

Supplementary information

Reported climate change impacts on cloud forest ants are driven by sampling bias: a critical evaluation of Warne et al. (2020)

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Appendix S1. Analyses.

We used the dataset BITR-19-251_Supplemental_Appendix_-_201106.xlsx loaded from Warne et al. (2020a), from Dryad at <https://datadryad.org/stash/dataset/doi:10.5061/dryad.zgmsbcc8q> (the updated version from November 9, 2020). We followed Warne et al. (2020b) in focusing on the species as those with unique BIN code (= a species boundary based on comparing COI barcodes in the BOLD database at www.boldsystems.org), rather than using taxonomical (and morphospecies) names. First, we excluded from the data all samples without BIN codes for the listed taxa (species or morphospecies). This yielded 342 individual ant records for the recent period (2008-2011) and 594 individual ant records for the historical period (1998-2001). The resulting species richness across all BIN fully matched those in Figure 1 in Warne et al. (2020b), including the species overlap between the two periods. Next, unlike Warne et al. (2020b), we considered the sampling methods used to collect each of the BIN incidences in the dataset (the individual ant records across the samples: barcoded individuals hereafter). We used the “Notes” column in the datasheets with the sampling information to assign a sampling methodology to each species and its records in each time period dataset. Then, we split the species records (i.e. individuals) into three groups: (i) sampled by malaise traps in the historical period, (ii) sampled by Malaise traps in the recent period, and (iii) sampled by any other method in the recent period (i.e. divided by five methods: Bait, Hand-collecting, Winkler/Berlese, Sift, Pitfall trap). The details about field sampling protocols are available in the Appendix to Smith et al. (2014). Note that the historical period was sampled only using Malaise trapping (594 individuals, 2 traps), while the recent period has been sampled by one Malaise trap and the five further methods (Malaise trap = 72 individuals, Other methods pooled = 270 individuals) (Warne et al., 2020b).

We used R package iNext (Chao et al. 2014) and the above individual BIN records in the two time periods, and those only sampled by Malaise trap in the recent period, to construct species accumulation curves, with 95% CI intervals in our Figure 1a (999 runs). For comparison of the two periods sampled, we also extrapolated the number of species to the same sampling effort for the recent period (i.e. 594 individual records). We did not extrapolate for the 72 records sampled by Malaise trap, as the extrapolation is not recommended for over a double of the original sample size (Hsieh & Chao 2016). We constructed a Euler diagram for our Figure 1b using the tool at <http://eulerr.co> (Larsson 2020) and comparing the species (unique BIN) divided

between the historical period, and the recent period sampled by (i) Malaise trap and (ii) sampled by other methods, including the three groups' mutual overlaps, and compared the pattern with Figure 1c in Warne et al. (2020b).

We used the split of the dataset by sampling methods to test, if there was also an effect of the sampling method on the body colour patterns of the ant species (the BIN measure for lightness) compared with Warne et al. (2020b: Figure 2a). We reconstructed a similar chart to that figure, using a violin plot in ggplot2 package (Villanueva & Chen, 2019) and the data sheet “*Lightness*”, $n = 64$) (Warne et al., 2020a). Instead of splitting the species by the two periods, and the common species pool (i.e. species occurring in both periods measured for lightness) as in Figure 2a in Warne et al. (2020b), we compared the historical period to the recent period split by the species sampled by “Malaise trap” versus those sampled exclusively by “Other methods”. There was a significant difference among the three groups (Kruskal-Wallis = 12.036, $df = 2$, $p = 0.002$), and while the Malaise trap datasets overlapped, the species sampled by the additional sampling methods in the recent period were significantly lighter (Figure 2a). To examine whether the pattern also hold if individual species' numeric representation in the data are considered, we recreated the diagram by considering the lightness across the ant individual BIN records ($n = 936$). To do so, we used the quantitative data for the species pool (46 unique BIN) measured for the lightness resulting in a total number of individual ants with lightness data of 469, and reran the analysis. Note that lightness was scored by the species, and each species was measured only once for lightness in Warne et al. (2020a), so the same species, as well individuals of the same species, all have the same lightness value in the plots. The outcome was very similar (Figure S1). Finally, we also looked at the variance in this lightness dataset across the individual six sampling methods (Figure S1b).

We used the data sheet “*Community*” used by Warne et al. (2020b) for their multivariate analysis, to create our Figure 2b: for the two cloud forest sites (i.e. Cacao1300, Cacao1500), we used both CacaoHist (historical period) and Cacao1500 (recent) data, but the latter we also divided by the sampling methods as above (i.e. Cacao1500-Malaise, Cacao1500-OtherMethods). Further, we examined the individual species elevational ranges occurring in those mountain cloud forest datasets (i.e. > 1200 m), using the same BIN occurrences but checking for their presence across the low elevational (10 to 600 m) and mid-elevational sites (700 m to 1200 m) in

the “*Community*” datasheet. For simplicity, we pooled the individual sites and estimated the corresponding observed ranges for each ant species occurring in one, or multiple cloud forest sites and periods, as following: species occurring in all elevations (across the three classes), only high-elevation, mid-elevation + high elevation, and low elevation + high elevation (Figure 2b). Note that the species occurring exclusively in lowlands are not considered, as only cloud forest sites are being compared here. Finally, we also examined in the two core BIN datasets (Warne et al., 2020a) the case study taxa (species in the ant genera not resampled = “lost”, or sampled only = “gained”, in the historical period), and their ranges and individual abundances (Figure 2c and Table S1).

Regarding phylogenetic measures of ant assemblages among the two time periods, we reran the analyses conducted in Warne et al. (2020b) using the phylogeny from Warne et al. (2020a) and the Picante R package (Kembel et al. 2010). We rerun the calculation for PD and NTI indices. In addition, we calculated the mean phylogenetic distance (MPD) index that captures better the clustering across the phylogeny than the near taxon distance (NTI) index. In all calculations, we split again the data from the recent period into the assemblage sampled by Malaise trap only, and the assemblage sampled by other five methods.

We did not attempt to calculate statistics for the functional diversity measure (FDis) for Malaise trap-sampled groups. First, the difference in Warne et al. (2020b) was not statistically significant, and it has been not clear what test was used (only two data points). Second, after we explored the original data, we found that of the 67 unique BIN species measured in Warne et al. (2020a), 44 of the 79 species (57%) were measured for morphological traits in the historical assemblage, while a higher proportion (61 of the 69 spp., 88%) were measured in the recent period, making further comparisons problematic. That is, an increase in functional diversity might be attributed (in addition to the methodological variance) to a larger species pool measured using workers in the latter period, probably due to a higher presence of winged individuals (sexuals) in the historical period (Warne et al., 2020b). However, we could not quantify this bias mentioned by authors, as the cast was not clearly marked for all individuals in a reproducible way in the data (Warne et al., 2020b). Original their statement for historical period “*the majority of these ants were winged males or queens (27/652)*” is also confusing, perhaps with an error (27 is not a majority, so probably it has been much higher proportion).

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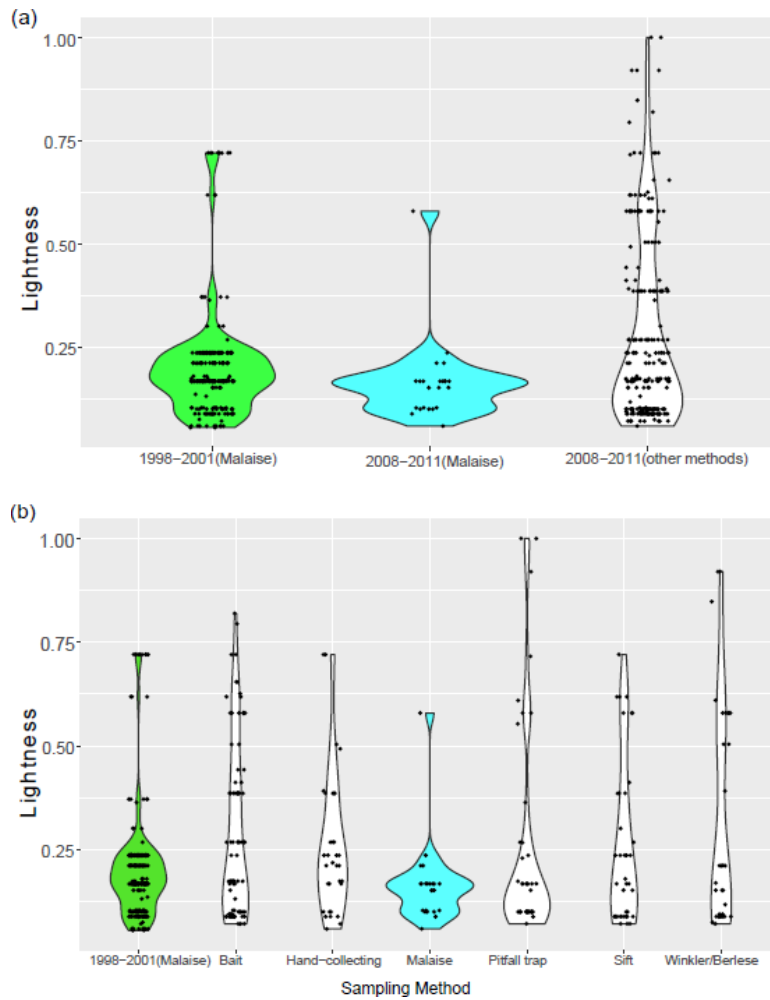


Figure S1. Changes in lightness of ants using individuals caught by different sampling methods in the historical (1998-2001, violin plots in green) and the recent (2008-2011, the other plots) period in Cacao Volcán. (a) Lightness violin plot follows Figure 2 from Warne et al. (2020) but with 2008-2011 data split into Malaise and non-Malaise samples for comparability and using individual ant as replicates. The pattern replicates that found for the ant species in Figure 2. (b) Lightness plots split by the different sampling methods (only Malaise trap in 1998-2001, but Malaise and five more methods used in 2008-2011). Most of the species (black points) with higher lightness were sampled by other sampling methods, in particular using baits, Winklers and soil sifting all conducted at the ground level of the cloud forest (see Appendix S1).

Table S1. Overview of the genera and their species (BIN) ranges sampled uniquely only in one of the two time periods according Warne et al. (2020a), Dryad, doi:10.5061/dryad.zgmsbcc8q. The case studies species from Figure 1 in Warne *et al.* (2020b), in bold letters. BIN refer to unique molecular species codes based on COI gene barcoding from <http://www.boldsystems.org>.

(a)

Missing species (BIN, 1998-2011 period)	Taxon name	Genus	N of indiv.	Elevational range (based on the “community” datasheet from Warne et al. (2020a))
BOLD:AAV6175	<i>Acropyga</i> MAS003	<i>Acropyga</i>	3	1500-hist
BOLD:AAV6028	<i>Basiceros</i> sp.	<i>Basiceros</i>	1	1500-hist
BOLD:AAL7224	<i>Belonopelta</i> MAS002	<i>Belonopelta</i>	1	1500-hist
BOLD:AAC5364	<i>Eciton burchellii</i>	<i>Eciton</i>	5	Whole range
BOLD:ACA2865	<i>Leptanilloides</i> MAS001	<i>Leptanilloides</i>	1	1500-hist
BOLD:AAI1241	<i>Leptogenys</i> MAS001	<i>Leptogenys</i>	1	Cacao-700, Cacao-1300, 1500-hist
BOLD:AAI1240	<i>Leptogenys</i> MAS003	<i>Leptogenys</i>	1	1500-hist
BOLD:AAP9703	<i>Myrmelachista nigrocotea</i>	<i>Myrmelachista</i>	2	1500-hist
		Rest	579	
		Total	594	

(b)

New species (BIN, 2008-2011)	Taxon name	Genus	N of indiv.	Elevational range (based on the “community” datasheet from Warne et al. (2020a))
BOLD:AAC2782	<i>Anochetus mayri</i>	<i>Anochetus</i>	1	Cacao-1000, 1500 (Malaise trap)
BOLD:AAG6736	<i>Azteca</i> MAS004	<i>Azteca</i>	3	Cacao-700, 1000, 1500 (other methods)
BOLD:AAG0936	<i>Ectatomma</i> MAS001	<i>Ectatomma</i>	2	Cacao-300, 600, 1500 (other methods)
BOLD:AAG9506	<i>Wasmannia</i> MAS001	<i>Wasmannia</i>	3	Cacao-10, 300, 600, 700, 1000, 1200, 1500 (other)
		Rest	333	
		Total	342	

Table S2. Results of analyses of phylogenetic diversity using Picante package. The indices calculated in Warne et al. (2020b: PD and NTI) and newly provide here (MPD) are in bold. Note NTI values slightly differ from Warne et al. (2020) due to the randomisation of the observed values (999 runs). Significant p-values (in italics) suggest a phylogenetic clustering among neighbouring species for MNTD (terminal structure), and clustering across the phylogeny for MPD (basal structure). The recent period (2008-2011) is split to Malaise trap and other methods, and compared with historical data (1998-2001) that were sampled only using Malaise trapping.

(a)

Phylogenetic diversity	PD	Sp. richness (BIN)
Recent: All methods (2008-2011)	17.33	69
Historical: Malaise only (1998-2001)	18.72	78
Recent: Malaise trap (2008-2011)	8.28	26
Recent: Other Methods (2008-2011)	14.6	55

(b)

Mean Nearest Taxon Distance	Sp. richness (BIN)	mnt d.obs	mntd.rand. d.mean	mntd.rand. sd	mntd. obs.rank	mntd. obs.z	NTI^{*)}	p-value
Recent: All methods (2008-2011)	69	0.37	0.43	0.03	8	-2.36	2.36	<i>0.008*</i>
Historical: Malaise only (1998-2001)	78	0.32	0.41	0.02	1	-3.94	3.94	<i>0.001*</i>
Recent: Malaise trap (2008-2011)	26	0.50	0.55	0.05	160	-1.00	1.00	0.16
Recent: Other Methods (2008-2011)	55	0.39	0.46	0.03	8	-2.41	2.41	<i>0.008*</i>

^{*)}NTI = -1 times SES_{mntd} (mntd.obs.z)

(c)

Mean Pairwise Distance (MPD)	Sp. richness (BIN)	mpd .obs	mpd.rand. mean	mpd.rand. .sd	mpd.obs. rank	mpd.obs. z	p-value
Recent: All methods (2008-2011)	69	0.83	0.88	0.02	7	-2.46	<i>0.007*</i>
Historical: Malaise only (1998-2001)	78	0.88	0.88	0.02	451	-0.17	0.451
Recent: Malaise trap (2008-2011)	26	0.84	0.88	0.04	157	-0.99	0.157
Recent: Other Methods (2008-2011)	55	0.83	0.88	0.03	21	-2.04	<i>0.021*</i>