COMMENTARY

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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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1 | INTRODUCTION

Long-term changes in tropical insect communities are largely unknown, in particular for mountain forest ecosystems (Basset & Lamarre, 2019; Chen et al., 2009). This is worrying, given the ongoing environmental changes including those concerning climate. Here, we present a reanalysis of a recent paper by Warne et al., (2020a), in which the authors reported changes in species richness, species composition, phylogenetic diversity, and trait distributions in a cloud forest ant assemblage in response to climate change. We show that these shifts over one decade are, however, due to major differences between sampling periods in terms of sampling methods and effort. Finally, we stress in our commentary the need for a fully standardized methodology to distinguish true climate change effects on communities from sampling bias (see also e.g., Klesse et al., 2018).

Anthropogenically driven climate change is a major ongoing threat to global biodiversity (Brondizio et al., 2019). Ants are a commonly used indicator taxon for monitoring changes along temperature gradients (Lach et al., 2010), which makes them suitable for studying climate-related community shifts. However, relatively little work has been conducted on ant assemblages in tropical cloud forest (e.g Mottl et al., 2019; Smith et al., 2014) despite predictions that highland assemblages are likely to change the most (Bishop et al., 2019). While the effects of habitat disturbance on ants have

been well documented (Andersen, 2019), long-term data to test for climate change effects on ants are scarce. Most studies on the interaction of climatic changes with elevation, and the consequent species range shifts, have focused on vertebrates, plants, and moths (e.g., Cheng et al., 2019, and review there). Hence, the effects of climate change on ants are limited to modeled predictions (e.g., Bishop et al., 2019), or small-scale experiments in temperate forests (e.g., Diamond et al., 2016). To our knowledge, only one study has monitored long-term changes in a rain forest ant community, via multiple resurveys over a decade at 850 m a.s.l. and did not find a directional trend (Donoso, 2017).

A recent study by Warne et al., (2020a) is the first to report such effects on cloud forest ant assemblages at high elevations over a one-decade time period. The authors compared ant diversity and composition using molecular-based species (BINs—Barcode Index Numbers) between historical (1998–2001) and recent (2008–2011) periods in a forest near the top of Volcán Cacao at 1500 m a.s.l., a mountain in the Área de Conservación Guanacaste (ACG) in Costa Rica (Warne et al., 2020a). They concluded that "*cloud forest ant communities are becoming more similar to communities from lower elevation forests,*" and these findings have been cited as "*major changes in ant diversity and composition with climate change*" (Hulshof & Powers, 2020). While the impacts of climate change on biodiversity and ecosystems are substantial and need to be thoroughly examined (Brondizio et al., 2019; Urban, 2015),

it is vital that the measurement of these impacts is done properly and account for any sources of methodological bias.

We focus our critique on the standardization of the sampling methods and effort across the sampling periods, and the subsequent interpretation of the differences found. The authors invest much less of their sampling effort in the recent period compared with the historical period in terms of the sampling events (11 vs. 59) (Warne et al., 2020a). Furthermore, only Malaise traps were used in the first sampling period (two traps), but five more sampling methods (bait, hand-collecting, Winkler/Berlese, Davis sifters, and pitfall trapping) were used in addition to a single Malaise trap in the recent period. This resulted in the majority of ants resurveyed in the recent period being collected by methods other than Malaise trapping (Figure 1a, Appendix S1). However, Warne et al., (2020a) did not consider any effects of these major differences in sampling methods and effort between the time periods. This surprised us, as the fact that a Malaise trap samples a rather specific part of the fauna is well known, likewise that a different sampling effort biases the estimates of overall diversity (see Longino & Colwell, 1997). In particular, Malaise traps are typically used to sample the mobile, flying invertebrate fauna from different forest strata (Delabie et al., 2021). The other methods used by Warne and colleagues (e.g., pitfall traps, litter sifting) instead focus on the ground and leaf litter-dwelling fauna (Longino & Colwell, 1997). Comparing samples collected using different methods is not a like-for-like comparison, and hence, observed differences may simply be the result of the different biases and designs of the methods.

Here, we present a reanalysis of the authors' published dataset (Warne et al., 2020b), in which we account for these methodological discrepancies and reassess the main findings from Warne et al., (2020a). Specifically, we reproduced the main analyses (the original

Figures 1 and 2a, and phylogenetic diversity measures from Warne et al., (2020a)) considering which sampling methods have been used to collect the barcoded species' incidences in each of the two sampled periods (Figures 1b and 2a, Table S2). Furthermore, we took into account the sampling effort (i.e., n of species incidences being compared), and the abundance and elevational ranges of the ant species in additional analyses (Figures 1a and 2bc, Table S1). The details of our analyses and the data used are available in the supporting information. We demonstrate that sampling biases can explain the reported changes in ant diversity across time, initially interpreted as climate-driven effects by Warne et al., (2020a). Furthermore, we show that the ant assemblages appear to be similar through time, rather than different, although small sample size precludes any strong inferences about community stability.

2 | REANALYSES AND CRITICAL EVALUATION OF THE FINDINGS OF WARNE ET AL (2020a)

Where our analyses contradict those of Warne et al., (2020a), we cite the original text from their study as a subtitle (i.e. 2.1-2.6 headings) and describe our relevant analyses below.

2.1 | Ant species richness was higher in the 1990s than in the 2000s

We demonstrate that if the different number of species incidences across all the samples (i.e., number of all barcoded ant individuals) is taken into account, sampling in the recent period predicts instead a
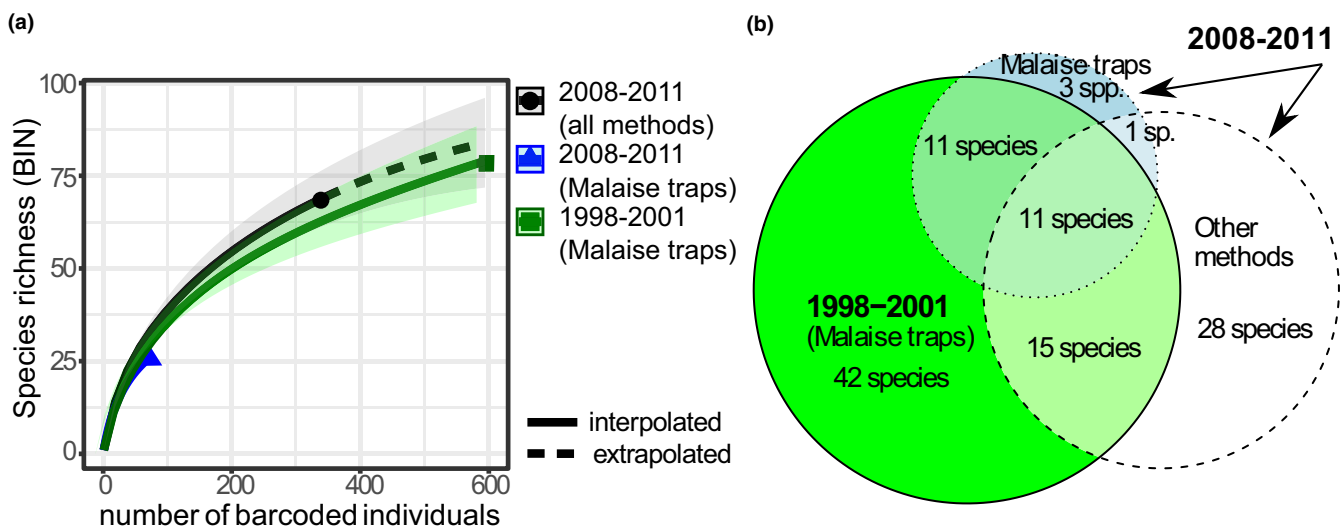

FIGURE 1 Ant species diversity sampled in historical (1998–2001) and recent (2008–2011) periods in Cacao Volcán. (a) Species accumulation curves with 95% confidence intervals showing increasing observed species richness (number of BIN) with number of barcoded ant individuals in Warne et al., (2020b). The green curve denotes the historical period (594 individuals sampled using single method: Malaise trap), while the black curve denotes the recent period (342 individuals sampled across six different sampling methods), and blue curve is for the recent period but with only Malaise traps data included (72 individuals; one trap only and with less sampling effort). (b) Euler diagram of the species richness based on Figure 1 from Warne et al., (2020a) but with the recent period (2008–2011) split by the sampling methods. Circles are proportional to the number of species sampled in each group, and the numbers refer to their exclusive parts (i.e., the unique and overlapping species)
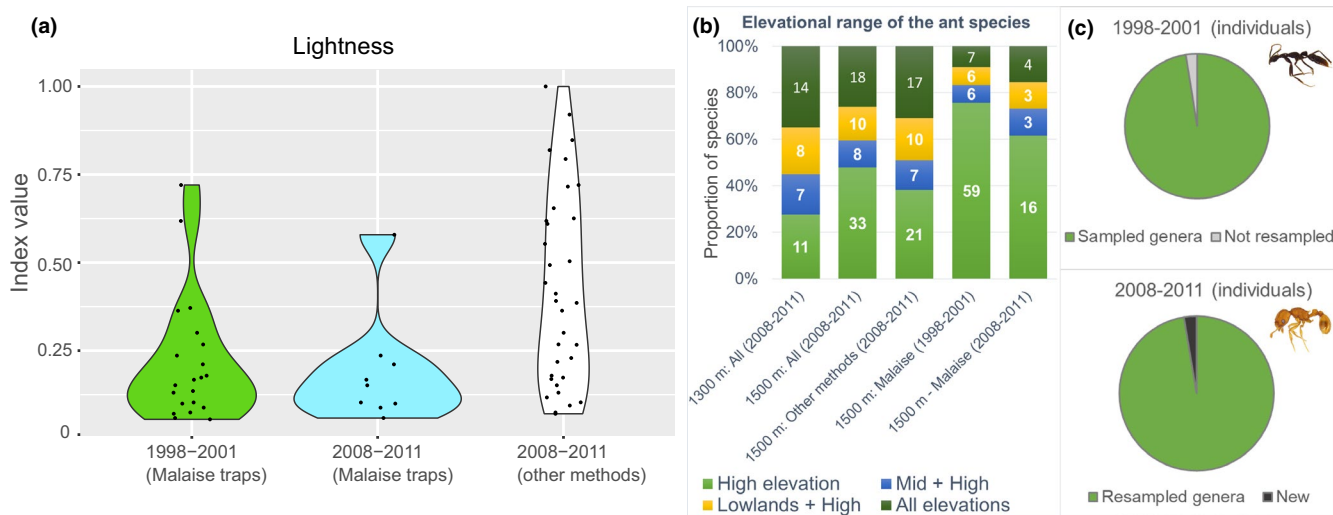
FIGURE 2 Changes in ant species' lightness and composition between historical (1998–2001) and recent (2008–2011) periods in Cacao Volcán. (a) Lightness violin plot following Figure 2 from Warne et al., (2020a) but split to allow comparison between Malaise trap and other sampling methods. Most of the species (black points) with higher lightness were sampled by other sampling methods, in particular using baits, Winklers, and soil-sifting on the ground (see Figure S1 and Figure S2 for further analyses across individual methods and ant individuals). (b) Proportions of the species by their main elevational ranges for the two cloud forest sites, 1300 m (Cacao-Derrumbe) and 1500 m (Cacao-Cima: the resampled site) from figure 3 in Warne et al., (2020a). Numbers inside the bars denote the number of species (BIN). The 1500-m site is split by the sampling periods and methods. (c) Pie chart of the sampled ant individuals (i.e., BIN species incidences) in the two sampling periods. The "case studies" genera presented by Warne et al., (2020a) for the species range shifts under climate change hypothesis not resampled (e.g., *Leptogenys*) or sampled only in the recent period (e.g., *Wasmannia*) made up less than 3% of all barcoded individuals across seven (1998–2001) and four (2008–2011) genera, respectively (see text and Table S1)

higher richness (Figure 1a). Although the difference is not large, that is, confidence intervals overlap, as they do for the Chao2 estimates presented by Warne et al., (2020a). This higher richness in the recent period is expected because a greater number of sampling methods were used. More importantly, the curves for the same method (Malaise trap) overlap. Hence, the null hypothesis that species richness does not change through time cannot be rejected.

2.2 | Species overlap between timeframes was low

If the species data (111 unique BIN) are split by time period and sampling method, there is a near complete overlap in species composition between the two periods when comparing only Malaise traps (Figure 1b). In other words, almost all novel species found in the recent period were sampled by methods not used in the first time period (28 of 32 spp.).

2.3 | The species assemblage in the collections from the 1990s was significantly phylogenetically clustered and functionally less diverse as compared to collections from the early 2000s

Warne et al., (2020a) focused mainly on the phylogenetic and functional measures of ant diversity. However, the differences in phylogenetic and functional diversity measures between the two

periods were rather small (and not significant for functional diversity). Furthermore, these diversity measures are dependent on the number of species compared in each group (Table S2a). The decrease in the Mean Nearest Taxon Distance (i.e., NTI index) was interpreted by Warne et al., (2020a), as a stronger phylogenetic clustering in the historical than the recent community. We confirmed that pattern for NTI, when only the assemblages sampled by Malaise traps are compared (Table S2b). However, this index is more sensitive to clustering at the tips of the phylogeny, while mean pairwise distance (MPD) is more effective at capturing clustering at deeper nodes (Kembel et al., 2010). We calculated SES_{MPD} and show that the latter measure suggests a different conclusion: neither recent nor historical communities are clustered when only Malaise traps, that is, a like-for-like comparison, are considered, but sampling by other methods (i.e., addition of the taxa sampled from the litter) led to a phylogenetic clustering in the recent community (Table S2c).

2.4 | The average lightness of the assemblage of cloud forest ant species became lighter through time

We show that for the Malaise trap assemblages' lightness did not change through time (Figure 2a, Figure S1 and Figure S2). Recent work has demonstrated that ant communities are about twice as dark in the forest understorey than on the ground (Law et al., 2020). As the sampling methods used exclusively in the recent period are biased toward capturing the leaf-litter and ground-foraging ant

species (see above), we argue that the greater sampling of lighter colored ants in that period compared with the historical period is expected (Figure S1b). Thus, sampling bias can once again explain this apparent assemblage-level change in lightness through time.

2.5 | Cloud forest ant communities are becoming more similar to communities from lower elevation

If we consider the elevational ranges of the individual ant species from the entire community data for the cloud forest sites (Smith et al., 2014; Warne et al., 2020b), all sites and periods contain a proportion of the species that extend their range across the whole elevational gradient, as well as species that are unique to cloud forests (Figure 2b). However, the proportion of high-elevation specialists is lower across all sampling methods at both of the cloud forest sites (at 1300 m and 1500 m) than for Malaise traps in both studied periods at 1500 m site (Figure 2b). The (slight) shift toward the mid-elevation communities presented in figure 3 in Warne et al., (2020a) might hence be explained by differences in sampling methods between the two periods.

2.6 | Gains and losses of particular species

Both the genera lost from the historical period and the genera gained in the recent period made up a minority of the sampled (barcoded) individuals (~2.5%, Figure 2c). Moreover, some of the species lost from the historical period were found at the lower elevations at Cacao gradient (Table S1), which would not be expected if these are high-elevation species that are being pushed off the top of their elevational ranges. For the two case study genera (*Leptogenys* and *Wasmania*) highlighted in Warne et al., (2020a), only *Leptogenys* MAS002 was limited to cloud forest. This species, not resampled in the recent period, was a singleton in the historical period, which is weak evidence for extinction. The second, *Wasmannia* MAS001 (three individuals sampled in the recent time period only), is a native species to the ACG region, widespread along the low and mid elevations (Ratnasingham & Hebert, 2007) (Table S1). A possibility that both species are rare in Cacao Volcán, but may be naturally co-occurring in both time periods cannot be excluded.

3 | DISCUSSION AND PERSPECTIVE

While climatically driven elevational shifts have been documented for some invertebrate groups (e.g., moths moving up by 67 m in 40 years (Chen et al., 2009)), recent studies highlight the importance of consistent sampling methods and timing: if such methodological variance is considered, range shifts can appear to be less pronounced (Cheng et al., 2019) or driven by natural seasonality (Maicher et al., 2020). For cloud forest ant assemblages, several recent studies that consider both habitat change and

elevation found that the mountain assemblages are more robust to vegetation changes than their lowland counterparts (Hethcoat et al., 2019; Mottl et al., 2019). This paints a more optimistic picture that mountain ants in these tropical environments might be perhaps also less sensitive to climatic changes. Our reanalysis of Warne et al. fails to reject the null hypothesis of stability in the Neotropical mountain ant community at this single site, although the small sample size of the reanalyzed data (*n* = 2 Malaise traps from the historical time period and *n* = 1 from the recent time period), precludes any strong inferences about this pattern. This contrasts with the high but unidirectional variation found in ant communities at lower elevations in the Neotropics (Donoso, 2017). However, more data are needed on the topic, as the Warne et al. dataset describes only a single location, and hence power to detect trends at large spatial scales is likely to be low. Furthermore, climate-related changes are expected to accelerate through time (Janzen & Hallwachs, 2020; Urban, 2015).

Our motivation for writing this commentary is to ensure that the ways that humans affect natural ecosystems are robustly documented in the scientific literature. If we are to combat ongoing threats such as climate change, we need a correct understanding of how and why ecosystems are changing. It would not be good for conservation if flawed studies are held up as flagships of climate change science. We would like to stress that as insect ecologists we are all very much concerned by the effects of humans on natural ecosystems. However, it is vital that climate change research is rigorous and as free from methodological bias as possible if we are to convince politicians to protect unique tropical ecosystems. While the climate change in the ACG may have already started to change cloud forest ecosystems, the wording "*we have not been able to measure this drying in any particularly "scientific" way; when the house is burning, a thermometer is not the thing to call for*" (Janzen & Hallwachs, 2020) potentially gives powerful ammunition to climate change skeptics. Likewise, "*happy accidents of collection*" (Warne et al., 2020a) taken post hoc and not carefully analyzed may have a similar effect. We suggest that calling for a thermometer is now more important than ever. The detailed documentation of ant and other insect communities at ACG is unique for a hyper-diverse tropical region and offers an opportunity to conduct a much more rigorous comparison, probably with less effort than would be possible in other, less accessible, little known, and pristine tropical rain forest communities elsewhere. Future studies should build on such an opportunity by conducting ongoing resampling of the sites using the same methodology. Only by doing this, can we all convince the world that our shared global house is burning.

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