1   Commentary

2   **Reported climate change impacts on cloud forest ants are driven by sampling bias (critical**

3   **evaluation of Warne et al., 2020)**

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5   Petr Klimes[1][*], Tom R. Bishop[2,3], Tom M. Fayle[1], Shuang Xing[1]

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7   [1]Biology Centre of the Czech Academy of Sciences, Institute of Entomology, Ceske Budejovice,

8   Czech Republic.

9   [2]Department of Earth, Ocean and Ecological Sciences, University of Liverpool, Liverpool, UK.

10   [3]Department of Zoology and Entomology, Centre for Invasion Biology, University of Pretoria,

11   Pretoria, South Africa

12

13   [*]Correspondence: Petr Klimes, Biology Centre of the Czech Academy of Sciences, Institute of

14   Entomology, Branisovska 1160/31, 370 05, Ceske Budejovice, Czech Republic. Email:

15   peta.klimes@gmail.com

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23 **Abstract**

24 Long-term changes in tropical insect communities are largely unknown, in particular for

25 mountain forest ecosystems, which is worrying given the ongoing environmental changes

26 including those concerning climate. We present a re-analysis of recent paper by Warne et al.

27 (2020), in which the authors reported changes in species richness, genetic diversity and trait

28 distributions in a cloud forest ant assemblage in response to climate change. We show that these

29 reported shifts over one decade are due to major differences between sampling periods in terms

30 of sampling methods and effort. We stress the need of a fully standardized methodology to

31 disentangle true climate change effects on communities from sampling bias.

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33 **Key words:** climate change, elevational gradients, sampling bias, tropical forests.

Main text

Anthropogenically driven climate change is a major ongoing threat to global biodiversity (Brondizio, Settele, & Díaz, 2019). Ants are a commonly used indicator taxon for monitoring changes along temperature gradients (Lach, Parr, & Abbott, 2010), which make them suitable model for 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, Hallwachs, & Janzen, 2014) despite to predictions that highland assemblages are likely to change most (Bishop et al., 2019). While the effects of habitat disturbance on ants have been well documented (Andersen, 2019), the long-term data to test for the 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 modelled 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 rainforest ant community via multiple resurveys over a decade at 850 m and did not find a directional trend (Donoso, 2017).

A recent study by Warne, Hallwachs, Janzen, & Smith (2020b) 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 (BIN) between historical (sampled between 1998-2001) and recent (i.e. 2008-2011) periods in a forest near the top of Volcán Cacao at 1500 m, a mountain in the Área de Conservación Guanacaste (ACG) in Costa Rica (Warne et al., 2020b). 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 and Powers (2020). While the impacts of climate change on biodiversity and ecosystems are

58 substantial and need to be thoroughly examined (Brondizio et al., 2019; Urban, 2015), it is vital

59 that the measurement of these impacts is done properly and account for any sources of

60 methodological bias. In this commentary, we present analyses demonstrating that sampling

61 biases and inconsistency can explain changes in ant diversity across time which were initially

62 interpreted as climate driven effects by Warne et al. (2020b).

63   We focus our critique on the interpretation of that study's outcomes, considering the

64 standardisation of the sampling methods and effort across the sampling periods. The authors

65 invest much less of their sampling effort in the recent period compared with the historical period

66 in terms of the sampling events (11 vs. 59) (Warne et al., 2020b). Furthermore, only Malaise

67 traps were used in the first sampling period (two traps), but five more sampling methods (bait,

68 hand-collecting, Winkler/Berlese, Davis sifters, and pitfall trapping) were used in addition to a

69 single Malaise trap in the recent period. This resulted in the most resurveyed ants being caught

70 by methods other than Malaise trapping (Figure 1a, Appendix S1). However, Warne *et al.* (2020)

71 did not consider any effects of these major differences in sampling methods and effort between

72 the time periods. This surprised us, as the fact that a Malaise trap samples rather a specific part of

73 fauna is well known, likewise that a different sampling effort biases the estimates of overall

74 diversity (see Longino & Colwell, 1997). In particular, Malaise traps are typically used to sample

75 the mobile, flying invertebrate fauna from different forest strata (Delabie et al., 2021). The other

76 methods used by Warne and colleagues (e.g. pitfall traps, litter sifting) instead focus on the

77 ground and leaf litter-dwelling fauna (Longino & Colwell, 1997). Comparing samples collected

78 using different methods is not a like-for-like comparison. Rather, observed differences may

79 simply be the result of the different biases and designs of the methodologies themselves.

80   Here we present a reanalysis of the authors' dataset (Warne, Hallwachs, Janzen, & Smith,

81   2020a), in which we account for these methodological discrepancies. We reassess the main

82   findings from Warne et al. (2020b) (cited text presented as subtitles). We show that the ant

83   assemblages appear to be then similar through time, rather than different. The details of our

84   analyses and the data used are presented in Figure 1 and the Supporting Information.

85

86   *Ant species richness was higher in the 1990s than in the 2000s.*

87   We demonstrate that if the different number of species incidences across the samples (i.e.

88   number of barcoded ant individuals) is taken into account, sampling in the recent period predicts

89   instead a higher richness (Figure 1a). Although the difference is not large (confidence intervals

90   overlap, as they do for the Chao2 estimates presented by Warne et al. (2020b)), higher richness

91   would be expected in the recent period because a greater number of sampling methods are used.

92   More importantly, the curves for the same method (Malaise trap) overlap. Hence the null

93   hypothesis that species richness does not change through time cannot be rejected.

94

95   *Species overlap between timeframes was low.*

96   If the species data (111 unique BIN) are split by time period and sampling method, there is a

97   near complete overlap in species composition between the two periods when comparing only

98   Malaise traps (Figure 1b). In other words, almost all novel species found in the recent period

99   were sampled by methods not used in the first time period (28 of 32 spp.).

100

101   *The species assemblage in the collections from the 1990s was significantly phylogenetically*

102   *clustered and functionally less diverse as compared to collections from the early 2000s.*

103   Warne et al. (2020b) focused mainly on the phylogenetic and functional measures of the ant

104   diversity. However, the differences in phylogenetic and functional diversity measures between

105   the two periods were rather small (and not significant for functional diversity). Furthermore,

106   these diversity measures are dependent on the number of species compared in each group (Table

107   S2a). The decrease in the Mean Nearest Taxon Distance (i.e. NTI index) was interpreted by

108   Warne et al. (2020b), as a stronger phylogenetic clustering in the historical than the recent

109   community. We confirmed that pattern for NTI, when only the assemblages sampled by Malaise

110   traps are compared (Table S2b). However, this index is more sensitive to clustering at the tips of

111   the phylogeny, while Mean Pairwise Distance (MPD) is more effective at capturing clustering at

112   deeper nodes (Kembel et al., 2010). We calculated SES_{MPD} and show that the latter measure

113   suggests a different conclusion: neither recent nor historical communities are clustered when

114   only Malaise traps (like for like comparison) are considered, but sampling by other methods (i.e.

115   addition of the taxa sampled from the litter) led to a phylogenetic clustering in the recent

116   community (Table S2c).

117

118   *The average lightness of the assemblage of cloud forest ant species became lighter through time.*

119   We show that for the Malaise trap assemblages lightness did not change through time (Figures

120   2a, S1 and S2). Recent work has demonstrated that ant communities are about twice as dark in

121   the forest understorey than on the ground (Law et al., 2020). As the sampling methods used

122   exclusively in the recent period are biased towards capturing the leaf-litter and ground-foraging

123   ant species (see above), we argue that the greater sampling of lighter coloured ants in that period

124   compared to the historical period is expected (Figure S1b). Thus, sampling bias can once again

125   explain this apparent assemblage-level change in lightness through time.

126

127   *Cloud forest ant communities are becoming more similar to communities from lower elevation.*

128   If we consider the elevational ranges of the individual ant species from the entire community

129   data for the cloud forest sites (Smith et al., 2014; Warne et al., 2020a), all sites and periods

130   contain a proportion of the species that extend their range across the whole elevational gradient,

131   as well as species which are unique to cloud forests (Figure 2b). However, the proportion of

132   high-elevation specialists is lower across all sampling methods at both of the cloud forest sites

133   (at 1300 m and 1500 m) than for Malaise traps in both studied periods at 1500 m site (Figure 2b).

134   The (slight) shift towards the mid-elevation communities presented in Figure 3 in Warne et al.

135   (2020b) might hence be explained by differences in sampling methods between the two periods.

136

137   *Gains and losses of particular taxa*

138   The genera lost from the historical period and the genera gained in the recent period, each made

139   up a minority of the sampled (barcoded) individuals (~2.5%, Figure 2b). Moreover, some of the

140   species lost from the historical period were found at the lower elevations at Cacao gradient

141   (Table S1), which would not be expected if these are high elevation species that are being pushed

142   off the top of their elevational ranges. For the two case study genera (*Leptogenys* and *Wasmania*)

143   highlighted in Warne et al. (2020b), only *Leptogenys* MAS002 was limited to cloud forest. This

144   species, not resampled in the recent period, was a singleton in the historical period, which is

145   weak evidence for extinction. The second, *Wasmannia* MAS001 (three individuals sampled in

146   the recent only), is a native species to the ACG region, widespread along the low and mid

147   elevations (Ratnasingham & Hebert, 2007) (Table S1). A possibility that both species are rare in

148   Cacao Volcán, but may be naturally co-occurring in both time periods cannot be excluded.

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Discussion and perspective

152 While climatically driven elevational shifts have been documented for some invertebrate

153 groups (e.g. moths moving up by 67 m in 40 years (Chen et al., 2009)), recent studies highlight

154 the importance of consistent sampling methods and timing: if such methodological variance is

155 considered, range shifts can appear to be less pronounced (Cheng et al., 2019) or driven by

156 natural seasonality (Maicher et al., 2020). For cloud forest ant assemblages, several recent

157 studies that consider both habitat change and elevation found that the mountain assemblages are

158 more robust to vegetation changes than their lowland counterparts (Hethcoat et al., 2019; Mottl

159 et al., 2019). This paints a more optimistic picture that mountain ants in these tropical

160 environments might be perhaps also less sensitive to climatic changes. Indeed, our reanalysis of

161 Warne et al. suggests rather surprising stability in the Neotropical mountain ant community at

162 this single site in contrast to a high (but undirectional) variation in the lower elevations (Donoso,

163 2017). However, more data are needed on the topic, as the Warne et al. dataset describes only a

164 single location and hence power to detect trends at large spatial scales is likely to be low.

165 Furthermore, climate related changes are expected to accelerate through time (Janzen &

166 Hallwachs, 2020; Urban, 2015).

167 Our motivation for writing this commentary is to ensure that the ways that humans affect

168 natural ecosystems are robustly documented in the scientific literature. If we are to combat

169 ongoing threats such as climate change, we need a correct understanding of how and why

170 ecosystems are changing. It would not be good for conservation if flawed studies are held up as

171 flagships of climate change science. We would like to stress that as insect ecologists we are all

172 very much concerned by the effects of humans on natural ecosystems. However, it is vital that

173 climate change research is rigorous and as free from methodological bias as possible if we are to

174 convince politicians to protect unique tropical ecosystems. While the climate change in the ACG

175 may have already started to change cloud forest ecosystems, the wording "*we have not been able*

176 *to measure this drying in any particularly "scientific" way; when the house is burning, a*

177 *thermometer is not the thing to call for*" (Janzen & Hallwachs, 2020) potentially gives powerful

178 ammunition to climate change sceptics. Likewise "*happy accidents of collection*" (Warne et al.,

179 2020b) taken post hoc and not carefully analysed may have a similar effect. We suggest that

180 calling for a thermometer is more important than ever. The detailed documentation of ant and

181 other insect communities at ACG is unique for a super-diverse tropical region, and offers an

182 opportunity to conduct a much more rigorous comparison, probably with less effort than would

183 be possible in other, less accessible, little known, pristine tropical rainforest communities

184 elsewhere. Future studies should build on such an opportunity by conducting ongoing resampling

185 of the sites using the same methodology. Only by doing this can we all convince the world that
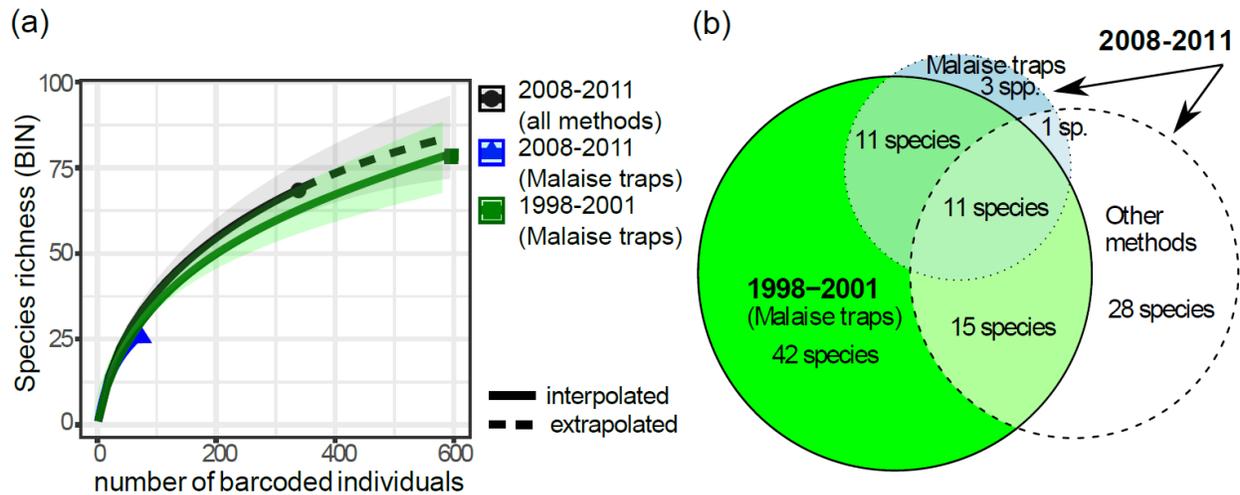
186 our shared global house is burning.

187

188 **Acknowledgements**

Figures and legends:

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. (2020a). 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.

(2020b) 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).
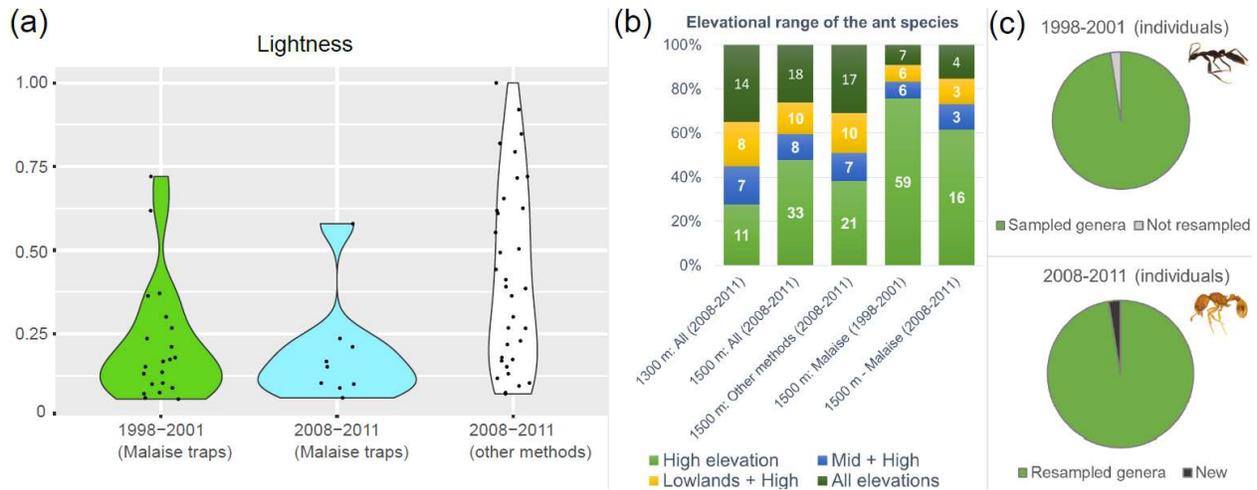
Figure 2 Changes in ant species' lightness and composition between historical (1998-2001) and

recent (2008-2011) periods in Cacao Volcán. (a) Lightness violin plot following Figure 2 from

Warne et al. (2020b) 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

Supplementary Information Figure S1 and 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. (2020b). 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. (2020b) 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).

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