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Integrative taxonomy reveals multiple cryptic species within Central American *Hylomyrma* FOREL, 1912 (Hymenoptera: Formicidae)

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Abstract

Advances in molecular methodology allow for higher-resolution analyses of evolutionary relationships and species limits. To clarify species boundaries in the ant genus *Hylomyrma* FOREL, 1912 within Central America, we take an integrative approach to taxonomy, combining data from thousands of nuclear loci (Ultra-Conserved Elements), the mitochondrial gene Cytochrome Oxidase I, and morphological traits. We reveal potential cryptic species within named taxa and two morphologically distinct new species: *Hylomyrma montana* and *H. plumosa*. *Hylomyrma montana* is the first definitive montane species in the genus, with populations in Costa Rica, Panama, and the Pacific slope of Ecuador. We show that the Ecuadorian and Central American populations form a single clade and are not the result of convergent, montane specialization. We discuss our findings in relation to both montane diversification and Neotropical biogeography.

Key words: Biogeography, montane specialization, phylogenomics, target enrichment, new species.

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Introduction

Hylomyrma FOREL, 1912 is an exclusively Neotropical ant genus that occurs from southern Mexico to Argentina. Originally described as a subgenus within *Pogonomyrmex* MAYR, 1868, it was elevated to generic status by WHEELER (1922). *Hylomyrma* has always been considered a close relative of *Pogonomyrmex*, and this was convincingly demonstrated in recent molecular studies (BRADY & al. 2006, WARD & al. 2015, BRANSTETTER & al. 2017). The only thorough species-level revision was by KEMPF (1973), in which twelve species were recognized. The genus has received little attention since Kempf's revision, with only one additional species being described (KUTTER 1977). Despite there being few studies, there is little doubt of the monophyly of *Hylomyrma*.

Little is known about the biology and natural history of *Hylomyrma*. The genus is restricted to wet forests, mostly lowland rainforest, where it is a member of the cryptic leaf litter ant community. It is known almost exclusively from Winkler and Berlese samples (J.T. Longino, unpubl.), and observations of nesting or foraging habits are extremely limited. A colony was discovered by E.O. Wilson in Veracruz, Mexico, and kept in an artificial nest for observation. He observed that "In captivity workers of this species captured *Drosophila* spp., *Isotoma viridis* BOURLET [Collembola], and a few other small insects offered them in the food chamber, and fed them directly to the larvae" (quoted in WHEELER & WHEELER 1960: p. 4). As far as we know, this is still the only published reference to a colony of *Hylomyrma*. At the time of the 1973 revision, Kempf had perhaps fewer than 100 specimens to examine. Since that time, mass collecting techniques and large faunal surveys, mainly in Central America, have yielded thousands of specimens from many localities. Although basic features of feeding and nesting remain unknown, progress can be made on understanding species boundaries and habitat preferences. Here, we focus on resolving species boundaries within Central American *Hylomyrma*.

The greater part of *Hylomyrma* diversity is found in South America, with Central America historically being known to have only two species: *H. dentiloba* (SANTSCHI, 1931) and *H. versuta* KEMPF, 1973. At the time of his revision, Kempf had only a handful of collections from all of Central America. There were a few collections from Veracruz (Mexico) and Belize, which he described as the new species *H. versuta*. There were the types of *H. dentiloba* from central Panama and a conspecific collection from nearby Barro Colorado Island. A single specimen from near Bluefields, Nicaragua, was described as being somewhat morphologically intermediate, and Kempf entertained the possibility that *H. versuta* and *H. dentiloba* were part of a single geographically variable species. Longino failed to find any consistent morphological differences between *H. dentiloba* and *H. versuta*, and in faunal studies (e.g., LONGINO & COLWELL 2011) he treated *H. versuta* as an informal junior synonym of *H. dentiloba*.

In those faunal studies, Longino also recognized a cloud forest morphospecies of *Hylomyrma* in Costa Rica (as *Hylomyrma* JTL001). On an elevational gradient (the Barva

Transect) it was present in mid-elevation cloud forest and sharply parapatric with the common lowland *H. versuta*. The cloud forest form was subtly but consistently different from the lowland species, differing in some details of surface sculpture. More recently, we reviewed *Hylomyrma* specimens from Ecuadorian cloud forest and found that they had the same characters as the Costa Rican cloud forest form. This raised the question of whether the two cloud forest populations might represent a single species, or at least allopatric members of a single lineage of cloud forest specialists. The Costa Rican faunal studies also revealed a second morphospecies (*H.* JTL002), sympatric with *H. versuta* in the Atlantic lowlands. It differed only in the presence of distinctive plumose pilosity and shorter striations on the first gastral tergite, and otherwise was identical to the common *H. versuta*. It was uncertain whether the difference in pilosity was due to underlying species differences or some form of intraspecific variation (polyphenism or genetic polymorphism).

To improve upon the taxonomy of Central American *Hylomyrma* and to examine the potential biogeographic connection between Central and South American cloud forest sites, we employed an integrative approach to taxonomy (sensu SCHLICK-STEINER & al. 2010), combining molecular phylogenetics, morphology, and biogeography. *Hylomyrma* species are morphologically similar across a wide geographic range, and sequence data help clarify species boundaries and biogeographic history. This is particularly relevant to investigating the species status of the newly discovered montane morphospecies, for which morphological data alone are insufficient. A major goal of our study was to use molecular data to evaluate the alternative hypotheses of convergence or shared ancestry with regard to the montane morphospecies. We also sought to resolve species boundaries for the other Central American taxa, *H. dentiloba*, *H. versuta*, and the new plumose morphospecies *H.* JTL002.

For the molecular data, we used two approaches. We applied ultra-conserved element (UCE) phylogenomics to a set of ten specimens, which included the two populations of the cloud forest morphospecies, the plumose morphospecies, and multiple populations of Central American *H. dentiloba / versuta*. To gain further insight into species differences and to test the UCE results, we also skimmed mitochondrial Cytochrome Oxidase I (COI) barcode sequences from the off-target reads of UCE data, and we combined these with available barcode sequences for analysis. Finally, we integrated the molecular results with morphology and biogeography, evaluating congruence among them and the degree to which morphology can be used for species identification. Our results reveal potential cryptic species within *H. dentiloba* and provide justification for describing the montane and plumose morphospecies as new species: *Hylomyrma montana* and *H. plumosa*, respectively. *Hylomyrma montana* represents the first instance of montane specialization reported for the genus.

Methods

Material examined: A total of 1543 specimens of Central American *Hylomyrma* were examined, including the material used for morphological measurements (Fig. 1; Tab. S1, also including morphometric data, as digital supplementary material to this article, at the journal's web pages).

Repositories:

CAS California Academy of Sciences, San Francisco, CA, USA
JTLC John T. Longino, personal collection, University of Utah, Salt Lake City, UT, USA
MCZC Museum of Comparative Zoology, Cambridge, MA, USA
NHMB Natural History Museum of Basel, Basel, Switzerland
UCD University of California, Davis, CA, USA.
UCR Universidad de Costa Rica, San Pedro, Costa Rica
USNM National Museum of Natural History, Washington, DC, USA

Taxon sampling for sequencing: Ten *Hylomyrma* specimens and four outgroup species (*Myrmica* LATREILLE, 1804, *Manica* JURINE, 1807, *Pogonomyrmex*, *Patagonomyrmex* JOHNSON & MOREAU, 2016) were sequenced for UCEs (Fig. 2, Tab. 1). *Hylomyrma* specimens were three species from South America (*H. blandiens* KEMPF, 1961, *H. immanis* KEMPF, 1973, *H. reginae* KUTTER, 1977), four dispersed populations of *H. dentiloba / versuta* (from Costa Rica to Mexico), two specimens of the newly discovered cloud forest form (one from Costa Rica, one from Ecuador), and one specimen of the putative new species with plumose setae.

DNA sequence generation: To generate a highly supported phylogeny of *Hylomyrma*, we employed the UCE approach to phylogenomics (FAIRCLOTH & al. 2012), a target enrichment method that generates genome-scale data at a much lower cost than sequencing full-scale genomes. Although the core regions of UCEs are conserved, the flanking regions contain an increasing amount of sequence variability, permitting estimation of phylogenetic hypotheses at all levels of relatedness (FAIRCLOTH & al. 2012). UCEs have a noticeable advantage over other marker types. For one, the approach provides data from over 1,000 independent nuclear markers, which makes it robust to incomplete lineage sorting and other potential biases. Additionally, compared to mitochondrial genes (e.g., Cytochrome Oxidase I, COI), which are commonly used for species delimitation, UCEs do not have problems with pseudogenes, and are able to resolve both deep (e.g., BRANSTETTER & al. 2017), and shallow (e.g., WARD & BRANSTETTER 2017) divergences, with high confidence.

All UCE molecular work was performed following the UCE methodology described in BRANSTETTER & al. (2017). In brief, the following steps were performed: DNA extraction, library preparation, UCE enrichment using the ant-specific hym-v2 bait set (targets 2,524 loci), sample pooling (our samples were pooled into a set of 100 total samples), and sequencing on an Illumina HiSeq 2500 at the University of Utah genomics core facility.

UCE matrix assembly and analysis: After sequencing, the UCE data were demultiplexed by technicians from the University of Utah genomics core, and once received, the sequence data were cleaned, assembled, and aligned using the Phyluce package v1.5 (FAIRCLOTH & al. 2015) according to the process outlined in BRANSTETTER & al. (2017). To identify UCE contigs from the bulk of assembled contigs, we used the ant-specific hym-v2 bait file.

Following alignment, a Phyluce wrapper was used to trim poorly aligned regions using the program Gblocks (TALAVERA & CASTRESANA 2007). The program was run

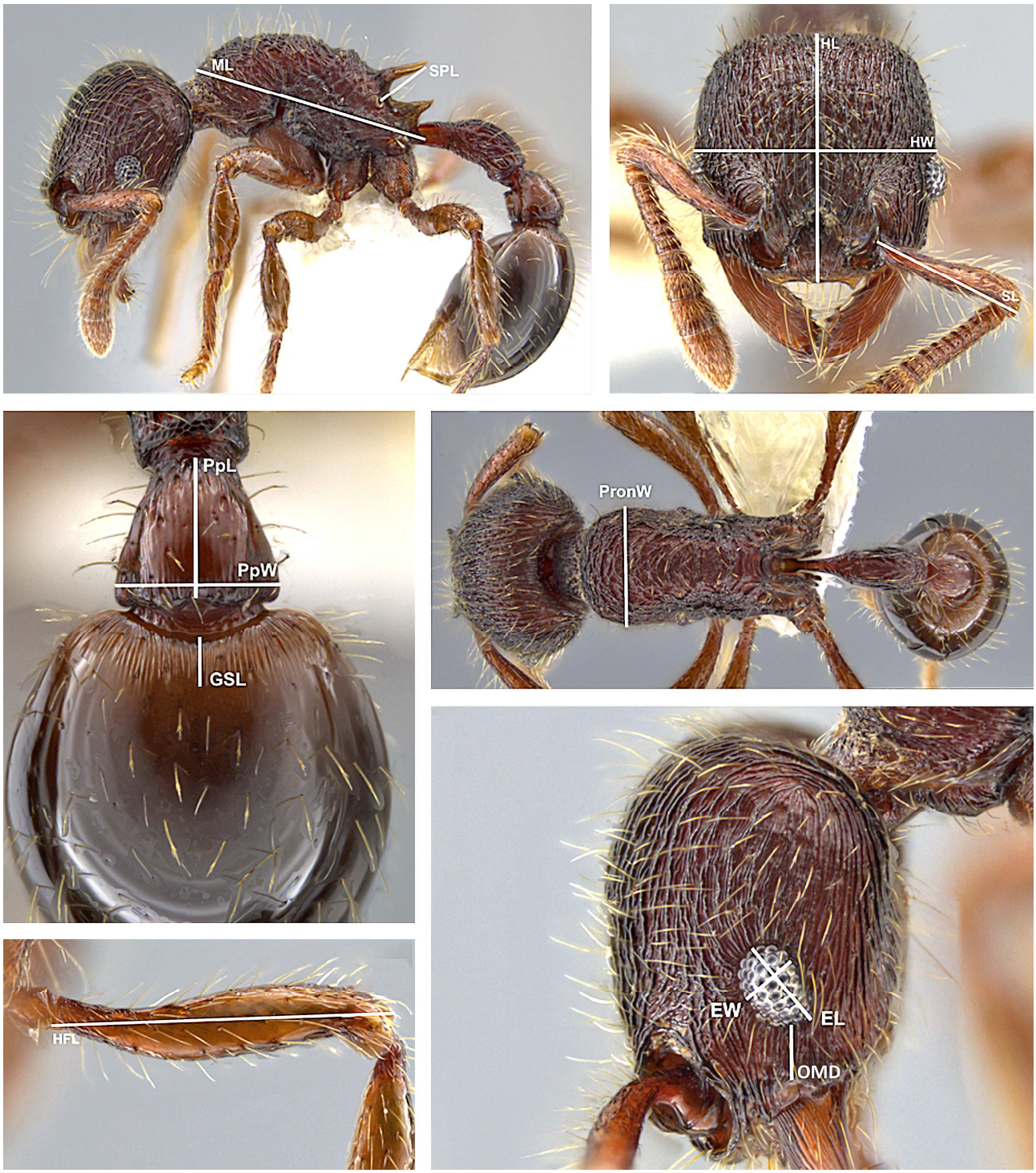

Fig. 1: Measurements included in morphological analysis, illustrated on a specimen of *Hylomyrma montana* (CASENT-0637306).

with reduced stringency parameters (b1:0.5, b2:0.5, b3:12, b4:7), because the default settings are overly conservative. We then used another script (phyluce_align_get_only_loci _with_min_taxa) to filter the initial set of alignments so that each alignment was required to include data for 100% of taxa. This resulted in a final set of 1,371 alignments (= loci) for analysis.

The filtered locus set was analyzed using the likelihood-based program RAxML v8.2 (STAMATAKIS 2014). A total of three concatenated analyses were performed in order to compare the results of different partitioning schemes: no partitioning, partitioning by locus, and partitioning under the hcluster algorithm in PartitionFinder v1.1.1 (LANFEAR & al. 2012; data pre-partitioned by locus). For each analysis, a best tree plus rapid bootstrap search was performed ("-f a" option; GTR + G model of sequence evolution; 100 bootstrap pseudoreplicates).

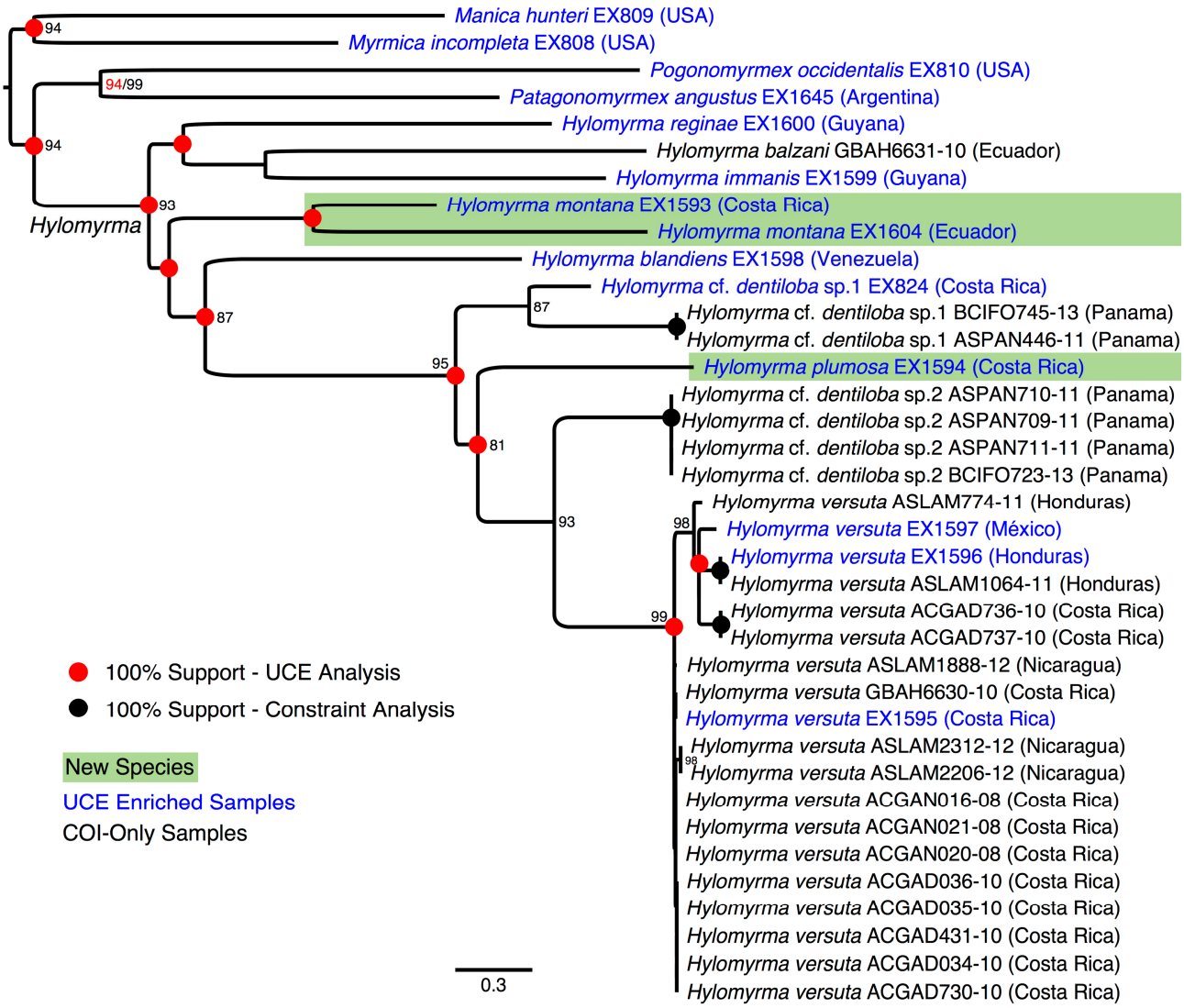

Fig. 2: Phylogeny of *Hylomyrma*, combining UCE and COI results. The tree was generated in RAxML by performing a constraint analysis in which placement of the taxa represented by COI data were constrained to the UCE topology. Red and black circles indicate nodes that received 100% bootstrap support in the UCE and constraint analyses, respectively. Red and black numbers indicate nodal support in the UCE and constraint analyses, respectively. Support values below 75% are not shown. Taxon names in blue were enriched for UCEs, whereas names in black represent COI samples only. Taxon names include the species name, either the extraction number ("EX") or BOLD accession number, and the country of origin. Taxa highlighted in green are new species described in this paper.

Raw Illumina reads and contigs representing UCE loci have been deposited at the NCBI Sequence Read Archive and GenBank, respectively (BioProject accession PRJNA-379610). The concatenated UCE matrix and all associated trees have been deposited at TreeBase (S20852).

COI matrix assembly and analysis: Due to the high abundance of mitochondrial DNA in samples, COI sequence data are generated as a byproduct of the UCE sequencing process (mtDNA is in effect naturally "enriched"). To extract COI sequences from UCE enriched samples, a COI sequence for *H. dentiloba* was taken from GenBank (GenBank Accesion#KC419949) and used as a bait to slice out matching sequences from the bulk of assembled contigs for each sample. These new COI sequences were combined with 23 *Hylomyrma* COI sequences available from the BOLD database (RATNASINGHAM & HEBERT 2007, Tab. 2). The combined data were aligned with MAFFT (KATOH & al. 2002), trimmed by eye to include only 657 bp from the barcode region, and then checked for evidence (e.g., indels, stop codons, etc) of nuclear pseudogenes (numts). The resulting matrix was partitioned by codon position and analyzed two different ways using RAxML: with and without a constraint tree. For the constraint analysis, the UCE topology was used as a fixed backbone. Both analyses were performed as best tree plus rapid bootstrap searches (GTR + G model of sequence evolution; 100 bootstrap pseudoreplicates).

The concatenated COI matrix and all associated trees have been deposited at TreeBase (S20852). The newly generated COI sequences have also been deposited at GenBank (Tab. 1).

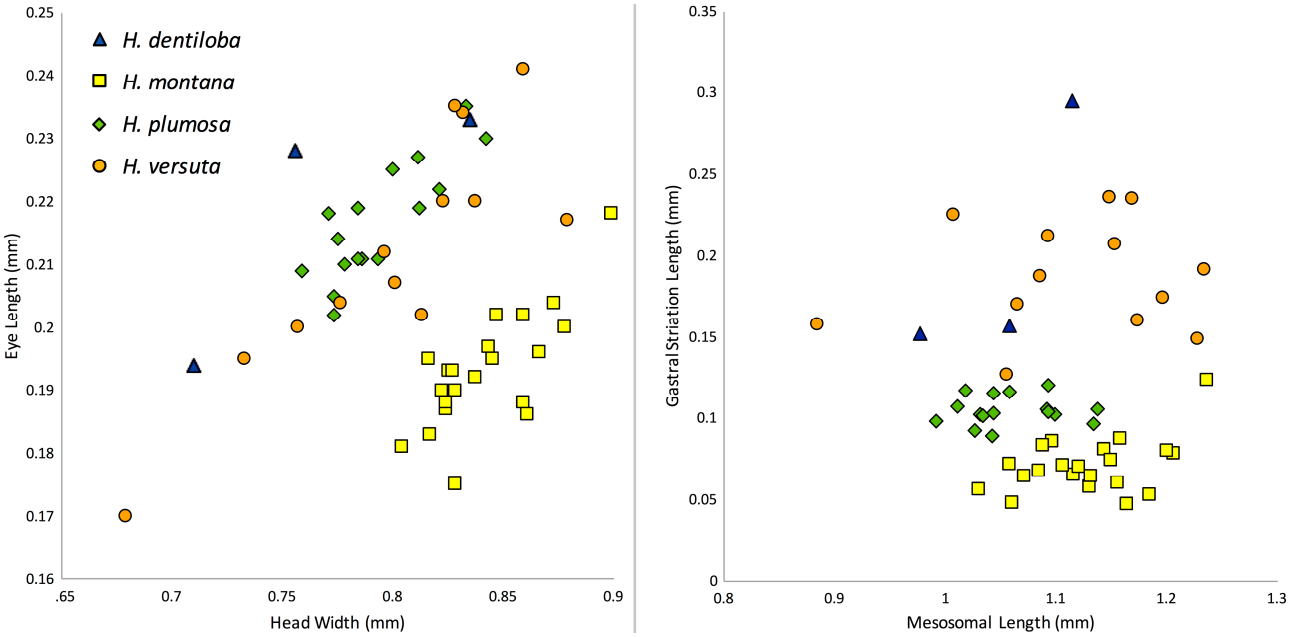

Fig. 3: Scatterplots of morphometric characters for Central American *Hylomyrma* species.

Tab. 1: Specimens used for UCE analysis. All specimens were adult workers. 1. Unique specimen identifier. Additional information at www.antweb.org. 2. Series = worker from same sample, but not necessarily nestmates. Specimen = same specimen, non-destructive DNA extraction. Nest = nestmate. * UCE data from BRANSTETTER & al. (2017).

Species	Extraction	Voucher[1]	Voucher type[2]	Country	Latitude	Longitude	COI GenBank #
Hylomyrma blandiens	EX1598	JTLC000015077	series	Venezuela	10.46097	-67.77406	KY907464
Hylomyrma immanis	EX1599	CASENT0637360	series	Guyana	5.01163	-59.64797	KY907458
Hylomyrma montana	EX1604	CASENT0633349	specimen	Ecuador	-0.39506	-78.981	KY907460
Hylomyrma montana	EX1593	CASENT0637306	series	Costa Rica	9.86732	-83.24131	KY907466
Hylomyrma plumosa	EX1594	INB0004100459	series	Costa Rica	10.40235	-84.03916	KY907463
Hylomyrma reginae	EX1600	CASENT0637359	series	Guyana	5.00917	-59.64047	KY907455
Hylomyrma versuta	EX1596	CASENT0617621	series	Honduras	15.48726	-88.23486	KY907461
**Hylomyrma* cf. *dentiloba* sp. 1	EX824	CASENT0636001	series	Costa Rica	8.40831	-83.32783	KY907459
Hylomyrma versuta	EX1595	INB0004099888	series	Costa Rica	10.41465	-84.02166	KY907462
Hylomyrma versuta	EX1597	JTLC000014425	series	Mexico	17.12347	-91.63816	KY907457
**Manica hunteri*	EX809	CASENT0633990	nest	United States	39.80893	-113.92689	KY907465
**Myrmica incompleta*	EX808	CASENT0635345	nest	United States	41.577	-111.56279	KY907456
**Patagonomyrmex angustus*	EX1645	CASENT0106125	nest	Argentina	-40.16667	-71.36667	KY907467
**Pogonomyrmex occidentalis*	EX810	CASENT0635633	nest	United States	39.7925	-113.24423	KY907454

Morphology: Eye. The shape, size, and relative location of the eye differs among species (Fig. 3a). It ranges from relatively small, round, and bluntly pointed anteriorly to larger, more elongate, lacking a blunt tip and instead forming a rounded lobe anteriorly.

Gaster striation. On tergum 1 of the gaster in all Central American species there are fine to coarse striations beginning at the postpetiolar insertion and continuing longitudinally toward the posterior margin (Figs. 1c, 4c). In some species, the striations are as long as or longer than the length of the postpetiole. In others, the striations are shorter, in some cases nearly absent.

Pilosity. *Hylomyrma* have abundant dorsal pilosity. In most species, the setae are simple, but in one species (*H. plumosa*) they are branched, usually trifid (Fig. 5c).

Measurements: A set of measurements was made for each species using a Leica MZ12.5 stereo-microscope at 63×. The list of measurements is presented below and illustrated in Figure 1. Two measurements in particular, eye length (EL) and gastral striation length (GSL), were most valuable for separating species. Measurements are in millimeters and are reported to three decimal places, but indistinct structure boundaries and differences in specimen orientation lead to a precision of about 0.03 mm (established by performing repeated measurements on a single specimen). Specimens collected from throughout the geographic range were measured to encompass geographic variation.

EL eye length; measured along the maximum diameter
EW eye width; maximum width perpendicular to EL

Tab. 2: Specimens used for DNA barcode sequences, exclusive of specimens in Table 1.

Species	BOLD #	GenBank #	Country	Latitude	Longitude
Hylomyrma balzani	GBAH6631-10	FJ824420	Ecuador	1.2861	-77.8906
Hylomyrma cf. *dentiloba* sp. 1	ASPAN446-11	n/a	Panama	6.1000	-79.8300
Hylomyrma cf. *dentiloba* sp. 1	BCIFO745-13	n/a	Panama	9.1547	-79.8481
Hylomyrma cf. *dentiloba* sp. 2	ASPAN709-11	n/a	Panama	6.1000	-79.8300
Hylomyrma cf. *dentiloba* sp. 2	ASPAN710-11	n/a	Panama	6.1000	-79.8300
Hylomyrma cf. *dentiloba* sp. 2	ASPAN711-11	n/a	Panama	6.1000	-79.8300
Hylomyrma cf. *dentiloba* sp. 2	BCIFO723-13	n/a	Panama	9.1547	-79.8481
Hylomyrma versuta	ACGAD034-10	HM919650	Costa Rica	10.9720	-85.4860
Hylomyrma versuta	ACGAD035-10	HM919651	Costa Rica	10.9720	-85.4860
Hylomyrma versuta	ACGAD036-10	KC417791	Costa Rica	10.9720	-85.4860
Hylomyrma versuta	ACGAD431-10	HQ545724	Costa Rica	10.8470	-85.3270
Hylomyrma versuta	ACGAD730-10	HQ545993	Costa Rica	10.7890	-85.3490
Hylomyrma versuta	ACGAD736-10	HQ545999	Costa Rica	10.7890	-85.3490
Hylomyrma versuta	ACGAD737-10	HQ546000	Costa Rica	10.7890	-85.3490
Hylomyrma versuta	ACGAN016-08	KC419074	Costa Rica	10.9690	-85.3180
Hylomyrma versuta	ACGAN020-08	KC417531	Costa Rica	10.9690	-85.3180
Hylomyrma versuta	ACGAN021-08	KC419949	Costa Rica	10.9690	-85.3180
Hylomyrma versuta	GBAH6630-10	FJ824421	Costa Rica	10.4167	-84.0667
Hylomyrma versuta	ASLAM1064-11	n/a	Honduras	15.4870	-88.2350
Hylomyrma versuta	ASLAM774-11	JN283739	Honduras	14.8710	-87.9000
Hylomyrma versuta	ASLAM1888-12	n/a	Nicaragua	12.6720	-83.7160
Hylomyrma versuta	ASLAM2206-12	n/a	Nicaragua	12.9580	-85.2250
Hylomyrma versuta	ASLAM2312-12	n/a	Nicaragua	12.9640	-85.2330

GSL gastral striation length; in dorsal view, the maximum length of the basidorsal striations on gastral tergum I
HFL hind femur length; in anterior view, maximum length of hind femur
HL head length; in full face view, the length of the head capsule excluding the mandibles, measured in a straight line from the mid-point of the anterior clypeal margin to the mid-point of the posterior margin
HW head width; in full face view, the maximum width of the head excluding the eyes
ML mesosomal length; in profile view, the diagonal length of the mesosoma from the point at which the pronotum meets the cervical shield to the posterior basal angle of the metapleuron
OMD oculomandibular distance; minimum distance between the anterior border of the eye and the anterior border of the head capsule as seen in lateral view
PpL postpetiole length; in dorsal view, the length of the postpetiolar node measured from anterior edge to posteriormost border along midline
PpW postpetiole width; in same view as and perpendicular to PpL, the maximum width of postpetiole
PronW pronotum width; in dorsal view, the maximum width of the pronotum
SL scape length; the maximum straight-line length of the scape, excluding the basal constriction that occurs just distal of the condylar bulb
SPL propodeal spine length; in profile view, the distance from the inner posterior margin of the propodeal spiracle to the propodeal spine apex

Indices:

CI cephalic index; 100 × HW / HL
GSI gastral striation index; 100 × GSL / ML
OI ocular index; 100 × EL / HW
PpWI postpetiole width index; 100 × PpW / PpL
SI scape index; 100 × SL / HW
SPI propodeal spine index; 100 × SPL / HW

Results

UCE phylogeny: After sequencing, assembly, and the extraction of contigs representing UCE loci, we recovered an average per contig coverage of 36x and a mean contig length of 944 bp (Tab. S2). Following alignment, trimming, and filtering of the UCE contigs, our UCE matrix consisted of 1,374 loci and 1,267,360 bp of sequence data, of which 167,340 bp were informative (Tab. S3). The matrix included only 8.8% missing data (including gaps).

The three specimens of *Hylomyrma dentiloba* / *versuta* from Mexico, Honduras, and the Caribbean side of Costa Rica form a clade that is the sister group to the plumose

morphospecies found sympatrically in Costa Rica (Figs. 2, S1). These in turn are sister to a *H. dentiloba / versuta* specimen from the Osa Peninsula on the Pacific side of Costa Rica. The sister group to this *H. dentiloba* clade is *H. blandiens* from Venezuela. The two cloud forest specimens from Costa Rica and Ecuador form a clade separate from the other Central American specimens. *Hylomyrma immanis* and *H. reginae*, both from South America, form a clade and fall outside the other eight sequenced *Hylomyrma* specimens.

COI phylogeny: A total of 23 unique COI sequences were taken from BOLD, resulting in a final COI matrix of 37 specimens, 657 bp of aligned sequence, and only 3.4% missing data (Tab. S3). The two COI phylogenies (constrained and unconstrained) have poor support at deeper nodes, but are congruent with the UCE phylogeny in terms of species boundaries (Figs. 2, S2). The two montane specimens cluster together, separate from the *Hylomyrma dentiloba* / *versuta* clusters. There are four specimen clusters that together form a well-supported *H. dentiloba / versuta* clade in both the constrained (Fig. 2) and unconstrained (Fig. S2) COI phylogeny: *H.* cf. *dentiloba* sp. 1, *H.* cf. *dentiloba* sp. 2, *H. plumosa*, and *H. versuta*. However, the relationships among the four clusters differs between the two phylogenies. In the unconstrained phylogeny, the support values for the two subclades are low (22% and 62%) and we disregard these relationships, instead relying on the constrained tree for further conclusions. In the constrained tree, the relationships are (*H.* cf. *dentiloba* sp. 1 (*H. plumosa* (*H.* cf. *dentiloba* sp. 2, *H. versuta*))). The BOLD sequences from Panama were all from Barro Colorado Island (BCI) and separated into two clusters, suggesting two sympatric species there. BCI is close to the type locality of *H. dentiloba*, and thus true *H. dentiloba* is probably one of these clusters.

Morphological measurements: Two morphological features were found that differed greatly between species: gastral striation length (measured using gastral striation index GSI) and eye size (measured using ocular index OI). OI was most relevant for *H. montana*, which has a small eye relative to the other species. GSI was relevant for both *Hylomyrma montana* and *H. plumosa*, both of which have significantly shorter GSL than *H. dentiloba* and *H. versuta*. Both of these characters are utilized in the key to Central American *Hylomyrma* given below. However, no morphological differences were found to separate *H. dentiloba* and *H. versuta*. Also, we were not able to locate vouchers for COI sequences yielding the *H.* cf. *dentiloba*_sp. 1 and *H.* cf. *dentiloba*_sp. 2 clusters, so we could not investigate morphological differences between these clusters.

Taxonomic conclusions: We use the name *Hylomyrma versuta* to refer to all *H. dentiloba*-like specimens from the Caribbean lowlands of Costa Rica northward to Mexico. In Costa Rica it is sympatric with the two new species *H. plumosa* and *H. montana*. In the case of *H. plumosa*, both species occur together in lowland rainforest. In the case of *H. montana*, the two species segregate by elevation, with a narrow zone of contact around 1000 m. The status of *H. dentiloba* remains unresolved pending further study of the Panama fauna. At present, *H. versuta* and *H. dentiloba* are morphologically indistinguishable. We use the name *H. dentiloba* to refer to the Panamanian and Osa (Costa Rica) specimens. COI results show two separate clusters, potentially revealing cryptic species, and we refer to the two clusters as *H.* cf. *dentiloba* sp. 1 and *H.* cf. *dentiloba* sp. 2 (Tabs. 1, 2, Fig. 2).

Taxonomic synopsis of Central and South American *Hylomyrma*

[1] Species treated in this manuscript. [2] Species used in UCE and COI analysis. [3] Species used in COI analysis only.

H. balzani[3] (EMERY, 1894). French Guiana, Ecuador, Brazil, Paraguay, Argentina.
= *speciosa* BORGMEIER, 1937.
H. blandiens[2] KEMPF, 1961. Panama to Brazil.
H. columbica (FOREL, 1912). Colombia.
H. dentiloba[1,2] (SANTSCHI, 1931). Costa Rica to Panama.
H. dolichops KEMPF, 1973. Colombia, Ecuador, Brazil.
H. immanis[2] KEMPF, 1973. Ecuador, Peru, Brazil, Guyana, French Guiana.
H. longiscapa KEMPF, 1961. Ecuador, Brazil, Guyana, Suriname, French Guiana.
H. montana[1,2] n.sp. Costa Rica, Panama, Ecuador.
H. plumosa[1,2] n.sp. Costa Rica.
H. praepotens KEMPF, 1973. Ecuador, Peru, Brazil, Colombia, French Guiana.
H. reginae[2] KUTTER, 1977. Guyana, French Guiana, Brazil.
H. reitteri (MAYR, 1887). Colombia, Venezuela, Brazil.
= *goeldii* FOREL, 1912.
H. sagax KEMPF, 1973. Ecuador, Colombia, Brazil, French Guiana.
H. transversa KEMPF, 1973. Colombia, Peru.
H. versuta[1,2] KEMPF, 1973. Mexico to Costa Rica.

Key to Central American species of *Hylomyrma*, based on worker caste

1 At least some dorsal setae branched (Fig. 5c). ..... ***Hylomyrma plumosa* sp.n.**

– Pilosity simple, never branched 2

2 Postpetiolar dorsum densely sculptured, not smooth and shining; gastral striae long (GSI 12 - 26); eye relatively large (OI 27 - 30, Fig. 3).

a Atlantic slope of Costa Rica northward. ..... ***Hylomyrma versuta***

b Panama to Osa region of Costa Rica. ..... ***Hylomyrma dentiloba* complex**

– Postpetiolar dorsum smooth and shining or weakly striate (Fig. 4c); gastral striae short (GSI 4 - 10); eye relatively small (OI 21 - 24, Fig. 3) ..... ***Hylomyrma montana* sp.n.**

Species accounts

***Hylomyrma dentiloba* (SANTSCHI, 1931)** (Figs. 3, 6, 7)

Lundella dentiloba SANTSCHI, 1931: p. 271. Holotype worker: Panama, France Field, 2 June 1930 (A. Bierig) [NHMB, unique specimen identifier CASENT0913528] (AntWeb image examined). Combination in *Hylomyrma*: KEMPF (1960: 430).

Geographic range: Panama, Costa Rica (Osa Peninsula).

Measurements (n = 3): EL 0.194 - 0.233, EW 0.121 - 0.126, GSL 0.152 - 0.295, HFL 0.706 - 0.783, HL 0.778 - 0.855, HW 0.71 - 0.835, ML 0.977 - 1.115, OMD

Fig. 4: *Hylomyrma montana*, holotype, worker (CASENT0637306): (a) profile view; (b) full-face view; (c) gaster and postpetiole dorsum; (d) dorsal view.

0.092 - 0.104, PpL 0.227 - 0.251, PpW 0.293 - 0.35, PronW 0.512 - 0.625, SL 0.548 - 0.591, SPL 0.196 - 0.241, CI 91 - 98, GSI 15 - 26, OI 27 - 30, PpWI 129 - 139, SI 71 - 82, SPI 28 - 31.

Comments: The delimitation of *Hylomyrma dentiloba* remains unresolved, and for convenience we use a geographic definition of the species. Among the four clusters defined by sequence data, *H. plumosa* is morphologically distinct, but we have found no morphological differences among the remaining three clusters. We use the name *H. dentiloba* to refer to *H. dentiloba*-like material from the Osa Peninsula of Costa Rica south through Panama, and *H. versuta* for material from the Atlantic slope of Costa Rica northward. Thus one scenario is that with further investigation of the Panamanian fauna, cryptic species within "*H. dentiloba*" will be resolved. An alternative scenario is that *H. dentiloba* and *H. versuta* together are a single paraphyletic species, with a low level of gene flow, yet having spawned at least one fully differentiated species (*H. plumosa*) from within the range.

***Hylomyrma montana* sp.n.** (Figs. 3, 4, 7)

urn:lsid:zoobank.org:act:42EA005C-05E5-44D0-B7ED-2AB10FCE7AAF

Type material: Holotype worker: Costa Rica, Limón, Cerro Platano, 9.86732 - 83.24131 ± 20 m, 1130 m above sea level (a.s.l.), 18 June 2015 (Project ADMAC, collection code Wm-E-03-1-01) [CAS, unique specimen identifier CASENT0637306]. Paratype workers (n = 12): same data as holotype [MCZC, CASENT0638686; UCD, CASENT-0638689; UCR, CASENT0638690; USNM, CASENT-0638691; JTLC, CASENT0638721, CASENT0638702, CASENT0638694, CASENT0638692, CASENT0638699, CASENT0638696, CASENT0638719, CASENT0638695].

Geographic range: Costa Rica, Panama, Ecuador.

Diagnosis: Postpetiolar dorsum smooth and shining or weakly striate; eye relatively small (OI 21 - 24).

Description of worker: Measurements (holotype): EL 0.195, EW 0.134, GSL 0.065, HFL 0.796, HL 0.833, HW 0.845, ML 1.116, OMD 0.133, PpL 0.278, PpW 0.295, PronW 0.563, SL 0.676, SPL 0.282, CI 101, GSI 6, OI 23, PpWI 106, SI 80, SPI 33. Measurements (range, n = 20): EL 0.175 - 0.218, EW 0.124 - 0.149, GSL 0.047 - 0.123, HFL 0.775 - 0.945, HL 0.813 - 0.948, HW 0.804 - 0.899, ML 1.03 - 1.236, OMD 0.108 - 0.151, PpL 0.246 - 0.314, PpW 0.289 - 0.371, PronW 0.544 - 0.645, SL 0.621 - 0.742, SPL 0.248 - 0.329, CI 94 - 102, GSI 4 - 10, OI 21 - 24, PpWI 106 - 124, SI 75 - 83, SPI 31 - 39.

Head subquadrate; in full-face view sides and dorsum relatively straight, occipital corners rounded. Clypeus bidentate, denticles moderately long and projecting; clypeal

Fig. 5: *Hylomyrma plumosa*, holotype, worker (CASENT0638700): (a) profile view; (b) full-face view; (c) plumose setae on gaster; (d) dorsal view.

margin between denticles slightly concave. Clypeus and frontal area rugose. Frontal lobes rather large, with convex sides projecting over antennal insertions. Head in full-face view densely longitudinally striatorugose. Rugae beginning at mandibular insertions and frontal lobes, and continuing uninterrupted to occipital margin, occasionally intersecting and forming a sub-reticulate pattern. Area between rugae micro-punctatostriate. Head with moderate pilosity, hairs golden in color and pointed at ends. Eye consisting of more than 40 facets, relatively short and distant from anterolateral margin of head. Antennal scapes rugose, curved, thinner towards antennal insertions and thicker towards apices. Pilosity of scapes consisting of sub-erect to erect hairs. Funicular segments of antennae gradually increasing in size, and covered with sub-erect hairs.

Mesosoma in profile view convex from neck to propodeal spines, profile interrupted by rugae. Sides of mesosoma densely reticulate-rugose, interrugal spaces micro-punctatostriate, subopaque. Pronotal and metanotal grooves absent. In profile view, dorsum of mesosoma with long and pointed erect hairs. Propodeal spines medium sized and projecting over inferior propodeal plates.

Petiole elongated, lacking differentiated peduncle and node. Sides and dorsum of petiole rugose. Postpetiole in profile subquadrate; ventral surface flat, dorsal surface slightly convex. In dorsal view, postpetiole sub-triangular, sides flaring from anterior margin to posterior margin. Dorsal surface of postpetiole largely smooth and shining, with at most a few faint longitudinal costulae. Gaster largely smooth and shining. Tergum 1 of gaster with short longitudinal costulae, less than 0.1 mm in length, in some specimens nearly absent.

Overall body color dark reddish-brown. Legs and antennae somewhat lighter brown.

Etymology: The name is in reference to the montane distribution of the species.

Comments: *Hylomyrma montana* is the first recorded species in the genus known to be exclusively restricted to cloud forest habitat. Given the phylogenetic relationships of the sequenced specimens, we can reject the hypothesis that the Costa Rican and Ecuadorian populations of *H. montana* are independently derived from nearby extant lowland relatives. There is no way to rule out the possibility of independent evolution from lowland relatives that are now extinct. It may also be that other lowland species or populations that have not been sequenced will break the clade with the two *H. montana* populations. But the most likely scenario is that *H. montana* is a single clade and represents a single colonization of the montane habitat. The sister group to *H. montana* is a clade that contains all the remaining Central American species and a South American species, *H. blandiens*. Thus *H. montana* could have

Fig. 6: *Hylomyrma dentiloba* from Osa Peninsula (CASENT0636001): (a) full-face view; (b) profile view. *Hylomyrma versuta*, holotype, worker (MCZ-ENT00035424): (c) full-face view; (d) profile view.

originated in Costa Rica and dispersed to the Andes, or vice versa, the latter possibility being most likely.

***Hylomyrma plumosa* sp.n.** (Figs. 3, 5, 7)

urn:lsid:zoobank.org:act:C9B43ABB-CF42-45F1-81D3-AA0EF64D0296

Type material: Holotype worker: Costa Rica, Limón, Hitoy-Cerere, 9.66480 - 83.02346 ± 10 m, 250 m a.s.l., 10 June 2015 (collection code: ADMAC#Wa-E-02-2-38) [CAS, unique specimen identifier CASENT0638700]. Paratype workers (n = 13): Same data as holotype [MCZC, CASENT0638687; UCD, CASENT0638688; UCR, CASENT0638693; USNM, CASENT0638697; JTLC, CASENT0638720, CASENT0638701, CASENT0638703, CASENT0638725, CASENT0638698, CASENT0638700, CASENT0638722, CASENT0638724, CASENT0638735].

Geographic range: Costa Rica.

Diagnosis: At least some of dorsal setae branched, usually trifid.

Description of worker: Measurements (holotype worker): EL 0.211, EW 0.13, GSL 0.089, HFL 0.735, HL 0.799, HW 0.784, ML 1.042, OMD 0.109, PpL 0.253, PpW 0.3, PronW 0.573, SL 0.591, SPL 0.228, CI 98, GSI 9, OI 27, PpWI 119, SI 75, SPI 29. Measurements (range, n = 15): EL 0.202 - 0.235, EW 0.109 - 0.138, GSL 0.089 - 0.12, HFL 0.709 - 0.801, HL 0.793 - 0.871, HW 0.759 - 0.842, ML 0.991 - 1.137, OMD 0.095 - 0.12, PpL 0.227 - 0.285, PpW 0.293 - 0.331, PronW 0.55 - 0.618, SL 0.544 - 0.622, SPL 0.212 - 0.253, CI 93 - 99, GSI 8 - 11, OI 26 - 28, PpWI 114 - 133, SI 70 - 77, SPI 27 - 31.

Head subquadrate, occipital margin very slightly convex, occipital corners rounded. Clypeus bidentate, area between denticles mostly straight. Frontal lobes convex, ending anteriorly in a rounded angle, projecting over antennal insertions. Rugae on head running longitudinally and parallel, not intersecting; rugae interrupted occasionally by short gaps. Interrugal spaces microstriate, subopaque. Eye large, ending anteriorly in elongate tapering lobe. Scape tapered toward insertions and covered by abundant suberect non-branched hairs.

In profile view mesosoma convex, profile uninterrupted. Sides and dorsum of mesosoma reticulate rugose, interrugal spaces microstriate, subopaque. Propodeal spines relatively short, projecting posteriorly.

Peduncle of petiole present but not well developed. Sides and dorsum of petiole rugulose. Postpetiole subquadrate, covered on dorsum by longitudinal striae. Gaster mostly smooth and shining, striations on dorsum of tergum 1 short, but longer than *H. montana*.

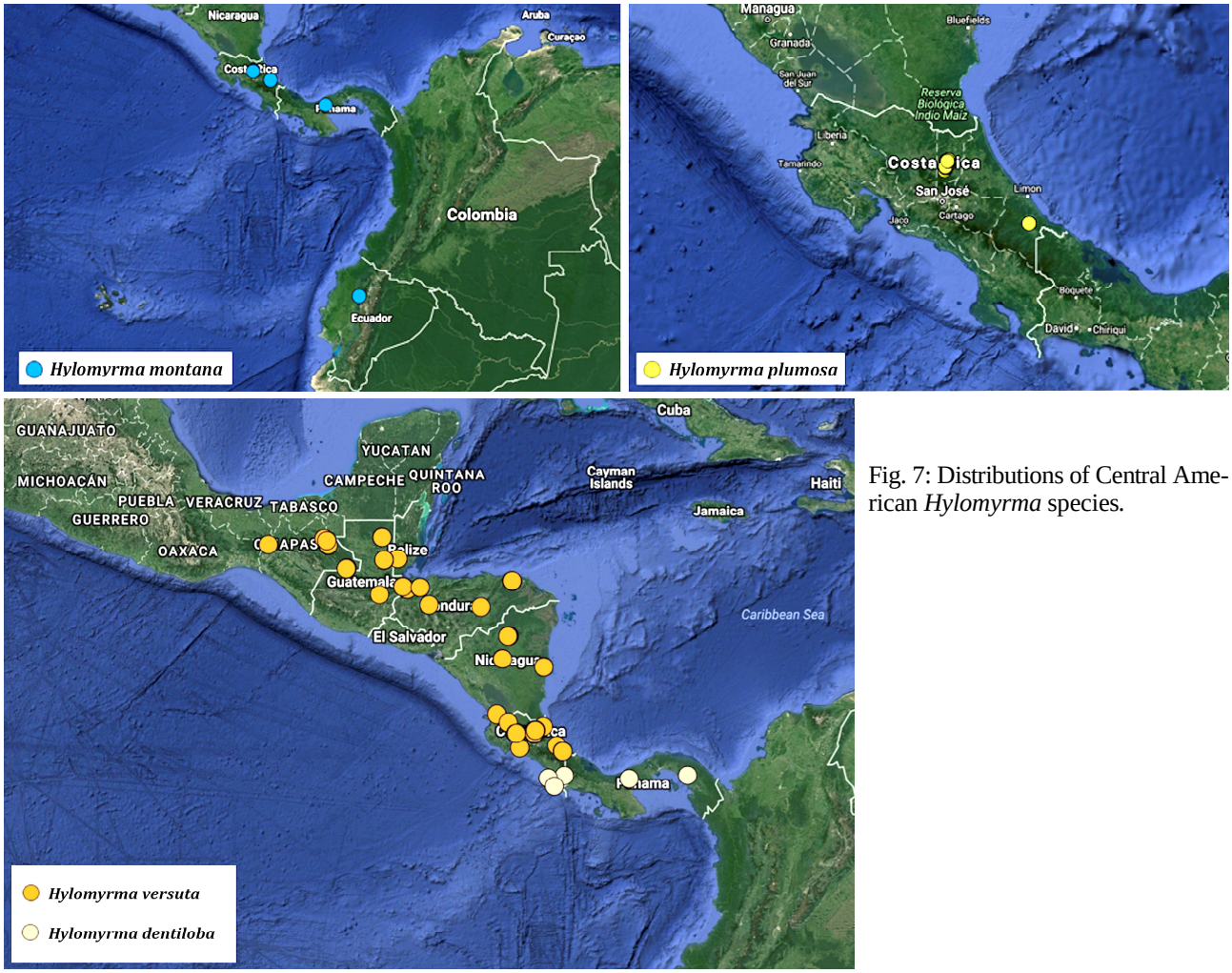

Fig. 7: Distributions of Central American *Hylomyrma* species.

Body color reddish to reddish-brown, legs somewhat lighter, gaster darker brown. Dorsum of head, mesosoma, and gaster abundantly setose, at least some setae branched, bifid or more often trifid.

Etymology: This species is named for the distinctive branched hairs that distinguish it from all other *Hylomyrma*.

Comments: Though the plumose setae and short gastral striation of *Hylomyrma plumosa* are distinctive, the species is otherwise extremely similar to *H. dentiloba* and *H. versuta*. Nevertheless, the morphological differences, the sequence divergence, and the sympatry with *H. versuta* at multiple sites in Costa Rica all support the hypothesis of separate species. The three specimens of *H. versuta* included in the UCE analysis emerge as a single clade with low sequence divergence, despite being from widely separated localities (Mexico, Honduras, and Costa Rica). Conversely, *H. plumosa* falls outside of this clade and with much higher sequence divergence, even though it is found sympatrically with the Costa Rican population of *H. versuta*. These results strongly suggest reproductive isolation between *H. plumosa* and *H. versuta*.

***Hylomyrma versuta* KEMPF, 1973** (Figs. 3, 6, 7)

Hylomyrma versuta KEMPF, 1973: 253, fig. 6. Holotype worker, paratype alate queen: Belize, Belmopan, 7 Aug 1972, in second growth forest (S. & J. Peck, berlesate no. 244) [MCZC, unique specimen identifier of holotype worker MCZ-ENT00035424, of paratype workers and alate queen MCZ-ENT00594451] (examined).

Geographic range: Costa Rica to Mexico.

Measurements (holotype worker): EL 0.194, EW 0.132, GSL 0.185, HFL 0.749, HL 0.86, HW 0.812, ML 1.146, OMD 0.092, PpL 0.265, PpW 0.315, PronW 0.59, SL 0.567, SPL 0.245, CI 94, GSI 16, OI 24, PpWI 119, SI 70, SPI 30. Measurements (range, n = 13): EL 0.17 - 0.241, EW 0.108 - 0.147, GSL 0.127 - 0.236, HFL 0.62 - 0.854, HL 0.734 - 0.99, HW 0.679 - 0.879, ML 0.884 - 1.234, OMD 0.071 - 0.124, PpL 0.231 - 0.3, PpW 0.244 - 0.349, PronW 0.453 - 0.646, SL 0.532 - 0.676, SPL 0.191 - 0.309, CI 87 - 98, GSI 12 - 22, OI 25 - 28, PpWI 105 - 130, SI 71 - 82, SPI 28 - 36. Measurements (paratype queen): EL 0.246, EW 0.144, GSL 0.271, HFL 0.789, HL 0.886, HW 0.823, ML 1.301, OMD 0.118, PpL 0.284, PpW 0.371, PronW 0.765, SL 0.592, SPL 0.304, CI 93, GSI 21, OI 30, PpWI 131, SI 72, SPI 37.

Comments: At present, there are no known morphological differences between *Hylomyrma versuta* and *H. dentiloba*. We differentiate the two species geographically, with *H. versuta* occurring from the Atlantic slope of Costa Rica northward to Mexico, and *H. dentiloba* occurring from the Osa Peninsula of Costa Rica eastward through Panama. See further discussion under *H. dentiloba*.

Discussion

Prior to this study, Central American *Hylomyrma* were placed into two species, the more northerly *H. versuta* and the Panamanian *H. dentiloba.* Relying largely on molecular evidence, we have revealed that there is greater species diversity within Central America than previously realized, including two new species (*H. montana* and *H. plumosa*) and possibly several cryptic species within *H. dentiloba*. This diversity has been overlooked because of the overall rarity of the genus as well as a large degree of morphological homogeneity among species. Our results suggest that morphology alone can be inadequate to identify specimens and to place species within an evolutionary context. By using UCE phylogenomics we were able to elucidate some of the cryptic diversity within Central American species and to gain a greater understanding of the evolutionary history among species. It is clear based on our geographically limited study that a thorough revision of *Hylomyrma* as a whole will require broad sampling of individuals across all species ranges for both morphological and molecular analysis. An increasing number of studies have demonstrated that morphological homogeneity, similar to what we have observed in *Hylomyrma*, is present within many ant lineages (SCHLICK-STEINER & al. 2006, ROSS & al. 2010, BLAIMER 2012), and that combining molecular data with morphology is often necessary to identify species and accurately reconstruct phylogeny.

The Central American *Hylomyrma* represent at least two distinct clades with South American relatives. The greater species diversity within South America and the presence of South American species in both branches from the root node of the UCE phylogeny suggest a South American origin for the genus. The *H. dentiloba* clade (*H. dentiloba*, *H. plumosa*, and *H. versuta*) perhaps evolved from a *H. blandiens*-like ancestor in South America and moved north into Central America, generating cryptic species along the way, with *H. versuta* being the northernmost and also being the northerly limit of the genus. *Hylomyrma montana* is possibly a second South American clade that colonized montane habitats and then spread through Andean, Panamanian, and Costa Rican cloud forest. Alternatively it could be a formerly widespread species that evolved in place during both Andean and Talamancan orogeny, and was then replaced in the lowlands by other *Hylomyrma* clades.

Hylomyrma montana represents a clear case of montane specialization from lowland ancestors. It also represents a biogeographic connection between what are now disjunct cloud forest habitats in Central and South America, separated by a lowland barrier in Panama. Biogeographic connections between cloud forests of Costa Rica and the Andes have been noted for plants (e.g., GENTRY 1982). In ants, several groups also show connections between Costa Rica and western Ecuador, including *Hylomyrma montana* (described here), species in the genus *Stenamma* (e.g., *S. alas, S. felixi, S. schmidti*; BRANSTETTER 2013), and *Pheidole innupta* (J.T. Longino, unpubl.). Several of these species are unknown from the Colombian Andes and it is uncertain whether this is a true gap or the result of undersampling. More work is needed to document the true distributions of cloud forest ants in the Andes, but there is an undoubted distribution gap across the lowlands of eastern Panama.

Andean uplift is correlated with a rapid diversification of Neotropical organisms and the establishment of montane communities (HOORN & al. 2010), and given the South American provenance of *Hylomyrma*, Andean orogeny would be a possible origin of *H. montana*. But the historical distribution of low cloud forest habitat is difficult to determine. Although the high Andean orogeny is relatively young (GREGORY-WODZICKI 2000) and the Talamancan uplift of Costa Rica even younger (GRÄFE & al. 2002), mid-elevation cloud forest may have been more extensively available before, during, and after this uplift, on lower mountain systems or even ephemeral volcano slopes. Much of the Pleistocene was cooler than our current interglacial conditions, with downward-shifted plant communities (BUSH & al. 2009). Cloud forest habitat was undoubtedly at lower elevations and thus more extensive and connected, with greater opportunities for dispersal. Exploring the phylogeny and biogeography of lineages like *H. montana* and linking this with orogeny will help to determine how and when montane-specialization occurs.

Establishing accurate evolutionary histories and species ranges is pivotal to evaluating the effects of local and global environmental change (FITZPATRICK & al. 2011). As habitat is destroyed and the climate altered, species ranges will move over space both laterally and vertically (COLWELL & al. 2008). A warming climate will allow tropical species to extend northwards, and lowland species will move up elevational gradients (LONGINO & al. 2011, LAURANCE & al. 2011). This process may result in the replacement of montane species. These effects will only be observable, however, if accurate species boundaries and ranges have been established. If a great deal of diversity within a group is not seen because of morphological homogeneity, these replacements will not be observed and the effects of climate change underestimated. Arthropods are already largely ignored in conservation studies, and it is likely that arthropod diversity is disappearing at an alarming rate (CARDOSO & al. 2011). Arthropod conservation studies should not underestimate diversity loss by failing to recognize true species boundaries.

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Data availability

Raw Illumina reads and contigs representing UCE loci are available from the NCBI Sequence Read Archive and GenBank, respectively (BioProject#PRJNA379610). DNA matrices and associated trees have been deposited at TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S20852). All newly generated COI data are available from GenBank (KY907454 - KY907467; Tab. 1).

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