Dear editor and reviewers,

We read carefully your comments and tried to answer all your requests. The text was modified accordingly.

Reviewer 1 (Alex Smith)

We have done all the modifications, corrections suggested by the reviewer except for the one bellow since we did not understand what the reviewer wanted or meant. The sampling started at 200m not at 1000m as we explained in the material and method.

One element that I think needs to be included in your discussion relates to your use of LOW or LOWEST and the elevational range you sampled. "At the lowest elevation (< 1000 m) the" and "The greatest plant diversity occurs at the lowest elevations with three other pla" – I think that some comment on the author's capacity to identify mid-elevational peaks when sampling begins at 1000m is warranted. This may, in fact, be a mid-elevation peak with the lowest elevation sites not present in the inventory.

I believe that what Dr. Smith is referring to here relates to wording as per the fragment that he quoted. A more complete quote from your paper is:

"At the lowest elevation (< 1000 m) the tropical rainforest is dominated by Dipterocarpaceae, the average daily temperature fluctuates between 25 and 30°C, and rainfall is greater than 4000 mm/year."

The use of "low" and "lowest" inconsistently is confusing for the reader. So, a sentence like the one shown here can perhaps be better written as:

"At elevations lower than 1000 m the tropical rainforest is dominated by Dipterocarpaceae, the average daily temperature fluctuates between 25 and 30°C, and rainfall is greater than 4000 mm/year."

I think that this is the main incident of this issue, though the authors may find more as they complete their editing.

[quote from Rahbek (2005): For example, a hump-shaped altitudinal species-richness pattern is the most typical (c. 50%), with a monotonic decreasing pattern (c. 25%) also frequently reported, but the relative distribution of patterns changes readily with spatial grain and extent. "About half of the studies evaluated" – be specific with the percentage.

Reviewer 2 (Gunnar Brehm)

All requests and corrections of the reviewer were followed, and the text was modified according to them. When the reviewer asked us some further justifications we answered them in bracket bellow

Concerning the improvement of our English, we believe that our American colleague and coauthor Steve Wilson know how to write his mother tongue.

### Introduction:

[Second sentence is a transition leading the reader into the remainder or the paragraph ..] Currently is not well focused, lacks important references and a clear hypothesis to be tested. For example, the second sentence is more or less meaningless ('One type of pattern that emerges is the change in species richness and differences in the composition of insect communities with increasing altitude').

Authors' changes have improved the introduction.

[see Fig 4B, E ... for example] I cannot think of any environmental gradient that will not result in such a change.

Can you point to others, besides in this study, that give similar evidence?

Suggested: Rewrite and focus, for example like this: 1) rain forests are very species rich ["The high organismic diversity of tropical rainforests ... "], 2) elevational gradients even increase regional diversity [... or not ... "One type of pattern ..." is a transition leading to "3)"], 3) the most common pattern found are hump shaped patterns or linear decreases (Rahbek etc.), but there are exceptions (increase, constant diversity) [we note the different patterns],

[I have not been able to find any other than those we already cited ... 5) continue with line 46 but find more references of current work conducted at Mt. Wilhelm (Novotny etc.); there have certainly been some very important studies on herbivorous insects in PNG in the last years.

With a quick search, I found at least one that's not cited here:

Novotny, V., Miller, S. E., Basset, Y., Cizek, L., Darrow, K., Kaupa, B., Kua, J. and Weiblen, G. D. (2005), An altitudinal comparison of caterpillar (Lepidoptera) assemblages on Ficus trees in Papua New Guinea. Journal of Biogeography, 32: 1303–1314. doi: 10.1111/j. 1365-2699.2005.01225.x

Perhaps there are others?

[I think I addressed this by mentioning that many are monophagous ... ] 6) from line 51 on: can you think of any group of herbivores in which feeding is not linked to the vegetation? I

cannot follow why Auchenorrhyncha are more suitable than other groups because 'they include species that feed xylem, phloem, or mesophyll'. Many groups of herbivores were used as 'indicators' and the arguments delivered are not convincing. What about specialisation? This could be an argument why a group is particularly closely linked to vegetation – everything else appears trivial. I cannot imagine that nobody has ever performed diversity studies of the study organisms in other places in the world. What were the results? What is then the expectation for PNG – use this as a working hypothesis.

Dr. Brehm asked a number of questions here. Could you answer them each specifically in terms of either how the MS currently addresses these issues (be specific), or in terms of why the stated concern is not actually concerning (again, please be specific).

**[Why? The point of the study was to examine species richness as numerous other studies have done]** Furthermore, the introduction does not introduce diversity measures that were performed in this study (concept of alpha diversity, beta diversity / turnover), and more meaningful measures must be applied.

- [Map was free and I modified it]: Figure 1: Map lacks longitude/latitude information. Better would be a map of the whole island of Guinea with the two major sampling areas, and an inserted map of Mt. Wilhelm with the precise location of the sampling sites in this area. There may be copyright issues with the graph as currently presented.
- [The spot selected in the forest for the sampling where located in the forest that was preserved]: forest: are all forst sites intact primary forests or are they disturbed to a varying extent?

This could be stated in the methods section with a single sentence. It is a valid concern, and helps to set the scene for our work. How were they preserved, etc.? If you just say that you chose areas that were intact primary forests (if that was the case) then it would provide the reader with good and useful information. It would also provide some historical data in terms of the current state of that forest.

- [true, but we did not examine correspondence between temperature and species richness, etc. Data from Leponce concerning the average temperature at the lowest elevation is 27.2°C which is as written in the text between 25 and 30°c. Those measurements were taken during the year 2010-2011] Temperature data are remarkably inprecise. There will be exact data from weather stations in PNG from similar sites (e.g. average temperature at 200 m a.s.l. is 25.2°C and not between 25 and 30°C), and this should be presented in a table.

I agree that the temperature descriptions in the paragraph could be written more precisely, indicating max., min., and mean daily temperatures. Similar to the above comment on a good

description of the site characteristics, this not only helps the reader to better understand it, but also may serve as historical information for the area.

- ["(Hope, 1976; Munzinger, 2013; Duvot, 2013)"] References are lacking or not precisely assigned to support information on the vegetation.
- [Yes, given in the material and method and in the supplementary data, code for each specimen is provided]: The sampling period was rather short. Is coverage similar in all samples? Data on coverage must be provided.

Please mention the supplementary data in the results section, and please provide a key for deciphering your coding for readers who are unfamiliar. I assume that dates can be ascertained from the codes? I also assume that the data will be deposited in this form with PeerJ? Or do you plan to send it to a DOI-based repository such as figshare or Dryad? If the latter (and that's not a bad idea), then be sure to include the DOI information in the text of your paper.

- [Parataxonomists from the Binatang research center were collecting the samples everyday in all the altitudes]:Logistics: How did you manage to to to every sampling location at every of the 16 days of the sampling period between 200 and 3700 m? Is there a road or a helicopter? Difficult to understand how this was possible (many field assistants?)

It's perhaps not vital to include this information, but it would be helpful for readers wondering how the work was done (perhaps planning similar work of their own). It also is one way of acknowledging the help that was received to make this possible.

- [pictures allowed to help us to distinguish the different morphospecies since most of them are likely to be new species and do not have any name yet.]: How could photographs of the insects facilitate the identification? Did other specialists check the material or was the material later re-examined?
- [the focus of the study was to specifically examine species richness ... results of Shannon-Weiner added Table 5]: Using simply observed species richness is certainly not sufficient. Tropical arthropod communites are usually undersampled (and certainly in this short-termed study), so other measures must be applied. Use for example EstimateS by Rob Colwell, freely available on the web, for rarefaction/extrapolation and think about using diversity measures.

I might mistaken, but I assume you mean Table 6 (as per the naming of your figure files and as mentioned in your text)?

.

- [data not available ... beyond scope of study ... ] Try to gather quantitative environmental data (temperature, tree species diversity, rainfall etc.) and consider to formally apply regression and / or multiple regression to find out which factor could be the most important.

Tables / Figures:

[constructed graph but not as informative and requires too much information in figure caption] Table 3 should be shown as graph.

### Other notes:

- -Perhaps I'm missing something in all of the files, but I cannot find any of the figure or table headings.
- -I assume that the specimens have been vouchered at the Muséum National d'Histoire Naturelle? This is not clear from the text (I am only reading between the lines). Could you specify vouchering please?

Elevational Gradient of Hemiptera (Heteroptera, Auchenorrhyncha) on a Tropical Mountain in Papua New Guinea

Running Title: Hemiptera in Papua New Guinea

**Keywords:** biodiversity, Cicadomorpha, elevational gradient, Fulgoromorpha,

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### **ABSTRACT**

Malaise trap sampling of Hemiptera (Heteroptera; Auchenorrhyncha) was conducted at 500 m intervals along an elevational gradient from 200 m to 3700 m on the east slope of Mount Wilhelm, Madang Province, Papua New Guinea. Hemiptera morphospecies richness and overall abundance decreased with increasing elevation, however, the Heteroptera did not exhibit either pattern. A few species were relatively abundant at each elevation whereas the majority of species were represented by  $\leq 5$  specimens. Morphospecies richness of Auchenorrhyncha, Cicadomorpha, Fulgoromorpha, Cicadellidae, Cixiidae, and Derbidae also decreased with increasing elevation but abundance decline was not significant due to the large number of specimens captured at 200 m relative to those captured at higher elevations. The percentage of Cicadomorpha specimens decreased with increasing elevation relative to that of the Fulgoromorpha which increased with increasing elevation. Environmental factors that may influence patterns of species richness along the elevational gradient are discussed.

### INTRODUCTION

The high organismic diversity of tropical rainforests has been the focus of numerous studies including those that document the diversity of selected taxa and others that seek to elucidate patterns. One type of pattern that emerges is the change in species richness and differences in the composition of insect communities with increasing elevation (Whittaker, 1952; Brühl *et al.*, 1999; Van Ingen *et al.*, 2008). Some studies found a decrease in species richness with increasing elevation (Hunter & Yonzon, 1992; Vázquez & Givnish, 1998) whereas some found the opposite – an increase in species richness with increasing elevation (Sanders *et al.*, 2003; Hodkinson, 2005). About half of the studies evaluated by Rahbek (2005) indicate that species richness increases, reaches a peak, then declines with increasing elevation, but this or other patterns can result from differences onin spatial grain and sampling methodology (Janzen *et al.*, 1976; Grytnes & Vetaas, 2002; Grytnes *et al.*, 2006; Wiens *et al.*, 2007; Guo *et al.*, 2013). Suggested reasons for these patterns include size of the habitat; isolation from similar communities; primary productivity as affected by temperature, length of the growing season, organism response to changing environmental conditions, and resource and habitat suitability (Hodkinson, 2005; McCain & Grytnes, 2010)

Papua New Guinea, which has the third largest expanse of tropical rainforest in the world (Brooks *et al.*, 2006), provides opportunities to examine patterns of species richness and elevation in numerous taxa. Mount Wilhelm (4509 m) has been the focus of studies of plant communities and elevational gradients (Brass, 1964; Hope, 1976; Munzinger *et al.*, 2013), but few studies of the structure of phytophagous insect communities relative to their host plants have been conducted (Dem, 2011).

Studies of taxa within the Hemiptera can provide useful insights about the ecological bases for distribution. Members of the Suborder Auchenorrhyncha are particularly suitable for

study because, with the exception of some fungivores, almost all species are sap-feeders on xylem, phloem, or mesophyll as larvae and adults (Tonkyn & Whitcomb, 1987; Della Giustina, 1989; McKamey, 1999; Attié *et al.*, 2008). Furthermore, they are basal heterotrophs which, relative to their host plant associations, have been treated as members of sap-feeding guilds (Denno, 1980), and many are monophagous or have a limited host plant range (Wilson *et al.*, 1994). As well, measures of their species richness and diversity have been used as indicators of habitat quality – the "Auchenorrhyncha Quality Index" (Wallner *et al.*, 2012; Spagnolo *et al.*, 2014). However, relatively few studies have focused on the Auchenorrhyncha (McCoy 1990; Novotný 1992, 1993; McKamey 2010).

Studies of biodiversity and elevational gradients are "natural experiments" that can evaluate ecological theories on climate change as they are keys to understanding how changes in abiotic factors, especially temperature, can affect faunal and floral distribution (Guo *et al.*, 2013; Sundqvist *et al.*, 2013).

The focus of our study is to document the distribution of Auchenorrhyncha and Heteroptera along a rainforest elevational gradient, to determine the effect of elevation on species richness and abundance, and to discuss the factors affecting distribution.

### **MATERIALS AND METHODS**

**Study area**. The study was conducted along an elevational transect on the northeast aspect of Mount Wilhelm in Papua New-Guinea (Figure 1). The transect followed the crests of the east slope of the mountain from 5°44′14.89″S, 145°19′56.13″E to 5°47′27.23″S, 145°3′29.58″E and began at 200 m elevation and extended to 3700 m (Table 1), which represents the limit of

the forest. The zonation of vegetation along the mountain slope (Hope 1976) corresponds to changes in temperature and humidity. At the lowest elevation (elevations less than< 1000 m) the tropical rainforest is dominated by Dipterocarpaceae, the average daily temperature fluctuates between 25 and 30°C, and rainfall is greater than 4000 mm/year. Between 1000 m and 2500 m, Lauraceae and Fagaceae are dominant and the average daily temperature ranges from 15 to 20°C. From 2500 m to 3000 m, Podocarpaceae become increasingly abundant and the average daily temperature is <12°C. Above 3000 m the sub-alpine vegetation is dominated by tree ferns, Cyatheaceae, the average daily temperature is ca. 8°C, and rainfall is < 3400 mm/year (Hope, 1976; Munzinger, 2013; Duvot, 2013).

**Study material**. We focused on collecting specimens of the hemipteran suborders Heteroptera and Auchenorrhyncha, although we also collected a few Sternorrhyncha (Table 2). The Auchenorrhyncha consists of the Fulgoromorpha (planthoppers) with 21 families, and the Cicadomorpha (leafhoppers, froghoppers, treehoppers, and cicadas) with 12 families (Cryan, 2005; Bourgoin, 2013; Soulier-Perkins, 2013), one of which, the Cicadellidae (leafhoppers), represented the majority of collected specimens.

Sampling method. Sampling was conducted for 16 days, from 25 October to 10 November 2012 at Seight sites placed every 500 m along the elevational transect on the east aspect of Mount Wilhelm. Four Malaise traps (Gibb & Oseto, 2006) were placed at random at each site; after placing- the first Malaise trap the three others were set up every 100 m following the same contour line. After we observed numerous ants on the Malaise traps set up at 200 m, we established a ninth sampling site employing the same protocol at Wanang from 18 November to 4 December at 200 m in order to provide samples untouched by ants, if needed. The contents of each trap were collected each day and preserved in 90% ethyl alcohol and placed in a zip-lock bag. The material was sorted to family before being exported from Papua New Guinea under permit number 012297.

All specimens were examined at the Muséum National d'Histoire Naturelle (Paris, France) using a Leica MZ16 stereo microscope and identified to morphospecies which is a useful means of identifying large numbers of specimens for ecological studies (Oliver & Beattie, 1993; New, 1998). Photographs using a Canon EOS 50D of representatives of each morphospecies were taken in order to facilitate identification. Recognition of morphospecies was based on morphological characters of the head, thorax, abdomen and legs.

**Data analysis**. The relationship between elevation and morphospecies richness and abundance was examined using Pearson product moment correlations (Roscoe, 1975) and the Shannon-Weiner Diversity Index (Krebs, 1989). A factorial correspondence analysis (FCA) was also used (ade4 package, Dray & Dufour, 2007) in R, version 3.0.2 (R development Core Team, 2013) to study the arrangement of morphospecies along the elevational gradient (Benzécri, 1964). For this multivariate analysis, each morphospecies was coded 1 if present and 0 if not; each line represented a trap and each column a morphospecies.

## **RESULTS**

In total, 4,205 specimens were sorted and 713 morphospecies identified; 3,318 specimens representing 596 morphospecies were from the collecting stations on Mount Wilhelm, the remainder were collected in Wanang and used as reference material for our study (Table 2).

**Morphospecies distribution**. A succession of morphospecies was observed along the elevational gradient. Each morphospecies was rarely collected at more than one elevation (Table 3); one cicadellid species was collected from 200 to 2200 m but in diminishing numbers. From 6% to 19% of the morphospecies consisted of more than five5 specimens at any given

elevation whereas 81% to 94% of morphospecies were represented by five5 or fewer specimens (Table 4).

Analysis via Factorial Components Analysis suggested that there was a succession of morphospecies along the elevational gradient and that there were few species that occurred at more than one elevation (Table 3; Figures 2, 3).

Morphospecies richness. Overall species richness declined with increasing elevation (Figure 4A; Table 5). Examining the relationship between elevation and each taxon revealed that there was no relationship between elevation and Heteroptera species richness (Figure 4B). A pattern of decreasing richness with increasing elevation was found for the Auchenorrhyncha (Figure 4C) as well as for the Cicadomorpha, Fulgoromorpha, Cixiidae, and Derbidae (Table 5).

Morphospecies abundance. The number of specimens captured by the traps appeared to decrease with increasing elevation; however, the correlation was not significant (Figure 4D; Table 5). As with species richness, no relationship was found between elevation and the abundance of Heteroptera (Figure 4E). Similarly, species abundance appeared to decrease with increasing elevation for the Auchenorrhyncha (Figure 4F), the Cicadomorpha, Fulgoromorpha, Cicadellidae, Cixiidae, and Derbidae (Table 5); however, the correlations were not significant. The large number of specimens captured at 200 m relative to those captured at higher elevations resulted in correlations that were not significant.

**Shannon-Weiner Diversity Indices.** The highest diversity indices were at the two lowest elevations, which corresponds to the patterns of morphospecies richness and abundance (Figs. 4A, D; Table 6). Between 1200 m and 3700 m the diversity indices increased then declined.

Cicadomorpha and Fulgoromorpha species richness. As indicated above, the Auchenor-rhyncha species richness decreased with increasing elevation which led us to further examine species richness patterns in the Cicadomorpha and Fulgoromorpha. Comparison of the proportions of Cicadomorpha relative to Fulgoromorpha suggested that the proportion of Fulgoromorpha increased with increasing elevation. The number of cicadomorph specimens collected at 3200 m appears to refute this suggestion; however, one cicadellid morphospecies represented 73.5% of all Cicadomorpha collected at this elevation. After removing this morphospecies from the analyses, we found that the Fulgoromorpha represented an increasing proportion of Auchenorrhyncha from ca. 10% at the lowest elevations to ca. 40% at 2700 m (Figure 5).

### DISCUSSION

Morphospecies distribution, richness, and abundance. Factorial Components Analysis indicated that there was a sequence of morphospecies corresponding to the elevational gradient of Mount Wilhelm. There was a -negative correlation of species richness and elevation for the Hemiptera. There was no relationship between species richness and elevation for the Heteroptera, which may be because of weak association with plant taxa as some were polyphagous and others predaceous (Schuh & Slater, 1995). The Auchenorrhyncha, Cicadomorpha, Fulgoromorpha, Cicadellidae, Cixiidae, and Derbidae all had the highest species richness at the lowest elevation and richness generally decreased with increasing elevation. As noted above, Auchenorrhyncha species are phytophagous (Mitter *et al.*, 1988), Cicadomorpha are phloem, xylem, or mesophyll feeders, Fulgoromorpha feed on phloem or fungi (Tonkyn & Whitecomb 1987; Wilson *et al.*, 1994) and numerous species of these taxa are mono- or oligophagous (Wilson *et al.*, 1994; Attié *et al.*, 2008). The greatest plant diversity occurred at the lowest elevations with three other plant communities occurring at ca. 1000,

2500, and 3000 meters in elevation, respectively (Hope, 1976; Hodkinson, 2005). Differences in plant diversity and communities are likely factors that explain, in part, the observed elevation gradient in species richness.

Abiotic factors that may explain the distribution of the hemipteran taxa include the climatic changes that occur with increasing elevation. Temperature and rainfall decrease significantly from 25-30°C and ca. 4000 mm/year at lower elevations to <8°C and ca. 3400 mm/year at the highest elevation. These factors directly affect insect development and survival and correspond to the zonation of the vegetation which indirectly affects the distribution of hemipterans (McCain & Grytnes, 2010; Régnière et al., 2012; Savopoulou-Soultani et al., 2012). Species richness decreased with increasing elevation which is similar to patterns observed in several studies (Hunter & Yonzon, 1992; Vázquez & Givnish, 1998). The abiotic factors cited above can explain, in part, the decrease in species richness with increasing elevation that we observed. Although species richness generally decreased with elevation, there were slight increases in richness at 2200 m and 3200 m (Figures 4A, C). An increase in hemipteran species richness at these two elevations was also observed by Dem (2011). This humpshaped pattern was also inferred from the Shannon-Weiner Diversity Indices between 1200 and 3700 m (Table 6) and has been found in more detailed studies of other insect taxa (Mc-Coy, 1990; Brehm et al., 2007). Slightly higher species richness at these elevations could correspond to regions where plant communities from lower and higher elevations intergrade, or it could be a response to the distribution of insectivores (Sam et al., 2014). At every taxonomic level evaluated there was no correlation between abundance and elevation (Figures 4D, 4F) with very large numbers of specimens captured at the lowest elevations and substantially fewer at higher elevations (except for the Heteroptera). For the Auchenorrhyncha species abundance increased at 2200 m and 3200 m (Figure 4F) which could corre-

spond to regions where plant communities from lower and higher elevations intergrade.

Species richness and elevation and sampling methodology. Malaise trap sampling is a very effective means of sampling a portion of an insect community, but as with any single collecting technique, it cannot provide a complete survey of the insect fauna (Leather & Watt, 2005; Ozanne, 2005). Apterous and brachypterous insects, those that do not leave their host plants, and those that live in the forest canopy are less likely to be captured in the traps. Placement of traps in areas where vegetation is too dense or too sparse will affect capture rate (Ozanne, 2005). This was addressed in our study by random placement of the four traps at each site with the expectation that traps placed in areas with an adequate representation of the hemipteran fauna will compensate for those placed in less suitable areas (Smith, 2013). Papua New Guinea has a tropical climate with alternating wet and dry seasons. Accurate sampling of hemipterans is a function of the linkage of life cycles to this seasonality. We collected for a short period of time; however, it was done during the optimum collecting period for planthoppers and leafhoppers (Novotny & Basset, 1998).

Cicadomorpha and Fulgoromorpha species richness and abundance. The Cicadomorpha and Fulgoromorpha were the dominant taxa in terms of species richness and abundance (Table 2). The relative percentages of the numbers of Cicadomorpha and Fulgoromorpha collected at each elevation (Figure 5) indicated that the proportion of Cicadomorpha decreased with increasing elevation whereas the Fulgoromorpha increased. However in order to show this general pattern, one morphospecies of cicadellid was removed from the analysis. Its outbreaks in the Malaise traps tended to mask the inversely proportional tendencies observed between Cicadomorpha and Fulgoromorpha along the altitudinal gradient.

In the Cicadomorpha, the Cicadellidae consisted of 80% of all Hemiptera collected, f. In addition few Cercopidae, only one Aphrophoridae, two Membracidae, and three Cicadidae were collected.

In the Fulgoromorpha, the families with the highest species richness and abundance were the Achilidae, Cixiidae, and Derbidae. The remaining six families included substantially fewer morphospecies and individuals (Table 2). The Achilidae have been associated with species in 27 families of plants, the Cixiidae with species in 88 families, and the Derbidae with species in 28 families (Wilson et al., 1994; Bourgoin, 2013). Three of the plant families associated with Cixiidae and Derbidae, Cyatheaceae, Podocarpaceae and Fagacae, are major components of the three highest plant communities along our elevational transect.

As well, these three planthopper families, which represented 79% of morphospecies (N = 170) and 90% (N = 404) of individual planthoppers, have larvae that feed underground on plant roots (Cixidae) or, it is presumed, fungal hyphae (Achilidae, Derbidae) (Wilson et al. 1994).

Our inventory of Hemiptera along an elevational gradient on Mt. Wilhelm resulted in finding no pattern of morphospecies distribution and abundance among Heteroptera but declines in morphospecies richness with increasing elevation in the Auchenorrhyncha and its subgroups. The decreasing proportion of Cicadomorpha morphospecies relative to Fulgoromorpha with increasing elevation may be due to differences in host plant communities or larval habitats and therefore warrants further study.

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