

# Ground dwelling pygmy grasshoppers (Orthoptera: Tetrigidae) in Southeast Asian tropical freshwater swamp forest prefer wet microhabitats

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## Abstract

Tetrigidae are an ancient group of grasshoppers and, similar to many other insects, have associations and preferences for specific microhabitats and habitats. The ecology of pygmy grasshoppers in Southeast Asia is generally under studied, especially in threatened habitats such as freshwater swamp forests. A study in Nee Soon Swamp forest, Singapore, was conducted to investigate association of limno-terrestrial pygmy grasshoppers with waterbodies and microhabitat. Specifically, we looked at the abundance and species assemblage of all pygmy grasshoppers. We correlated the abundance with major gradients of variation summarizing substrate and vegetation types along belt transects where sampling was performed. We found that pygmy grasshoppers in general are associated with wetter microhabitat conditions rather than the main streams in the swamp forest (i.e., water bodies). This is despite differences in microhabitat conditions of belt transects nearer to and further away from the main streams. We also found that pygmy grasshopper abundance is associated with the wetness of dicot leaf litter. We inferred that the abundance of food resources and suitability for egg development may explain their preference for wet microhabitats. We also found that the same patterns applied for adults and juveniles, suggesting that there is no demographic difference or ontogenetic shift in microhabitat association. Lastly, the adult assemblage can also be correlated to microhabitats. Based on our findings, we propose that pygmy grasshoppers can also be suitable bio-indicators for the freshwater swamp forest, owing to their sensitivity to microhabitat conditions.

## Key words

abundance, ecology, limno-terrestrial, microhabitat association, Singapore

## Introduction

Insects are highly dependent on their environment. Plant communities within an area can vary in diversity, assemblage and productivity, and such heterogeneity can drive dynamics among arthropod communities and influence arthropod-plant interactions (e.g. Knops et al. 1999, Schaffers et al. 2008, Haddad et al. 2009). At a smaller scale, insects are also known to have preference for specific microhabitat conditions (e.g. Wardhaugh et al. 2013,

2014, Pincebourde and Casas 2015, Thom and Daniels 2017). Likewise, orthopterans have associations with vegetation and microhabitat conditions (Joern 1982, Badenhauer et al. 2015).

Orthoptera (grasshoppers, crickets and katydids) are among the largest group of terrestrial insects, comprising more than 27,000 described species (Cigliano et al. 2017). The Tetrigidae, or pygmy grasshoppers, are a monophyletic clade and forms a unique lineage among the Caelifera (grasshoppers) (Song et al. 2015). These grasshoppers are known to prefer moist areas and semi-aquatic habitats like marshes, margins of waterbodies and floodplains. Amédégnato and Devriese (2008) considered most species of pygmy grasshoppers as limno-terrestrial that require an aqueous matrix (moist areas) in strictly terrestrial habitats while some are more dependent on water (such as for egg laying and nymphal development). They appear to be good swimmers and/or divers (Paranjape et al. 1987, Gröning et al. 2007, Amédégnato and Devriese 2008). Different levels of dependency on water correspond to varying degrees of adaptations to swimming, including expanded hind tibia with reduced spines in more water dependent species (Amédégnato and Devriese 2008). Being a group of terrestrial invertebrates which are associated with water, they thus provide an important trophic exchange between aquatic and terrestrial food webs (Bastow et al. 2002, Balian et al. 2008).

However, there is still a dearth of information on the pygmy grasshoppers from Southeast Asia. This is made worse with the taxonomy being particularly problematic, rendering accurate species identification difficult (Tumbrinck 2014). Most of our current understanding of the ecology of pygmy grasshoppers is limited to their diet, ranging from observational (e.g. Paranjape and Bhalerao 1985, Blackith and Blackith 1987, Reynolds et al. 1988) to quantitative data (e.g. Kuřavová et al. 2017b). Quantitative studies on the habitat/microhabitat association of pygmy grasshoppers are rare and restricted to a few species (e.g. Hochkirch et al. 2000, Gröning et al. 2007, Kuřavová and Kočárek 2015, Musiolek and Kočárek 2016), and even fewer in Southeast Asia. Southeast Asia consists of three biodiversity hotspots (Myers et al. 2000), and is threatened by large-scale deforestation and land use conversion (Brooks et al. 2002, Sodhi et al. 2010, Wilcove et al. 2013). At the same time, Southeast Asia is also rich in orthopter-

ans, possibly equally affected by the crisis as are other organisms (Tan et al. 2017a). Along with fragmentary understanding of the orthopteran diversity in Southeast Asia (Tan et al. 2017a), there is a pressing need to study the ecology of Southeast Asian orthopterans.

Here, an attempt was made to investigate the association of the Southeast Asian pygmy grasshoppers with microhabitats and proximity to water bodies. Specifically, such ecological association of the pygmy grasshoppers from freshwater swamp forest were examined for the first time. Studies on pygmy grasshoppers in aquatic habitats focus mostly on species occurring in salt marshes, ponds and rivers (e.g. Hochkirch et al. 2000, Gröning et al. 2007, Amédégno and Devriese 2008). There has not been a study on species from a tropical region and hence tropical freshwater swamp forests up to now. Our study of the water association of the pygmy grasshoppers can provide us with a better understanding of the ecological preferences and association of these tropical orthopterans.

To investigate the association of the pygmy grasshoppers with the microhabitats in the freshwater swamp forests, two hypotheses were tested. Firstly, we investigated if the pygmy grasshoppers in freshwater swamp forest were associated with the water body by comparing the abundance of pygmy grasshoppers near vs. further away from the main streams. If the unique semi-aquatic lifestyle of the pygmy grasshoppers required a close association with existing waterbodies in the swamp forest, this would suggest they may tend to concentrate near streams. Secondly, we also aimed to investigate if the pygmy grasshoppers preferred specific microhabitat conditions. The microhabitats were quantified by a series of vegetation and surface types, as well as surface temperature and relative humidity. Nee Soon Swamp Forest (NSSF), Singapore, was used as the study site. NSSF is the last remnant of freshwater swamp forest in Singapore but remains ecologically significant (Ng and Lim 1992, O'Dempsey and Chew 2013). It also holds unique and endemic biodiversity (e.g. Gorochoy and Tan 2012, Jäch et al. 2013). As the pygmy grasshoppers were usually small and had small dispersal range, we expected that they would form close associations with their preferred microhabitats.

## Materials and methods

**Study area.**— Four sites, situated along the main stream within the NSSF were opportunistically sampled. In each site, eight 20 × 10 m belt transects were opportunistically demarcated based on accessibility. Within each site, four belt transects were situated along the stream banks less than 1 m away from the main stream and four other belt transects were situated at least 5 m from the main stream. In total, 32 belt transects were surveyed. Each belt transect was surveyed once.

**Sampling of pygmy grasshoppers.**— Sampling was conducted between late August 2013 and early February 2014, during the cooler and wetter part of the year in Singapore. Surveys were conducted between 7:30 pm (after last light) and 10 pm. Nocturnal survey was found to be more appropriate for representative estimation of pygmy grasshopper abundance as they are more easily sighted at night than in the day. Two belt transects from a single site were opportunistically sampled per survey night. Each site was surveyed across the entire span of the sampling period, rather than restricted to a single month, to minimize the effect of temporal autocorrelation. Each belt transect was surveyed systematically for 20 min by two equally trained and efficient surveyors (MKT and HY) working together at the same time, from one end of the belt transect to the other. Adults and juveniles were actively searched using both headlamps and hand torches. The ground, low-lying vegetation and dead logs were searched. Pygmy grasshoppers were thor-

oughly sampled within the time frame and belt transect. Abundance was quantified as the total number of individuals found in each belt transect. Adult specimens were tentatively identified to morpho-species because Tetrigidae in Singapore are generally not well studied with species delimitation remaining unresolved and many species undescribed (Tan et al. 2017b). All adults were likely to be capable of flight owing to the presence of developed hind wings whereas the nymphs were flightless.

**Microhabitat quantification.**— At every 1 m along the belt transect, the surface and vegetation types were recorded. Surface types include: (1) dry dicot leaf litter, (2) wet dicot leaf litter, (3) dry muddy (fine and claying substrate), (4) wet muddy, (5) dry sandy (coarser and porous substrate), (6) wet sandy, (7) gravel and rocks, (8) wet dead woody structure (i.e. log and exposed roots), (9) dry woody structure (i.e. log, trunk and exposed roots), (10) water-logged, (11) wet ceramics (e.g. broken ceramic tiles left over from past settlement) and (12) dry monocot leaf litter. We distinguished dicot leaf litter, muddy surface and sandy surface between wet and dry. For surface types 7 to 12, they were always either wet or dry and hence not distinguished between wet and dry. We summarized different plant forms as vegetation types that included: (1) herbaceous plants and grass, (2) woody plants (i.e. trees, woody shrub), (3) creepers and vines, (4) rattans (including *Pandanus*), (5) ferns and (6) palms. The prominence of each microhabitat (both surface and vegetation type) was quantified by counting the number of times they were recorded within the belt transect. Within each belt transect, the ambient surface temperature and ambient surface relative humidity were also recorded three times, at approximately the two ends and midpoint, and averaged.

**Data analyses.**— As the different types of substrate and vegetation were not mutually exclusive, they were likely to be highly intercorrelated. To summarize substrate and vegetation into major gradients of variation, non-metric multidimensional scaling (NMDS) using Bray-Curtis distance was performed using the 'metaMDS' function implemented with the community package vegan version 2.3-1 (Oksanen et al. 2015). Stress values of 0.20 and below were deemed acceptable for interpretation of patterns (Clarke 1993), and where this was exceeded, the plots were ordinated on three dimensions. NMDS axes representing the microhabitat variation were labelled Microhabitat NMDS1 and Microhabitat NMDS2. The scores for the NMDS axes were scaled and checked for collinearity. Variance inflation factor (vif) values of below 3.0 were deemed acceptable (Zuur et al. 2010).

To test if the pygmy grasshoppers were associated with specific microhabitat axes, the abundance of pygmy grasshoppers was fitted as a generalized linear mixed-effects model using Poisson error structure using the 'glmer' function. We included the scores on the two scaled NMDS axes and proximity to main stream (either close or far) as fixed effects and the site location as random effect.

We postulated that pygmy grasshoppers had habitat preferences, and thus occurred at higher abundances associated with specific microhabitat conditions. Additionally, owing to their preference for aquatic habitat, we also postulated that more pygmy grasshoppers would be found on belt transects closer to the main stream. The following models were proposed and ranked accordingly to Akaike information criterion with adjustments (AICc) using the 'MuMIn' package (Barton and Barton 2015):

1. ~ Microhabitat NMDS1
2. ~ Microhabitat NMDS2

3. ~ Microhabitat NMDS1 + Microhabitat NMDS2
4. ~ Proximity to main stream
5. ~ Microhabitat NMDS1 + Proximity to main stream
6. ~ Microhabitat NMDS2 + Proximity to main stream
7. ~ 1 (null model)

We interpreted the models with delta (difference in the AICc of a particular model and that of the best model) less than 2.0 (Burnham and Anderson 2002) in tandem. Subsequently, we also selected surface and vegetation types based on the NMDS plot that might have been important to explain the abundance. To better understand how each of these environmental variables correlated with the abundance, we proposed various models containing each of these environmental variables as fixed effect and ranked accordingly to Akaike information criterion with adjustments (AICc).

Since adults and juveniles could have different microhabitat requirements and dispersal abilities, we performed separately the same analyses using the subset of (i) adult and (ii) juvenile abundances to investigate if the same patterns persisted. The assemblage (or beta-diversity) of adults were correlated with major gradients of variation in microhabitat conditions (i.e., Microhabitat NMDS1 and NMDS2). This was done by performing canonical analysis of principal coordinates (CAP) with Euclidean distance using the 'capscale' function implemented with the community package vegan version 2.3-1 (Oksanen et al. 2015).

All statistical analyses were done in the R software version 3.3.3 (R Development Core Team 2017).

## Results

A total of 94 adult and juvenile pygmy grasshoppers were collected during the sampling of 32 belt transects (19 from Site 1 (= Woodcutter Trail), 13 from Site 2 (= Nee Soon interior), 28 from Site 3 (= Nee Soon main pond) and 13 from Site 4 (= Upper Seletar). In total, 62 adults and 32 juveniles were collected. We identified the adults into six morpho-species, with one dominant morpho-species. All morpho-species were collected across the entire sampling period and there appeared to be no clear evidence of species turnover with time.

The first two scaled NMDS axes summarized the microhabitat conditions (including vegetation and surface types) and had a stress value of 0.17 (below the threshold of 2.0), indicating that they were sufficient to represent the variation in the microhabitat conditions (Fig. 1). Subsequently, we obtained the scores for the two axes to test our hypotheses. The Microhabitat NMDS1 showed that belt transects with wetter microhabitats (waterlogged, wet muddy and wet dicot leaf litter) had higher scores than those with drier microhabitats (dry sandy, dry muddy and dry dicot leaf litter). The Microhabitat NMDS2 showed that belt transects with coarser surface type (i.e. sand, tiles, rock and gravel) had a lower score than those with finer surface type (i.e. mud). Microhabitat NMDS2 also summarized the vegetation types. A PERMANOVA also showed that the microhabitat conditions were dissimilar between the belt transects nearer to the main streams and those further away ( $F$ -value = 4.81,  $R^2$  = 0.14,  $p$ -value = 0.003) (Fig. 2).

When we correlated the total abundance of pygmy grasshoppers with the NMDS axes and proximity to main streams, the two best models with delta less than 2.0 both had NMDS1 as fixed effect (Table 1). The best model also had NMDS2 as fixed effect. The best model explained 35% of variance, about 10% more than that explained by the second best model (Table 1). Total abundance had a strong positive relationship with Microhabitat NMDS1

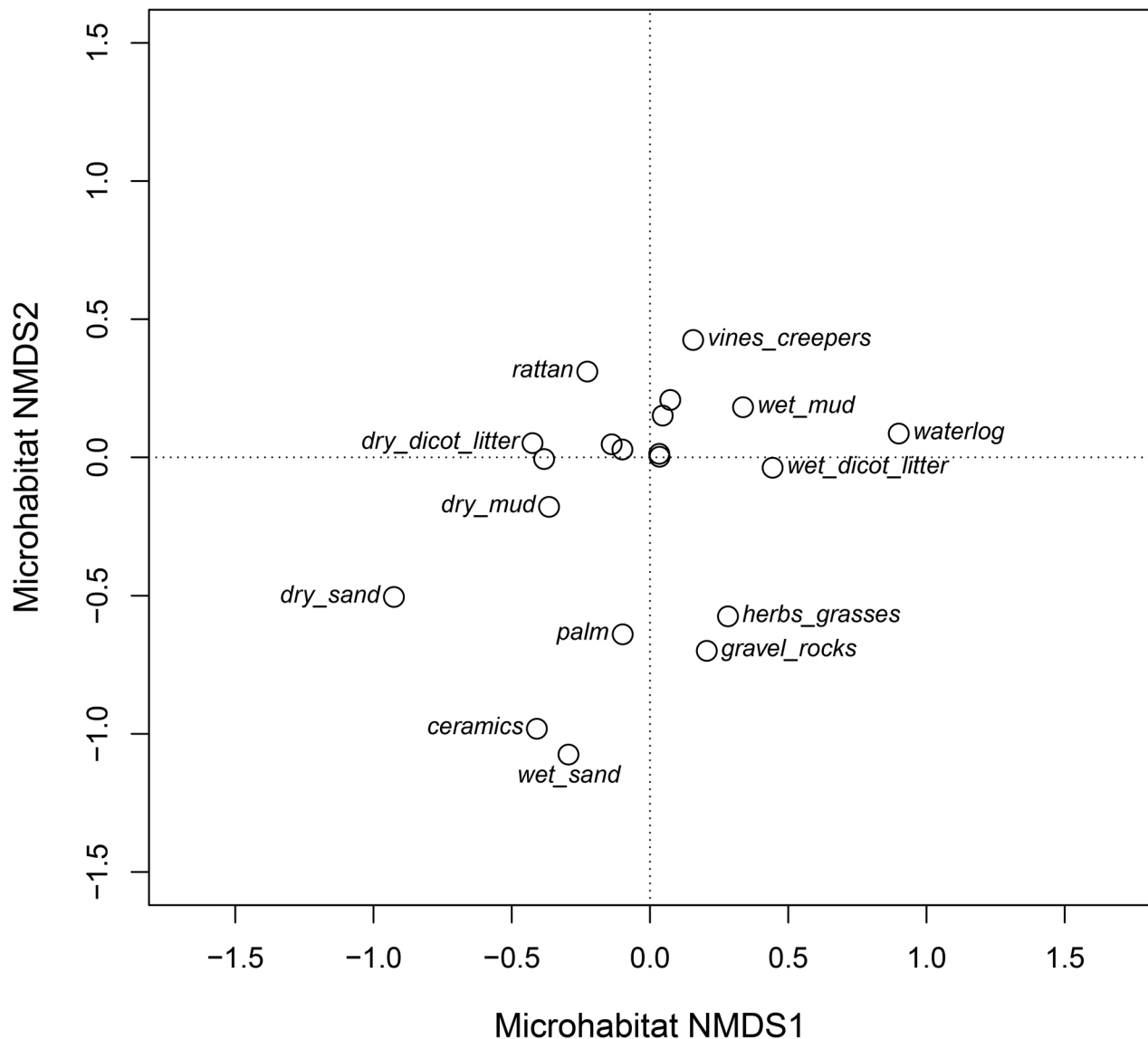
(97.5% CI[0.75, 2.06] in best model and 97.5% CI[0.71, 2.10] in second best model) and a weak negative relationship with Microhabitat NMDS2 (97.5% CI[-1.81, 0.09] in best model). This showed that higher abundance of pygmy grasshoppers could be found in wetter microhabitats (waterlogged, wet muddy and wet dicot leaf litter) than in drier microhabitats (dry sandy, dry muddy and dry dicot leaf litter). Proximity to main stream was not among the best models.

Since total abundance of pygmy grasshoppers had a strong positive relationship with NMDS1, we proposed further models, each model with a single environmental variable as a fixed effect: (1) dry dicot leaf litter, (2) wet dicot leaf litter, (3) wet muddy, (4) dry sandy, (5) waterlog and (6) herbaceous plants and grasses. Upon ranking them using AICc, the best models with delta < 2.0 had dry and wet dicot leaf litter as fixed effects and explained 40% and 37% of variance, respectively (Table 2). Total abundance had a strong negative relationship with dry dicot leaf litter (97.5% CI[-0.12, -0.05] as best model (Fig. 3) and a positive relationship with wet dicot leaf litter 97.5% CI[0.05, 0.11] as second best model). This showed that wetness of dicot leaf litter had a strong effect on the abundance of pygmy grasshoppers.

When we performed the analyses using adults and juveniles separately, the same patterns were observed. Higher abundance of adult and juvenile pygmy grasshoppers were found in wetter microhabitats than in drier microhabitats (Suppl. material 1: Tables 1 and 2). Juvenile abundance had a strong positive relationship with Microhabitat NMDS1 (97.5% CI[0.35, 2.63] in best model and 97.5% CI[0.39, 2.56] in second best model) and a weak negative relationship with Microhabitat NMDS2 (97.5% CI[-2.14, 0.94] in second best model). Likewise, adult abundance had a strong positive relationship with Microhabitat NMDS1 (97.5% CI[0.58, 2.15] in best model and 97.5% CI[0.59, 2.26] in second best model) and a strong negative relationship with Microhabitat NMDS2 (97.5% CI[-2.20, -0.03] in best model). Specifically, we found that wetness of dicot leaf litter had a strong effect on the abundance of both adults and juveniles (Suppl. material 1: Tables 3 and 4). Juvenile abundance had a strong relationship with dry dicot leaf litter (97.5% CI[-0.16, -0.03] and wet dicot leaf litter 97.5% CI[0.04, 0.15]). Adult abundance had a strong relationship with dry dicot leaf litter (97.5% CI[-0.14, -0.05]). Proximity to main stream was not among the best models. Lastly, we found the assemblage of pygmy grasshoppers was significantly correlated to Microhabitat NMDS1 and Microhabitat NMDS2 (proportion variance explained = 0.17, pseudo- $F$ -value = 2.89, number of permutations = 999,  $p$ -value = 0.013) (Fig. 4). No apparent difference was detected between the six morpho-species.

## Discussion

Our study in NSSF demonstrated that pygmy grasshoppers in general were not associated with the main streams in freshwater swamp forests. Instead, the pygmy grasshoppers were found to be associated with wetter microhabitats in general. This is despite the microhabitats between the belt transects nearer to and further away from the main streams being different. In Southeast Asia, many Scelimeninae can be found to occupy river banks and forage for submerged food resources (Kuřavová et al. 2017a). The pygmy grasshoppers from the freshwater swamp forest in Singapore, which include Scelimeninae, however, had different microhabitat associations. This was to be expected since the freshwater swamp forest is a different habitat to that of a typical river. The highly



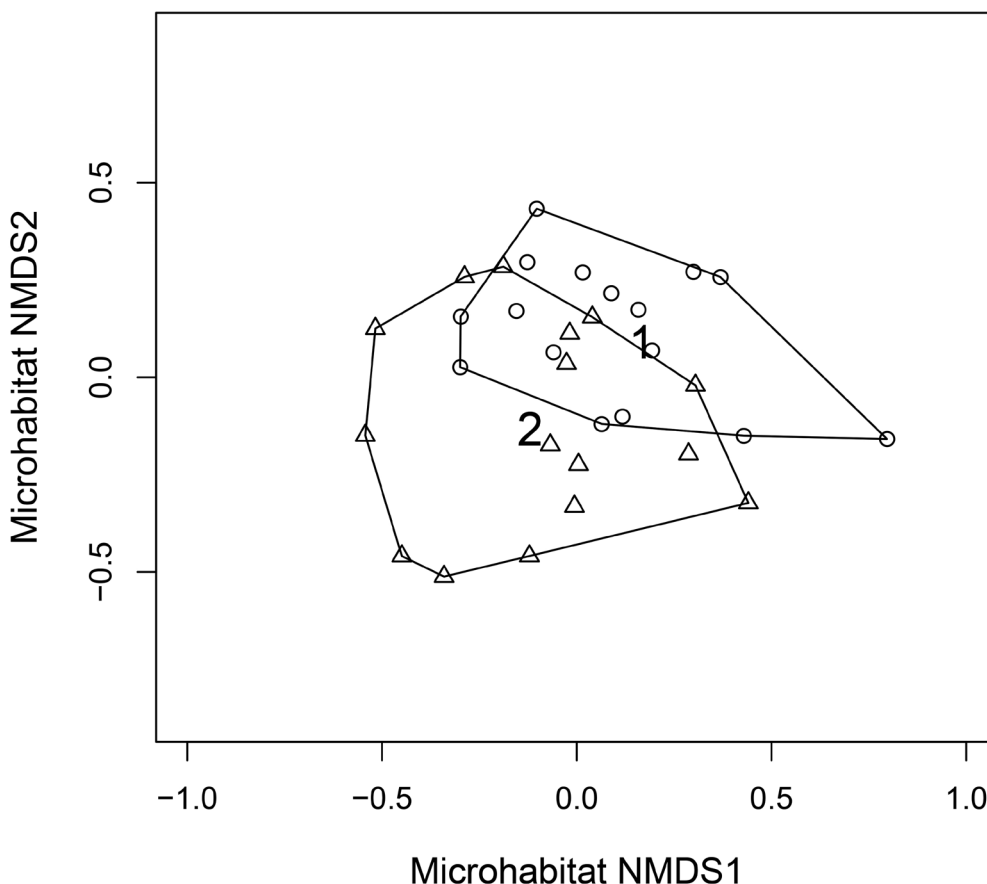
**Figure 1.** A non-metric multidimensional scaling (NMDS) using Bray-Curtis distance to summarize microhabitat condition data, indicating the different microhabitat conditions. Stress value for first two axes = 0.17.

dynamic water level within the freshwater swamp forest leads to the formation of a dense network of main streams and smaller ephemeral streamlets (O'Dempsey and Chew 2013). This also indicates that different pygmy grasshoppers from different habitats and lineages can have very different life histories.

As the diet of pygmy grasshoppers generally consists of algae, moss, fungi and lichen that thrive on wetter conditions (Paranjape et al. 1987, Hochkirch et al. 2000, Kočárek et al. 2008, Bidau 2014, Kuřavová and Kočárek 2015, Kuřavová et al. 2017a, 2017b), our findings also suggested that greater abundance of food sources in wetter microhabitats can account for higher abundance of pygmy grasshoppers in general. We also found that specifically, the wetness of dicot leaf litter is strongly positively correlated with the abundance of pygmy grasshoppers. We inferred that areas with wet dicot leaf litter provided suitable microhabitats for pygmy grasshoppers owing to the availability of food resources. Additionally, wet microhabitats could also provide suitable conditions for egg

development (Paranjape et al. 1987). Since pygmy grasshoppers are known to lay eggs on substrate (Hartley 1962, Paranjape et al. 1987), wet leaf litter, in particular, might reduce rate of drying of the underlying substrate. From our observational studies, we could only infer these behaviors, and how precisely pygmy grasshoppers from tropical Southeast Asia utilize their microhabitats should be further investigated under laboratory conditions. This will then provide better mechanistic understanding of the biology of these pygmy grasshoppers.

On the other hand, pygmy grasshoppers did not appear to be associated with the vegetation types in the swamp forest since their food sources were often not affected by the vegetation types (Paranjape 1985, Paranjape and Bhalerao 1985). This is contrary to previous studies showing diet preference of orthopterans is often closely linked to their association with specific vegetation or host plants (Joern 1982, Schaffers et al. 2008, Badenhauer et al. 2015), although these studies do not focus on pygmy grasshop-



**Figure 2.** A non-metric multidimensional scaling (NMDS) using Bray-Curtis distance to summarize microhabitat condition data, showing two hulls representing belt transects (1, circle) close to and (2, triangular) far from the main stream. Stress value for first two axes = 0.17.

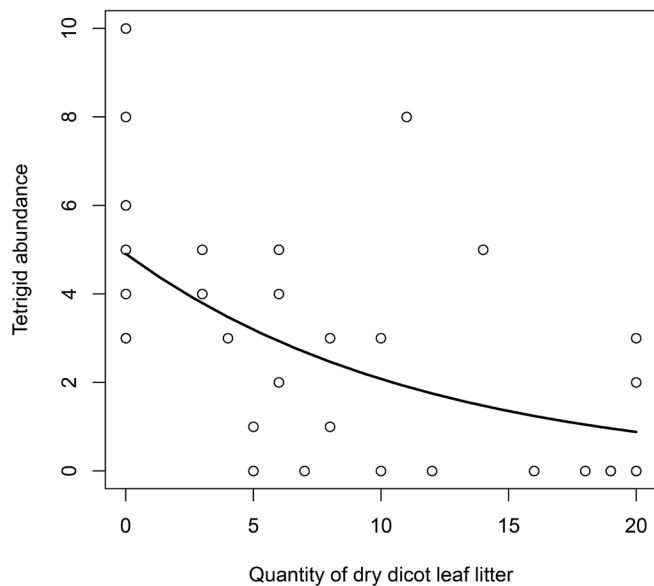
**Table 1.** Effect of Microhabitat NMDs1 and NMDs2 and proximity to main stream on total abundance of pygmy grasshoppers. Marginal  $R^2$  ( $R^2_m$ ) represents variance explained by fixed effects whereas conditional  $R^2$  ( $R^2_c$ ) represents variance explained by both fixed and random effects.

	df	logLik	AICc	delta	weight	$R^2_m$	$R^2_c$
~ Microhabitat NMDs1 + Microhabitat NMDs2	4	-70.70	150.9	0.0	0.51	0.35	0.35
~ Microhabitat NMDs1	3	-72.29	151.4	0.6	0.38	0.27	0.28
~ Microhabitat NMDs1 + Proximity to main stream	4	-72.24	154.0	3.1	0.11	0.27	0.28
~ Microhabitat NMDs2 + Proximity to main stream	4	-76.57	162.6	11.8	0.00	0.25	0.25
~ 1	2	-80.08	164.6	13.7	0.00	0.00	0.06
~ Microhabitat NMDs2	3	-79.25	165.3	14.5	0.00	0.10	0.10
~ Proximity to main stream	3	-79.55	166.0	15.1	0.00	0.03	0.10

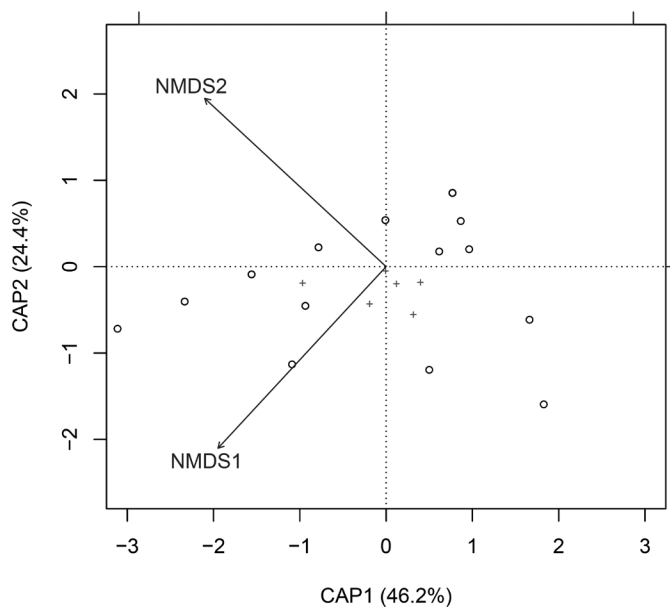
**Table 2.** Effect of specific environmental variables on total abundance of pygmy grasshoppers.

	df	logLik	AICc	delta	weight	$R^2_m$	$R^2_c$
~dry dicot leaf litter	3	-66.80	140.5	0.0	0.54	0.40	0.45
~wet dicot leaf litter	3	-66.97	140.8	0.3	0.46	0.37	0.45
~wet mud	3	-77.38	161.6	21.2	0.00	0.07	0.18
~dry sand	3	-77.54	161.9	21.5	0.00	0.06	0.16
~herbaceous plants and grasses	3	-77.99	162.8	22.4	0.00	0.14	0.14
~1	2	-80.08	164.6	24.1	0.00	0.00	0.06
~waterlog	3	-79.96	166.8	26.3	0.00	0.01	0.06

pers. Nonetheless, comparing our findings to other ecological studies of pygmy grasshoppers also revealed that association with the environment can be different among Southeast Asian species and with counterparts from other regions. For example, *Tetrix undulata* was shown to associate with habitats with preferred thermal properties (Ahnesjö and Forsman 2006) whereas *Tetrix tenuicornis* had differing preferences for microhabitats in accordance to changes in the weather conditions (Musiolek and Kočárek 2016). On the other hand, a riverine species, *Tetrix ceperoi*, prefers hotter, damper and bare areas, similar to species from NSSF (Gröning et al. 2007). Unlike previous studies (Ahnesjö and Forsman 2006, Gröning et al. 2007, Musiolek and Kočárek 2016) which focussed



**Figure 3.** Correlation between total abundance of pygmy grasshoppers and dry dicot leaf litter. The model was fitted using generalized linear mixed-effects model using Poisson error structure.



**Figure 4.** A canonical analysis of principal coordinates (CAP) with Euclidean distance to show association of adult assemblage with NMDS1 and NMDS2 representing microhabitat conditions. The circle represents belt transects and cross represents morpho-species.

on a single species of pygmy grasshopper, we investigated the overall abundance of all pygmy grasshoppers, while also examining possible differences between adults and juveniles. The main reason was because of the difficulty to identify closely related species and juveniles among Southeast Asian pygmy grasshoppers owing to unstable taxonomic status of many species (Blackith 1992, Kim and Kim 2004, Tan and Artchawakom 2015). Juveniles of many species were almost impossible to identify using morphology.

Interestingly, we did not find any difference in microhabitat association between the adults and juveniles. While adult and juvenile pygmy grasshoppers can have differing life history (e.g. dispersal ability, dependency on moisture), we did not find evidence for ontogenetic shift for the pygmy grasshoppers in NSSF. It appears that the species of pygmy grasshoppers had very similar association for specific microhabitat conditions since the assemblage of adults also showed similar trends as that of their abundance. We speculate that NSSF may be a unique ecosystem in which small insects such as the pygmy grasshoppers occupy very unique microhabitats.

Owing to the sensitivity of orthopterans to their environment, orthopterans have been proposed as bio-indicators of forests (Fartmann et al. 2012). Nevertheless, the tropical ecology of Southeast Asian orthopterans, along with many other invertebrates, are still under studied. Here, we demonstrated for the first time that pygmy grasshoppers from Southeast Asian freshwater swamp forests are not associated to waterbodies, unlike counterparts from the temperate and subtropical regions. Instead, these pygmy grasshoppers in general prefer wetter microhabitats. Loss or changes in these microhabitats owing to climate change or anthropogenic disturbances can potentially affect the populations (Inamke et al. 2016, Sueyoshi et al. 2016). This is especially so for the freshwater swamp forest which is currently small and isolated and for the pygmy grasshoppers with limited dispersal ability. We propose that pygmy grasshoppers can indeed be potential bio-indicators of freshwater swamp forest. Extending similar studies into other ecosystems can confirm if pygmy grasshoppers can also provide the same service. We hope that our study can help to fill in knowledge gaps in the ecology of pygmy grasshoppers in Southeast Asia, thus providing information for better management of threatened habitats, such as the NSSF.

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### Supplementary material 1

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Data type: Table

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