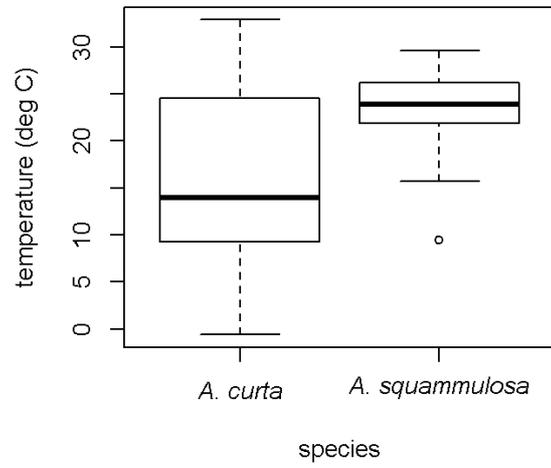
APPENDIX 2. Average bioclimatic variable values for *Anthophora curta* and *A. squammulosa*. The seasonality variables represent average change in either precipitation or temperature between seasons, with higher values indicating greater change between seasons. The mean diurnal range is defined as (mean of monthly temperature (maximum temperature–minimum temperature)).

Temperature Seasonality

Mean Temperature of Wettest Quarter

Mean Diurnal Range