

The Invasion of *Megachile polycaris* (Hymenoptera: Megachilidae) to Hawai'i

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Abstract

Islands are insular environments that are negatively impacted by invasive species. In Hawai'i, at least 21 non-native bees have been documented to date, joining the diversity of >9,000 non-native and invasive species to the archipelago. The goal of this study is to describe the persistence, genetic diversity, and natural history of the most recently established bee to Hawai'i, *Megachile polycaris* Say, 1831 (Hymenoptera: Megachilidae). Contemporary surveys identify that *M. polycaris* is present on at least O'ahu, Maui, and Hawai'i Island, with the earliest detection of the species in 2017. Furthermore, repeated surveys and observations by community members support the hypothesis that *M. polycaris* has been established on Hawai'i Island from 2017 to 2020. DNA sequenced fragments of the cytochrome oxidase I locus identify two distinct haplotypes on Hawai'i Island, suggesting that at least two founders have colonized the island. In their native range, *M. polycaris* is documented to forage on at least 21 different plant families, which are represented in Hawai'i. Finally, ensemble species distribution models (SDMs) constructed with four bioclimatic variables and occurrence data from the native range of *M. polycaris* predicts high habitat suitability on the leeward side of islands throughout the archipelago and at high elevation habitats. While many of the observations presented in our study fall within the predicted habitat suitability on Hawai'i, we also detected the *M. polycaris* on the windward side of Hawai'i Island suggesting that the SDMs we constructed likely do not capture the bioclimatic niche flexibility of the species.

Key words: solitary bee, invasion biology, floral host, cytochrome oxidase I, species distribution model

Hawai'i is home to exceptional biodiversity due to its insular nature, volcanism, and dynamic biogeographic history. However, of the seven extant bee families (Hymenoptera: Anthophila), only the Colletidae have successfully established without the assistance of humans (accidental or intentional) and diversified on the archipelago with a single genus *Hylaeus* Fabricius, 1793 (Hymenoptera: Colletidae). In fact, it is hypothesized that the radiation of the 63 described *Hylaeus* species occurred within 1 million years since the founders arrived on the islands (Magnacca and Danforth 2006, Magnacca 2007, 2011). In more recent times, non-native bees have also made their way to the archipelago, with the earliest documentation of the non-native European honey bee, *Apis mellifera* Linnaeus, 1758 (Hymenoptera: Apidae), in 1857 (Hopkins 1857, Roddy and Arita-Tsutsumi 1997). With the exception of the honey bee, all non-native bees to Hawai'i

were accidental human introductions, and likely arrived through the inbound shipments of horticultural and agricultural products (Howarth 1996). The first recorded accidental bee introduction was likely *Xylocopa sonorina* Smith, 1874 (Hymenoptera: Apidae) in 1874 (Snelling 2003). The lack of endemic familial diversity among the bees in Hawai'i is not unexpected as the archipelago is one of the most isolated islands on the planet as it is situated almost at the center of Pacific Ocean.

As of 2021, 21 non-native bee species have been documented in Hawai'i, including our subject species, *Megachile polycaris* Say, 1831 (Hymenoptera: Megachilidae) (Tabor and Koch 2021). The non-native bees of Hawai'i are solitary in nature (except for *A. mellifera*) and live either underground or in natural cavities (Snelling 2003). However, human-made structures are also a likely

resource for non-native bees to use for nesting purposes. With the increase of non-native bees in Hawai'i, there is significant potential for an increase of pathogen and parasite transmission across non-native wild bees, managed bees, and endemic *Hylaeus* bees (Villalobos and Shelly 1996, Koch and Sahli 2013, Mordecai et al. 2016, Aslan et al. 2019, Cortina et al. 2019, Ing and Mogren 2020). Therefore, it is critical to document the natural history of non-native bees to Hawai'i given the imperiled status of several *Hylaeus* bees (U.S. Fish and Wildlife Service 2015). Research on the interactions between *Hylaeus* and non-native bees at floral hosts are beginning to emerge, revealing potentially negative impacts on endemic *Hylaeus* bees (Koch and Sahli 2013, Aslan et al. 2014, Kuppler et al. 2017, Cortina et al. 2019, Ing and Mogren 2020).

Megachilid bees are a cosmopolitan and robust group of solitary bees, that are found on all continents except Antarctica and have been able to disperse across numerous oceanic islands, including islands in the South Pacific (Gonzalez et al. 2012, Davies et al. 2013). The Megachilidae are represented by at least 4,000 species worldwide, with at least 630 described species in North America (Michener 2007). These bees earn their common name of 'leaf cutters' as species within the family have the unique capacity to extract diverse materials from the environment for nest construction. Megachilid bees are documented to use petals, mud, leaves, resin, soil particles, gravel, and plant trichomes when constructing nests (Gonzalez et al. 2012). In fact, the materials used to prepare nests have been useful in identifying Megachilid bees to species from fossils originating during Pleistocene (Holden et al. 2014). In addition to using diverse materials from the environment, Megachilid bees are exceptionally flexible in the locations they may place their nests. These bees are documented to place their rolled nests on both surfaces of natural (e.g., branches) and human-made objects (e.g., fabricated walls), and may also use pre-existing cavities in the ground and in wood (Gonzalez et al. 2012).

Megachile polycaris is endemic to North America, with the vast majority of its documented distribution between 10°N (Guanacaste, Costa Rica) and 41°N (Nebraska, USA) (Figs. 1 and 2). In the continental United States, *M. polycaris* is documented from the Pacific shoreline of California east to the Atlantic shorelines of Florida. Thus, the geographic distribution *M. polycaris* traverses diverse climate zones, ecosystems, and floristic provinces throughout North America. Among these different environments, *M. polycaris* is likely accessing varied nesting materials, nesting sites, and floral resources

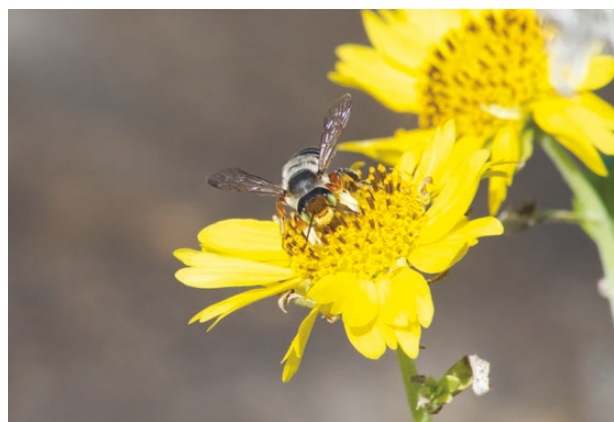


Fig. 1. Photograph of *Megachile polycaris* male documented in Kahului, Hawai'i, USA on 16 February 2019 at 14:07 PM HST on iNaturalist. Photo 31537359, (c) John Starmer, some rights reserved (CC BY-NC), <https://www.inaturalist.org/observations/20427517>.

that are locally abundant and available. Interestingly, *M. polycaris* females exhibit unique nesting construction strategies that are uncommon in other Megachilid bees (Michener 1962, 2007; Baker et al. 1985). In this species, nests can be either made up of a large brood chamber filled with a pollen provision where more than one larva is present or a single cell with one larva present with the pollen

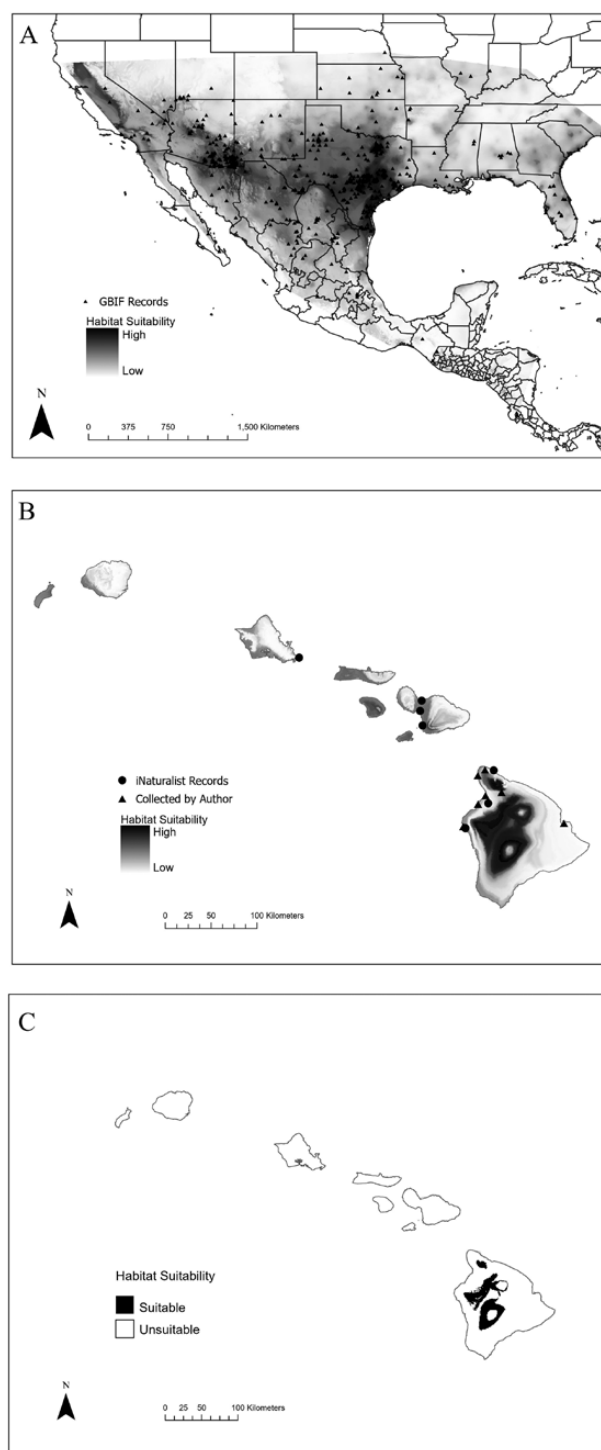


Fig. 2. Distribution of *Megachile polycaris* occurrence records and habitat suitability in their (a) native environment (b) non-native (Hawai'i) environment. (c) Suitable vs. unsuitable habitat and range of *M. polycaris* based on binary map analysis.

provision (Baker et al. 1985). In the case of the large brood chamber, there is an absence of partitions between cells, and the larvae are therefore reared in a common space where they all have access to a common resource mass (Michener 2007).

In this study, we combine published specimen records of *M. polycaris* on the Global Biodiversity Information Facility (GBIF) (<http://gbif.org>) and contemporary survey data from Hawai'i to describe the extent of the species' invasion. We also document general natural history observations of the species in Hawai'i and describe cytochrome oxidase haplotype diversity of sampled populations on Hawai'i Island. Finally, we present a species distribution model of *M. polycaris* in Hawai'i based on georeferenced records available from the GBIF.

Materials and Methods

Field Surveys in Hawai'i and Species Identification

Field surveys of *M. polycaris* were conducted at Anaeho'omalū Bay (17 June 2019), Keaukaha (13 November 2019), Māhukona (07 November 2017), and Kawaihae (07 November 2017) on Hawai'i Island. Surveys at Anaeho'omalū Bay, Māhukona, and Kawaihae were not timed and involved haphazard walks to floral resources where bees were foraging and where there was evidence for leaf cutting by Megachilid bees between 10:00 and 17:00 hours. In our study, we define a haphazard walk as the investigator walking in an area of <1 ha and observing bees foraging on blooming plants. *Megachile polycaris* that were observed foraging on flowers were collected with an insect net and placed in 1.5 ml microcentrifuge tube filled with 70% EtOH. Evidence for leaf cutting behavior was documented on damaged leaves with the characteristic half-circle cuts by Megachilid bees on ornamental *Bougainvillea* sp. Comm. Ex Juss. (Nyctaginaceae) and *Hibiscus tiliaceus* L. (Malvaceae), both which were growing in the coastal areas we surveyed. However, we are uncertain of whether the damage to petals and leaves is by *M. polycaris* as these bees were not observed cutting leaves in our field observations, and other non-native Megachilid bees are documented in Hawai'i (Snelling 2003). At Keaukaha, bee activity was observed by the staff of a local business during a visit by the authors. The staff reported that they routinely observe bees attempting to live inside spigots attached to liquid nitrogen regulator valves. The staff reported that they frequently had to evacuate the valves to clear bees and associated materials. Upon inspection, it was clear that *M. polycaris* females were attempting to nest in the spigot of the valve as evidenced by leaf material and pollen provisions.

All bees collected in this study were kept in a cooler and eventually stored at -20°C until needed for species determination of field identifications and genetic analysis. Field species determinations of *M. polycaris* by the authors were confirmed by Dr. Terry Griswold of the United States Department of Agriculture – Agricultural Research Service – Pollinating Insect – Biology, Management, and Systematics Research Unit (PIRU) using reference material and taxonomic resources housed at the National Pollinating Insect Collection (NPIC) (Mitchell 1937, 1962, 1980; Michener 2007). Ten pinned specimens are curated into the NPIC whereas eight specimens are stored at -20°C at PIRU. All specimens have been assigned a unique accession number and are associated with the NPIC and PIRU (BBSL1161868-BBSL1161885).

Publicly Available Data

To describe the distribution of *M. polycaris* in its native range and data on available floral-host use, we downloaded 1864 records from 25

published datasets from the Global Biodiversity Information Facility (GBIF.org 2021). Georeferenced specimen records were mapped and described using *ggmap* in R (Kahle and Wickham 2013) (Fig. 2). No specific filters were made on the total dataset with the goal of getting a full sense of the geographic distribution and floral-host use of the available data. Finally, floral-host use in the GBIF dataset does not distinguish between plants being visited for pollen, nectar, or nesting materials. Thus, we do not distinguish between these possible routes of floral use in our study.

Genetic Study

Total DNA was extracted from the mid-leg of the field collected specimens using a Quick DNA Miniprep Kit (Zymo Research) following the manufacturer's protocol with modifications. For 18 specimens from Hawai'i, we amplified the 532 nucleotides of cytochrome oxidase I, a mitochondrial gene fragment, using the following primers: AP-L-2176 5'-GGTACAGGTTGAAGTGTTCACCC-3' (forward) and AP-H-2650 5'-TCCGACTGTAAATATGTGATGTGCTC-3' (reverse) (Penderson 1996). The total 25 µl reaction volume consisted of 1–2 µl of template DNA (~50 ng/µl), 1× Promega (Madison, WI) reaction buffer, 0.6 mM dNTP mixture, 0.2 µM of each primer, 0.2 units Taq Polymerase (Promega, Madison, WI), 2 mM of MgCl₂ (Promega, Madison, WI), and molecular grade H₂O to final volume. The PCR conditions for each reaction included one 4 min cycle at 94°C, 30 cycles of 94°C for 55 s, annealing temperature 52°C for 70 s, 72°C for 55 s, and a final extension period of 10 min at 72°C. PCR products were confirmed on a 1.4% agarose gel. Sequencing reactions were performed for both forward and reverse DNA strands (<http://etonbio.com>). We edited and assembled reads, and aligned the DNA sequences with Geneious Prime 2021.0.1 (<http://geneious.com>) (Kearse et al. 2012). We confirmed the absence of stop codons in the cytochrome oxidase I gene fragments with the Geneious Prime software by selecting for 'Invertebrate mitochondrial' under the 'Genetic Code' dropdown menu in the Translation option menu. Haplotype identification was conducted using the *pegas* library in R (Paradis et al. 2017).

Species Distribution Modeling

Approximately 37% ($n = 691$) of the *M. polycaris* records available on GBIF represented unique locality records associated with their native range in North America. The occurrence data were collected by a diversity of collectors at various localities across the native range of *M. polycaris*. All occurrence records were aggregated into 1 km² cells corresponding to the resolution of the environmental variables. Identical presence locations were removed and only one unique presence location was retained.

In our study, we applied 19 bioclimatic variables (derived from temperature and precipitation measures), with each variable averaged between 1970 and 2000, with a spatial resolution of 30 arcsec (~1 × 1 km), from the WorldClim 1.4 database (<http://www.worldclim.org/>) (Fick and Hijmans 2017). The variables used in our analysis are described in Table 1. To reduce multicollinearity among the bioclimatic variables, a principal component analysis was conducted to highlight the relationship between the target species occurrences and the specific environmental combinations within the archipelago. Variables were chosen based on orthogonal direction and overall environmental variation following strategies implemented in the BIOMOD2 package (Thuiller et al. 2009).

In our study, we used an ensemble modeling approach to estimate the distribution of *M. polycaris* in Hawai'i based on the environmental conditions from its native range following Tabor and Koch

Table 1. Bioclimatic variables evaluated to construct an *Megachile polcaris* species distribution model

Variable code	Variable description
BIO 1	annual mean temperature
BIO 2	mean diurnal range (mean of monthly (max temp - min temp))
BIO 3	temperature seasonality (standard deviation *100)
BIO 4	isothermality (BIO2/BIO7) (*100)
BIO 5	max temperature of warmest month
BIO 6	min temperature of coldest month
BIO 7	temperature annual range (BIO5-BIO6)
BIO 8	mean temperature of wettest quarter
BIO 9	mean temperature of driest quarter
BIO 10	mean temperature of warmest quarter
BIO 11	mean temperature of coldest quarter
BIO 12	annual precipitation
BIO 13	precipitation of wettest month
BIO 14	precipitation of driest month
BIO 15	precipitation seasonality (coefficient of variation)
BIO 16	precipitation of wettest quarter
BIO 17	precipitation of driest quarter
BIO 18	precipitation of warmest quarter
BIO 19	precipitation of coldest quarter

The variables were compiled from the WorldClim 1.4 database (<http://www.worldclim.org/>) (Fick and Hijmans 2017).

(2021). Ensemble models account for inter-model variability and uncertainty in the predictions, thus resulting in a more robust estimate of habitat suitability across a species distribution. Our ensemble modeling approach was conducted in the R environment v 3.6.1 (R Core Team, 2019) using the BIOMOD2 package (Thuiller et al. 2009). The algorithms used to produce an ensemble model were as follows: three regression methods (GAM: general additive model; GLM: general linear model; and MARS: multivariate adaptive regression splines), three machine learning methods (ANN: artificial neural network; GBM: generalized boosting model; and RF: random forest), two classification methods (CTA: classification tree analysis; FDA: flexible discriminant analysis) and one maximum entropy approach (MAXENT) (Phillips et al. 2006). With these models, we estimated the bioclimatic niche of *M. polcaris* throughout its native range and projected that condition onto its invasive distribution in Hawai'i. As background and absence data is not available for *M. polcaris*, we used 10,000 pseudo-absences randomly generated in the native range environmental space. The models were calibrated by using 80% of the occurrence points (presence and pseudo-absence) as training data and evaluated by using the remaining 20% as testing data (Freeman et al. 2018). We repeated the process of pseudo-absence generation three times and repeated evaluation runs four times for *M. polcaris*, resulting in a total of 108 models (nine models, four evaluation runs, and three pseudo-absence selection procedures) under a current climate (1970–2000) scenario.

Model validation and predictive performance for the *M. polcaris* ensemble model were evaluated with the area under the curve (AUC) of receiver operating characteristics and true skills statistics (TSS). The AUC value represents the predictive power of a model (Allouche et al. 2006). According to the AUC value, the model was graded as 'poor' (if AUC = 0.6–0.7), 'fair' (AUC = 0.7–0.8), 'good' (AUC = 0.8–0.9) or 'excellent' (AUC = 0.9–1.0) (Allouche et al. 2006). TSS measure ranges from –1 to +1 where +1 indicates a

perfect agreement, and a TSS value below 0.4 indicates poor model discrimination (Allouche et al. 2006). From the 108 models, we built ensemble models using a weighted-mean approach in which weights are awarded for each model proportionally to their evaluation metrics scores. Only the models with greater than fair predictive accuracy (TSS > 0.5) were used to build an ensemble from the projection outputs (Thuiller et al. 2009, Bellard et al. 2013).

Finally, binary maps that differentiated between 'suitable' and 'unsuitable' habitat were produced using the optimal threshold that maximizes the TSS score as a cut-off value, which then converted the projected occurrence probabilities during the cross-validation procedure. These binary maps were used to measure the range size of *M. polcaris* as represented by the number of climatically suitable pixels across the Hawaiian archipelago. R scripts are available on GitHub (<https://github.com/jesseat12/megachilepolcaris>).

Results

Field Surveys

Hawai'i surveys observed *M. polcaris* foraging on native and non-native plants to Hawai'i. Specifically, *M. polcaris* males and females were observed foraging on flowers of *Morinda citrifolia* L. (Rubiaceae) in Māhukona, Hawai'i and on flowers of *Heliotropium arboreum* (Blanco) Mabb. (Boraginaceae) in Anaeho'omalu, Hawai'i. Both of these plants are non-native to Hawai'i. However, *M. polcaris* were also observed foraging on flowers of *Sida fallax* Walp (Malvaceae) in Kawaihae, Hawai'i, a plant native to the archipelago. *M. polcaris* was consistently documented in Māhukona, Hawai'i by the authors from 2017 to 2019, foraging specifically on flowers of *M. citrifolia*. In fact, *M. polcaris* females was attempting to break open buds of *M. citrifolia* to acquire nectar as they were not fully bloomed. Finally, male and female *M. polcaris* were documented in Kamuela (~800 m above sea level) by W.D. Pereira and D.A. Yee foraging on *Senecio madagascariensis* Poir. (1817) (Asteraceae), a noxious weed to Hawai'i (Table 2).

Global Datasets and Documented Floral Hosts

In addition to the collections of *M. polcaris* made in our study, we identified seven 'Research Grade' human observations of the species on Maui, O'ahu, and Hawai'i Island captured via iNaturalist users and reported on GBIF (Fig. 2) (Table 3). According to iNaturalist, 'Observations become 'Research Grade' when the [iNaturalist] community agrees on species-level ID or lower, i.e., when more than 2/3 of identifiers agree on a taxon' (<https://www.inaturalist.org/>) (Table 3). *M. polcaris* documented in these iNaturalist observations present bee-plant interactions, date, and geographic data (Fig. 1). The earliest reported observation on iNaturalist was on 3 November 2018 and the most recent reported observation is 10 March 2020 (as of the writing of this manuscript). Of the 1864 records on GBIF, 385 records (20.7% of the data) had an associated floral-host record. These data were located on the 'associated taxa' column of the GBIF dataset. From the GBIF dataset, 21 plant families were identified as associated taxa of *M. polcaris* (Table 4).

Genetic Study

In total, 489 nucleotides of cytochrome oxidase I were amplified, aligned, and analyzed across 17 specimens collected throughout Hawai'i Island. Assessment of the sequences with Geneious Prime software detected no evidence for any stop codons in the cytochrome oxidase I fragments that were sequenced. Of the 17 specimens examined, we identified two haplotypes (Table 2). However,

Table 2. *Megachile polycaris* observed and collected on Hawaii Island from 2017 to 2019

BBSL accession #	GenBank accession #	Location	Host	Behavior	Date	Collector	Haplotype Group	Codon #105	Identified By
BBSL1161868	MZ720773	Kohanaiki	<i>Heliotrope arboreum</i>	Foraging	13-Jun-19	J. Koch & J. Tabor	1	CAT	T. Griswold
BBSL1161869	MZ720774	Hapuna	<i>Heliotrope arboreum</i>	Foraging	13-Jun-19	J. Koch & J. Tabor	1	CAT	T. Griswold
BBSL1161870	MZ720775	Hapuna	<i>Heliotrope arboreum</i>	Foraging	13-Jun-19	J. Koch & J. Tabor	1	CAT	T. Griswold
BBSL1161871	MZ720776	Hapuna	<i>Heliotrope arboreum</i>	Foraging	13-Jun-19	J. Koch & J. Tabor	2	CAC	T. Griswold
BBSL1161872	MZ720777	Hapuna	<i>Heliotrope arboreum</i>	Foraging	13-Jun-19	J. Koch & J. Tabor	1	CAT	T. Griswold
BBSL1161873 ^a	-	Māhukona	<i>Morinda citrifolia</i>	Foraging	13-Jun-19	J. Koch & J. Tabor	-	-	J. Koch
BBSL1161874	MZ720765	Anaeho'omalu	<i>Heliotrope arboreum</i>	Foraging	12-Jul-19	J. Koch	1	CAT	J. Koch
BBSL1161875	MZ720766	Anaeho'omalu	<i>Heliotrope arboreum</i>	Foraging	12-Jul-19	J. Koch	2	CAC	J. Koch
BBSL1161876	MZ720767	Anaeho'omalu	<i>Heliotrope arboreum</i>	Foraging	12-Jul-19	J. Koch	1	CAT	J. Koch
BBSL1161877	MZ720768	Anaeho'omalu	<i>Heliotrope arboreum</i>	Foraging	12-Jul-19	J. Koch	1	CAT	J. Koch
BBSL1161878	MZ720769	Anaeho'omalu	<i>Heliotrope arboreum</i>	Foraging	12-Jul-19	J. Koch	1	CAT	J. Koch
BBSL1161879	MZ720770	Keaukaha	-	Nesting	30-Nov-19	J. Koch	1	CAT	J. Koch
BBSL1161880	MZ720771	Keaukaha	-	Nesting	30-Nov-19	J. Koch	1	CAT	J. Koch
BBSL1161881	MZ720772	Anaeho'omalu	<i>Sida fallax</i>	Foraging	12-Jul-19	J. Koch	1	CAT	J. Koch
BBSL1161882	MZ720778	Māhukona	<i>Morinda citrifolia</i>	Foraging	11-Aug-17	J. Koch	1	CAT	T. Griswold
BBSL1161883	MZ720779	Kamuela	<i>Senecio madagascariensis</i>	Foraging	5-Oct-19	W.D. Perreira & D.A. Yee	1	CAT	W.D. Perreira & D.A. Yee
BBSL1161884	MZ720780	Kamuela	<i>Senecio madagascariensis</i>	Foraging	5-Oct-19	W.D. Perreira & D.A. Yee	1	CAT	W.D. Perreira & D.A. Yee
BBSL1161885	MZ720781	Hawi	-	-	3-Sep-17	D. Zarders	1	CAT	D. Zarders

BBSL represents accession number of specimens curated at the National Pollinating Insect Collection (pinned specimen) or USDA-ARS Pollinating Insect Research Unit (specimen held at -20°C).
^aCytochrome oxidase I gene fragment did not amplify in BBSL1161873 specimen.

Table 3. *Megachile polycaris* human observations (non-museum deposited) reported on GBIF (<http://gbif.org>)

GBIF ID	Locality Reported	Latitude	Longitude	Behavior	Date	Rights Holder/ Observer	Identifier on iNaturalist	Link to observation
3018124357	Hawaii Kai, Honolulu, HI, USA	21.304389	-157.6514	Foraging	7-Nov-19	Peter Edelman	John Ascher	https://www.inaturalist.org/observations/36485547
3018108328	Kalaoa, HI 96740, USA	19.67595	-156.02279	Foraging	3-Nov-18	Steve Wells	John Ascher	https://www.inaturalist.org/observations/18083662
3018106387	Kealia Pond National Wildlife Refuge, Maui, Hawaii, USA	20.7951	-156.46616	Foraging	17-Oct-19	Sea-kangaroo	John Ascher	https://www.inaturalist.org/observations/35203386
3017950322	Hawaii County, HI, USA	20.22776	-155.74538	Foraging	21-Nov-18	Steve Wells	John Ascher	https://www.inaturalist.org/observations/18550419
2883053096	Waikoloa Village, HI, USA	19.913051	-155.79944	Foraging	10-Mar-20	kbbkash	kbbkash	https://www.inaturalist.org/observations/61629171
2802571376	Kahului, HI, USA	20.893513	-156.45103	Foraging	16-Feb-19	John Starmer	John Ascher	https://www.inaturalist.org/observations/20427517
1945462011	5022 Makena Rd, Wailea-Makena, HI, US	20.656116	-156.44245	Foraging	20-Nov-18	Lisa Hopp Robinson	John Ascher	https://www.inaturalist.org/observations/18533150

Table 4. Number of *Megachile polycaris* interactions documented across 21 plant families on GBIF (<http://gbif.org>)

Plant Family	# of interactions reported on GBIF
Acanthaceae	9
Amaranthaceae	2
Apocynaceae	1
Aquifoliaceae	1
Areaceae	1
Asteraceae	271
Boraginaceae	9
Brassicaceae	10
Cactaceae	7
Convolvulaceae	1
Fabaceae	12
Lamiaceae	15
Malvaceae	9
Papaveraceae	1
Rosaceae	2
Rubiaceae	1
Salicaceae	1
Solanaceae	1
Tamaricaceae	3
Verbenaceae	18
Zygophyllaceae	6

the two haplotypes detected in our study exhibited a difference in only one base pair, found at the third nucleotide position of a codon for Histidine at nucleotide position #316 on the aligned sequences (i.e., codon position #105). Haplotype 1 was documented in 2017 and 2019 across multiple locations on Hawai'i Island (Table 2). Finally, in addition to species confirmation with museum specimens deposited in the National Pollinating Insect Collection, we observed 100% identity with 324 overlapping nucleotides between our 17 sequences and a cytochrome oxidase I sequence of an *M. polycaris* specimen (HM422942) deposited on NCBI. Sequences generated in our study are deposited into GenBank at National Center for Biotechnology Information (NCBI) (<https://www.ncbi.nlm.nih.gov/genbank/>). GenBank accession numbers are provided in Table 2.

Species Distribution Modeling

Following our PCA-approach to selecting variables to construct SDMs, we identified a combination of four bioclimatic variables to construct the final SDMs: mean diurnal range (BIO 2), temperature seasonality (BIO 4), mean temperature of coldest quarter (BIO 11), and annual precipitation (BIO 12) (Fig. 3). Our goal in variable selection was to capture seasonal and annual variation (as opposed to single month extremes) among the correlated variables in our analysis. Mean diurnal range captures the mean difference between the highest and lowest recorded temperature within a single day of a study region. Thus, larger values of mean diurnal range estimates suggest a greater difference between day and night time temperatures. Temperature seasonality captures the difference or amplitude between the highest and lowest recorded temperature across a year. Therefore, larger values of temperature seasonality imply that the climate of a study region gives rise to greater seasonal variation than smaller values of temperature seasonality. Although there is a degree of variability across the nine modeling approaches, *M. polycaris* habitat suitability (HS) decreased as mean diurnal range increased to ~15°C, but then HS increased again as mean diurnal range increased towards ~20°C. Regarding temperature

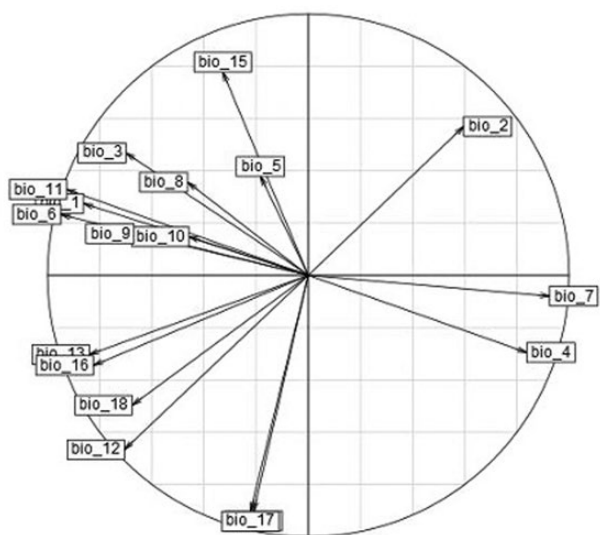


Fig. 3. Correlation circle of 19 bioclimatic variables used to identify four variables for the construction of the *Megachile polycaris* species distribution model as a function of principal components analyses implemented in BIOMOD2 (Thullier et al. 2009).

seasonality, *M. polycaris* HS increased with increasing temperature seasonality, and then remained relatively stable or declined after approaching ~65%, depending on the modeling approach employed. Furthermore, *M. polycaris* HS increased as the mean temperature of the coldest quarter increased towards ~10°C, and then HS declined as temperatures exceeded 10°C. Upon examination of the behavior of HS with the annual precipitation variable, it was evident that *M. polycaris* HS increased as annual precipitation increased to 1000 mm, however, HS decreased when annual precipitation exceeded 1000 mm. Figures for all relationships between bioclimatic variables and HS across the nine modeling approaches are available in [Supp Fig. 1 \[online only\]](#).

Based on AUC and TSS, the *M. polycaris* models exhibited high predictive accuracy across all nine modeling approaches ([Fig. 4](#)). Specifically, average AUC = 0.908 and average TSS = 0.627, suggests good predictive performance. Based on occurrence records associated with their native range ([Fig. 2A](#)), the ensemble SDM projects high HS on the leeward sides of the islands across the Hawaiian archipelago ([Fig. 2B](#)). However, on the windward sides of the islands, *M. polycaris* HS is predicted to be low. Furthermore, high elevation leeward locations on Hawai'i Island are estimated to exhibit high *M. polycaris* HS. Finally, binary maps produced in this modeling exercise demonstrated *M. polycaris* to have a high likelihood of HS across ~11% (2168 total pixels) of the terrestrial ecosystem in Hawai'i ([Fig. 2C](#)).

Discussion

Identifying native from non-native Hymenoptera is an important consideration for land management and conservation in Hawai'i. Complete taxonomic studies such as [Daly and Magnacca \(2003\)](#) and [Snelling \(2003\)](#) have established a framework for identifying both native *Hylaesus* and exotic bees respectively. Although many species are easy to distinguish from each other morphologically, there are some species such as *Ceratina arizonensis* (Cockerell, 1898) (Hymenoptera: Apidae) that may be mistaken for native *Hylaesus* species at first glance.

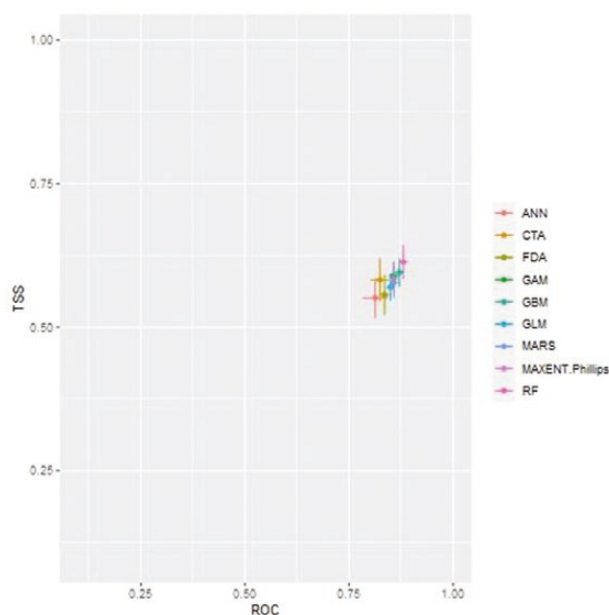


Fig. 4. Relationship between two measures of species distribution model predictive performance, the true skills statistic (TSS) and receiver operating characteristic (AUC) across nine modeling approaches (ANN = artificial neural network, CTA = classification tree analysis, FDA = flexible discriminant analysis, GAM = general additive model, GBM = generalized boosting model, GLM = general linear model, MARS = multivariate adaptive regression splines, MAXENT.Philips = maximum entropy approach, RF = random forest). Bars represents standard error.

Therefore, a taxonomic key by Snelling provided a framework for identifying many of the non-native species present in the Hawaiian Islands including *A. mellifera*, *C. dentipes* (Fries, 1914) (Hymenoptera: Apidae), *C. arizonensis*, *C. smaragdula* (Fabricius, 1787) (Hymenoptera: Apidae) and *X. sonarina* (Hymenoptera: Apidae) of the family Apidae. Also included were *Lithurgus scabrosus* (Fries, 1914) (Hymenoptera: Megachilidae), *M. diligens* (Smith, 1879) (Hymenoptera: Megachilidae), *M. fullawayi* (Cockerell, 1914) (Hymenoptera: Megachilidae), *M. gentilis* (Cresson, 1872) (Hymenoptera: Megachilidae), *M. chlorura* (Cockerell, 1918) (Hymenoptera: Megachilidae), *M. timberlakei* (Cockerell, 1920) (Hymenoptera: Megachilidae) and *M. umbripennis* (Smith, 1853) (Hymenoptera: Megachilidae) from the family Megachilidae as well *Lasioglossum impavidum* (Sandhouse, 1924) (Hymenoptera: Halictidae) from the family Halictidae ([Snelling 2003](#)). Additionally, non-native Colletidae *H. albonitens* (Cockerell, 1905) (Hymenoptera: Colletidae) and *H. leptoccephalus* (Morowitz, 1871) (Hymenoptera: Colletidae) were included in [Snelling \(2003\)](#). Moreover, recently introduced Colletidae *H. strenuus* (Cameron, 1897) (Hymenoptera: Colletidae) and *H. bisinuatus* Forster, 1871 (Hymenoptera: Colletidae) have also been found in Hawai'i. However, it's important to note that *H. bisinuatus* has only been collected twice in Hawai'i and has not been collected since 1994. Therefore, it is unlikely to have established populations ([Daly and Magnacca 2003](#)). Additionally, *L. imbrex* Gibbs 2010 (Hymenoptera: Halictidae), *L. microlepidoides* (Ellis, 1914) (Hymenoptera: Halictidae), and *L. puteulanum* (Gibbs, 2009) (Hymenoptera: Halictidae) of the family Halictidae have all been recently discovered in Hawai'i ([Magnacca et al. 2013](#)). However, since the publication of the [Snelling \(2003\)](#) taxonomic key, there have been a number of newly

discovered arrived non-native bees in Hawai'i that necessitate revisions to the key. Those additions include *M. lanata* (Fabricius, 1775) (Hymenoptera: Megachilidae) (Magnacca et al. 2013) and the study species, *M. polycaris*, which was not documented in Magnacca et al. (2013).

Based on available public data on GBIF, *M. polycaris* has been documented to forage on 21 flowering plant families, as well as numerous genera within each plant family. The capacity to forage on different types of flowering plant families positions *M. polycaris* to take advantage of novel flowering plant resources in its invasive range in Hawai'i. In our study, the plants we observed *M. polycaris* foraging on for nectar and pollen include *M. citrifolia* L. (non-native to Hawai'i), *S. madagascariensis* (Asteraceae) (non-native to Hawai'i), *H. arboreum* (non-native to Hawai'i), and *Sida fallax* (native to Hawai'i). All four plant species are not found in the native distribution of *M. polycaris*. However, they do belong to plant families that *M. polycaris* has been documented to forage upon in their native distribution. Specifically, one observation with *Hedyotis* (Rubiaceae), nine observations with *Phacelia* (Boraginaceae), one observation with *Callirhoe* (Malvaceae), and eight observations with *Sphaeralcea* (Malvaceae) (Table 4). All observations of *M. polycaris* by the authors occurred at flowering plants near sea-level along the coastline of Hāpuna, Māhukona, and Kohanaiki.

The ensemble SDM suggests that across all islands, the leeward side demonstrates the highest HS of *M. polycaris* (Fig. 2B). This SDM was based on four bioclimatic variables that summarize mean diurnal range, temperature seasonality, mean temperature of coldest quarter, and annual precipitation (Supp Fig. 1 [online only]). In our study, we report on the presence of *M. polycaris* primarily from habitats where the ensemble SDM predicts the species to occur on Hawai'i Island (Table 2) (Fig. 2B). However, *M. polycaris* has been detected on the windward side of Hawai'i Island as well. More standardized surveys of the species should be performed to determine whether *M. polycaris* has the capacity to invade habitats that fall outside of the bioclimatic niche they have invaded in Hawai'i. A recent study by Tabor and Koch (2021) demonstrated that SDMs that estimated HS of a bee species' invasive range performed better with records from the invasive range as opposed to the native range. Such a phenomenon would not be uncommon, given that invasive species, including bees, have demonstrated their capacity to invade novel bioclimatic niches (Strange et al. 2011, Graham et al. 2018). Based on their North American distribution, *M. polycaris* HS increases as annual precipitation approaches 1000 mm. However, HS declines after surpassing 1000 mm (Supp Fig. 1 [online only]). The windward side of the Hawaiian archipelago can receive a significant amount of annual precipitation. For example, Keaukaha receives an average of 3244 mm of annual precipitation (Giambelluca et al. 2013), a site where *M. polycaris* were detected (Table 2). Thus, even though high annual precipitation is associated with low *M. polycaris* HS in their native niche, it is clear that the annual precipitation may not be an effective predictor of invasive *M. polycaris* populations in Hawai'i. Thus, while bioclimatic variable-informed SDMs are useful in predicting the spread of an invasive species, it is critical to explore additional limiting factors such as nesting and floral resources that may predict the distribution of an invasive species in a novel environment.

In our study, we detected two haplotypes of *M. polycaris* (Table 2). Haplotype 1 was documented on both the windward and leeward side of Hawai'i Island, whereas haplotype 2 was only documented on the leeward side of Hawai'i Island. Furthermore, we documented haplotype 1 in 2017 and 2019 and haplotype 2 in 2019. Based on the available data, we suggest that there have been at least two founders, and potentially two founding events, that gave way to the invasive populations of

M. polycaris in Hawai'i. However, more extensive sampling of invasive populations and molecular work is needed to assess the invasion history of *M. polycaris* to Hawai'i. To our knowledge, *M. polycaris* is a recent invader, and was first documented in 2017 on Hawai'i Island. iNaturalist observations of *M. polycaris* have been made as early as 2018, also on Hawai'i Island (Table 3). As the species is large in body size and has conspicuous features (e.g., males have forelegs with long setae), it is likely that *M. polycaris* will continue to be documented on platforms like iNaturalist. Furthermore, it is also clear that the species can be identified by trained taxonomists through photograph evidence (Fig. 1 and Table 3), which will contribute immensely to the utility of iNaturalist to provide data on understudied insect species (Wilson et al. 2020).

In conclusion, we present evidence for the establishment of *M. polycaris* across the Hawaiian archipelago and their capacity to use novel floral resources to supplement their diet. Furthermore, based on a fragment of cytochrome oxidase I, we have detected at least two distinct haplotypes of *M. polycaris* present on Hawai'i Island. Haplotype 1 has been detected multiple years, suggesting the species establishment in Hawai'i. Finally, ensemble SDMs of *M. polycaris* based on occurrence records and bioclimatic data from their historic range suggest that HS is greatest on the leeward side of the archipelago and across low elevation and high elevation habitats. However, it is also clear that our modeling approach may not capture the true flexibility of the species to invade novel bioclimatic niches outside of their native range. The results of our study contribute to the knowledge of non-native bee ecology and persistence in Hawai'i and sets the pace for continued research on *M. polycaris* across its native and non-native distribution.

Supplementary Data

Supplementary data are available at *Journal of Insect Science* online.

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Author Contributions

JBUK: Conceptualization; Data Curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Writing – original draft; Writing – reviewing & editing. JAT: Data Curation; Formal analysis; Investigation; Methodology; Writing – original draft; Validation; Visualization; Writing – original draft; Writing – reviewing & editing. KM-A: Investigation; Writing – original draft; Writing – reviewing & editing. JAE: Investigation; Resources; Writing – reviewing & editing.

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