Received: 11 April 2017 | Revised: 19 June 2018 | Accepted: 20 June 2018

DOI: 10.1111/mec.14807

ORIGINAL ARTICLE

WILEY MOLECULAR ECOLOGY

Stream flow alone does not predict population structure of diving beetles across complex tropical landscapes

Athena Lam[1,2,3] | Emmanuel F. A. Toussaint[4] | Carolin Kindler[1] | Matthew H. Van Dam[1,2,3] | Rawati Panjaitan[5] | George K. Roderick[2] | Michael Balke[1,6]

[1]SNSB-Zoologische Staatssammlung München, Munich, Germany

[2]Department of Environmental Science, Policy and Management, University of California, Berkeley, California

[3]Institute for Biodiversity Science and Sustainability, California Academy of Sciences, San Francisco, California

[4]Florida Museum of Natural History, University of Florida, Gainesville, Florida

[5]Department of Biology, Faculty of Sciences and Mathematics, State University of Papua (UNIPA), Manokwari, West Papua, Indonesia

[6]GeoBioCenter, Ludwig-Maximilians-University, München, Germany

Correspondence
Athena Wai Lam, Institute for Biodiversity Science and Sustainability, California Academy of Sciences, 55 Music Concourse Dr., San Francisco, CA.
Email: AthenaWai@gmail.com

Funding information
Deutsche Forschungsgemeinschaft, Grant/Award Number: BA2152/11-1, BA2152/11-2, BA2152/19-1, DFG BA2152/17-1; NSF Graduate Research Fellow, Grant/Award Number: DBI #1402102; Department of Environmental Science Policy and Management, UC Berkeley

Abstract

Recent theoretical advances have hypothesized a central role of habitat persistence on population genetic structure and resulting biodiversity patterns of freshwater organisms. Here, we address the hypothesis that lotic species, or lineages adapted to comparably geologically stable running water habitats (streams and their marginal habitats), have high levels of endemicity and phylogeographic structure due to the persistent nature of their habitat. We use a nextRAD DNA sequencing approach to investigate the population structure and phylogeography of a putatively widespread New Guinean species of diving beetle, *Philaccolilus ameliae* (Dytiscidae). We find that *P. ameliae* is a complex of morphologically cryptic, but geographically and genetically well-differentiated clades. The pattern of population connectivity is consistent with theoretical predictions associated with stable lotic habitats. However, in two clades, we find a more complex pattern of low population differentiation, revealing dispersal across rugged mountains and watersheds of New Guinea up to 430 km apart. These results, while surprising, were also consistent with the original formulation of the habitat template concept by Southwood, involving lineage-idiosyncratic evolution in response to abiotic factors. In our system, low population differentiation might reflect a young species in a phase of range expansion utilizing vast available habitat. We suggest that predictions of life history variation resulting from the dichotomy between lotic and lentic organisms require more attention to habitat characterization and microhabitat choice. Our results also underpin the necessity to study fine-scale processes but at a larger geographical scale, as compared to solely documenting macroecological patterns, to understand ecological drivers of regional biodiversity. Comprehensive sampling especially of tropical lineages in complex and threatened environments such as New Guinea remains a critical challenge.

KEYWORDS
diving beetle evolution, Dytiscidae, habitat template concept, New Guinea biogeography, nextRAD phylogeography, *Philaccolilus*, population genomics

1 | INTRODUCTION

Understanding how environmental and geological factors shape the geographical distribution of biodiversity is a central theme in biology (Gaston, 2000). Despite its importance, many fundamental questions remain, including the degree to which the temporal and spatial nature of available habitats may impact the evolutionary trajectories of species or radiations thereof. The habitat template concept (originally

"templet," HTC) has been proposed to explain how intrinsic features and constraints imposed by a given habitat may drive the evolution of ecological traits and evolutionary strategies of its inhabitants (Southwood, 1977). These drivers, in turn, have scaled effects on the population structure and biogeography of species (Southwood, 1977, 1988; Korfiatis & Stamou, 1999; see Dijkstra, Monaghan, & Pauls, 2014 for a review). As a result, the habitat template concept predicts that species that occupy habitats characterized by different scales in time and space will exhibit different patterns of ecological and geographical diversification. It is, however, important to also consider Southwood's (1977: 359) conclusion, stating that one should "…not visualize habitat as a rigid causal templet…" and that "…an organism may evolve so that it is exposed to a different templet (…). Habitat and organism are thus parts of a system linked with 'feed-back'." The HTC and its predictions are also central to hypotheses underlying evolutionary diversification. For example, Wilson's (1959, 1961) taxon cycle operates through "…phases of range expansion and contraction coupled to ecological and evolutionary niche shifts" (Economo & Sarnat, 2012), providing an integrative framework to understand the evolution of narrow endemics out of widespread species.

According to the HTC, a fundamental factor influencing the selection for dispersal is the persistence of habitats through time (Bohonak, 1999; Denno, Roderick, Olmstead, & Döbel, 1991; Roff, 1994). Freshwater ecosystems, as spatially defined habitats with varied degrees of geological stability, are particularly well suited to testing the HTC (Hughes, 2007; Ribera, 2008; Ribera & Vogler, 2000). First, freshwater habitats can be readily categorized, allowing for direct evolutionary comparisons. The major abiotic factor that classifies freshwater habitats is their flow regime: Lotic habitats (i.e., streams and rivers) are those with running water, and lentic habitats (i.e., lakes, ponds) are those with standing water (Ribera, 2008), although it is important to note that stagnant water habitats do exist along stream beds and running waters can also dry out, as in intermittent streams (Shaverdo, Surbakti, Hendrich, & Balke, 2012). Second, these two types of habitats further differ in their ecological and spatiotemporal characteristics: Lotic habitats are generally considered to be more stable than lentic habitats as they are more continuous both spatially and temporally (Dijkstra et al., 2014; Ribera, Dolédec, Downie, & Foster, 2001). Thus, characters under direct selection as a result of habitat associations can be predicted a priori for lotic and lentic species: Lotic species are under decreased selection pressure for dispersal owing to the stability of lotic habitats and thus experience a lower risk of local extinction (Roff, 1986) than are species restricted to ephemeral standing water bodies (Dobson & Frid, 1998). Further, because dispersal is associated with gene flow among populations generally (Slatkin, 1985) and in aquatic species (Phillipsen et al., 2014), increased genetic differentiation of neighbouring habitat patches should be observed in lotic versus lentic species, a prediction that can be empirically tested (Ribera, Foster, & Vogler, 2003; Ribera & Vogler, 2004; Ribera et al., 2001). Finally, because reduced gene flow leads to an increased probability of peripatric and allopatric speciation, lineages with small geographical range sizes as assumed for lotic species will have a greater probability of extinction leading to higher species turnover in space and time (Ribera et al., 2001). We will refer to this set of hypotheses as the "habitat constraint hypotheses" herein. As noted above, lotic habitats are far from being homogenous. Many abiotic factors vary from headwaters to the lowlands, such as water salinity, temperature, nutrient load, substrate, and flow velocity, all in concert creating a vast number of different (micro) habitats. In addition, channels of highland streams are usually much more separated from each other and even more so from other catchments by the mountain ridges that fringe them, which in turn might limit dispersal between them. Lowland streams, on the other hand, are usually not separated by high mountains so more opportunities for dispersal between them exist (Bilton, Freeland, & Okamura, 2001; Bohonak & Jenkins, 2003; Hughes, 2007; Múrria, Bonada, Arnedo, Prat, & Vogler, 2013).

Several authors have documented associations between characteristics of aquatic habitats and dispersal traits and range size. For example, Arribas et al. (2012) demonstrated that lentic water beetles have larger wings when compared to closely related lotic species. Ribera and Vogler (2000) found that among 490 beetle species in the Iberian peninsula, only those with narrow distributions were lotic and that generally reduced vagility is associated with comparatively smaller range size in lotic species (Grewe, Hof, Dehling, Brandl, & Brändle, 2013; Ribera & Vogler, 2004; Ribera et al., 2001, 2003). Habitat characteristics also affect latitudinal patterns of diversity in aquatic species. For example, the proportion of lentic species increases towards the poles, a pattern thought to be the result of a greater dispersal by lentic species, leading to faster recolonization of areas glaciated during the Pleistocene (Ribera et al., 2003).

The genetic structure of freshwater aquatic macroinvertebrate populations is generally greater in lotic species when compared to their lentic counterparts (e.g., Ribera et al., 2001, 2003; Ribera & Vogler, 2004; Marten, Brandle, & Brandle, 2006; Hof et al. 2006; Monaghan et al., 2005). For example, Hjalmarsson, Bergsten, and Monaghan (2014) found that tropical stream margin beetle species had significantly greater population structure when compared to lentic and lotolentic (generalist) species. Papadopoulou et al. (2008), Papadopoulou, Anastasiou, Keskin, and Vogler (2009) showed that terrestrial beetles occupying habitat types differing in stability varied in coalescence of mitochondrial DNA (mtDNA), with lineages in more stable habitats having greater levels of population subdivision and geographical structure. However, predictions from the habitat constraint hypotheses have also been challenged by recent molecular genetic studies. A comprehensive phylogeny of Odonata found that lentic clades have higher diversification rates than lotic ones, with the explanation that larger range size resulted in a higher likelihood of vicariant events dividing an ancestral range, thereby creating a higher number of available habitats (Letsch, Gottsberger, & Ware, 2016). Désamoré, Laenen, Miller, and Bergsten (2018) found no evidence for increased net diversification rates between lentic and lotic species lineages in diving beetles (Dytiscidae), albeit with a reduced taxon sampling across the family. On larger spatial and temporal scales, Short and Caterino (2009) found different phylogeographic

patterns in three sympatric lotic beetle species from three different families, questioning the validity of habitat as a general predictor of evolutionary patterns. Finally, in tropical regions a large proportion of tropical lotic beetle diversity is found in stagnant water microhabitats at the edge of streams or around springs (Balke, Jäch, & Hendrich, 2004).

The *Philaccolilus* diving beetles (Dytiscidae, Laccophilinae) are endemic to New Guinea and strictly running water inhabitants (i.e., lotic sensu stricto), occupying smaller forest creeks, fast-flowing montane streams with heavy flooding and streaming, and mud free edges of lowland rivers (Balke, Larson, Hendrich, & Konyorah, 2000; Figure 1). There are twelve described species (Nilsson, 2016), and about five additional undescribed ones recently discovered in New Guinea (Balke, unpublished). Species in the genus vary in range size from limited endemics (*P. kokodanus*, *P. bicinctus*, *P. speciosus* on isolated mountain ranges of the Papuan Peninsula: *P. aterrimus* on Mount Gamey south of Nabire, and a new species from the Bewani Mountains), to wide ranges (e.g., *P. ameliae* across the central highland spine of the island and large parts of the Birds Head peninsula, *P. irianensis* along the western part of the north coast of New Guinea mainland; Balke et al., 2000; Balke unpublished).

Here, we use a nextRAD sequencing approach (nextera-tagmented, reductively amplified DNA) to examine the genomic population structure of the most widespread species, *P. ameliae* (Balke et al., 2000). This species includes two subspecies: *P. a. ameliae* (Balke et al., 2000) from eastern Papua New Guinea and the morphologically extremely similar *P. a. weylandensis* (Balke) from the Weyland Mountains of Papua (Figure 1), defined only by subtle differences in the male copulatory structure (Balke et al., 2000). With new localities reported here (purple in Figure 1), the geographical distribution of *P. ameliae*, as currently defined based on morphology, ranges across almost the entire island of New Guinea (Figure 1). New Guinea has a complex geotectonic history consisting of numerous geological elements such as continental fragments, former oceanic island arcs of Pacific origin and a massive central orogen mainly of Gondwanan origin (Toussaint et al., 2014), all of which are expected to have an impact on species ranges and population connectivity (e.g., Balke et al., 2009; Deiner, Lemmon, Mack, Fleischer, & Dumbacher, 2011; Toussaint, Sagata, Surbakti, Hendrich, & Balke, 2013; Toussaint et al., 2014). Specifically, we test predictions of the habitat constraint hypotheses for the widespread lotic *P. ameliae*, in the context of the complex geology of New Guinea that includes different geological formations, mountain ranges and stream systems.

2 | MATERIALS AND METHODS

2.1 | Taxon sampling

We sampled 60 individuals of *Philaccolilus ameliae* from seven localities across New Guinea (Figure 1, dots in purple and orange), representing both described subspecies (Balke et al., 2000). To test the monophyly of *P. ameliae*, we included 30 additional samples representing nine of the 12 described species of *Philaccolilus*, as well as three putative new species (Supporting Information Table S1). One individual from the closely related genus *Laccophilus* was included as an outgroup. DNA was extracted from whole beetles with punctured metacoxa, using the DNeasy Blood & Tissue Kit from Qiagen (Hilden, Germany). Voucher specimens are housed at the Museum Zoologicum Bogoriense, Cibinong, West Java, Indonesia and the Zoological State Collection, Munich.

2.2 | nextRAD sequencing

We obtained single nucleotide polymorphism (SNP) data by converting genomic DNA into nextRAD libraries as described by Russello, Waterhouse, Etter, and Johnson (2015). Briefly, genomic DNA was fragmented with Nextera reagent (Illumina, San Diego, CA, USA), which also ligates short adapter sequences to the ends of the fragments. The Nextera reaction was scaled for fragmenting 10 nanograms of genomic DNA. Fragmented DNA was then amplified, with one of the primers matching the adapter and extending nine nucleotides into the genomic DNA with the selective sequence GTGTAGAGC. Therefore, only fragments starting with a sequence that can be hybridized by the selective sequence of the primer were efficiently amplified by PCR. The nextRAD libraries were sequenced on an Illumina HiSeq 2500 (University of Oregon, USA). Custom scripts (SNPsaurus.com) were used to create a de novo reference from abundant reads, and all the reads were then mapped to the reference with an alignment identity threshold of 93% (BBMAP, Bushnell, 2016). Genotype calling was done using SAMTOOLS and BCFTOOLS (samtools mpileup -gu -Q 10 -t DP, DPR -f ref.fasta -b samples.txt | bcftools call -cv - > genotypes.vcf). The vcf files were converted to PHYLIP format by concatenating the de novo reference and substituting the called genotypes for each sample at the polymorphic positions.

Sequencing of the nextRAD library produced a total of 130,005,273 reads from 91 individuals, and the reads collapsed to 40,059 loci that were distinct from other loci by an identity threshold of at least 92%. These loci were used as a de novo reference for aligning the sequence reads from each sample using BBMAP (k = 9, slow mode, indel = 15, minid = 0.92). The resulting bam files were converted to a vcf genotype table using samtools mpileup. The putative variants in the vcf genotype table were then filtered using vcftools to remove variants not present in at least 85% of the samples and allowing variants with a population frequency of at least 0.05 to reduce artefactual variants. After filtering, the final data set consisted of 5,609 SNPs in 1,726 loci across 90 individuals (89 *Philaccolilus*, one outgroup). The vcf file was converted to PHYLIP format by concatenating the full sequence of each locus, ambiguity codes were used for heterozygote sites to create a sample-specific sequence. Thus, each sequence contained the variant and invariant nucleotides for each sample.

We tested all polymorphic loci for evidence of selection using the Bayesian simulation method of Beaumont and Balding (2004) as implemented in BAYESCAN 2.1 (Foll & Gaggiotti, 2008). Analyses were

FIGURE 1 Distribution of *Philaccolilus ameliae*, as defined morphologically, across New Guinea. Melanesia showing detailed sampling localities. Distributions based on Balke et al., 2000 and Museum collection data. Two pictures of *Philaccolilus ameliae* typical habitat are presented at the bottom (left, slow-flowing stream Kebar, valley floor; right, faster-flowing stream on mountain slope, Kebar). The base map was generated in the Google Maps API's StylingWizard (https://mapstyle.withgoogle.com/) and edited in a graphic design software [Colour figure can be viewed at wileyonlinelibrary.com]

run separately for each cluster studied in detail (described below). We used a prior odds value of 10, with 100,000 iterations and a burn-in of 50,000 iterations. We identified loci that were significant outliers at a *q*-value of 0.20. The analyses revealed that none of the loci displayed evidence of selection in any of the three clusters of interest outlined in the results section for the phylogenetic analysis. Therefore, all 5,609 SNPs were retained for population genetic analyses of *P. ameliae*. Raw nextRAD sequences, as well as input files for all major analyses, are available in DRYAD (https://doi.org/10.5061/dryad.hq77h24).

2.3 | Phylogenetic analyses

We conducted phylogenetic analysis on the full alignment of 172,600 bps including all specimens. The best model of nucleotide substitution was selected in IQ-TREE 1.5.3 (Nguyen, Schmidt, von Haeseler, & Minh, 2015) using the Auto function. All models included in IQ-TREE were tested including the ones relaxing the assumption of gamma-distributed rates (+R; Soubrier et al., 2012), and the fit of the different models was assessed using the Akaike information criterion corrected (AIC_c). To assess nodal support, we performed 1,000 ultrafast bootstrap replicates (UFBoot, Minh, Nguyen, & von Haeseler, 2013). We also performed SH-aLRT tests (Guindon et al., 2010), with 1,000 replicates. To fully explore alternative topologies and reduce the risk of local optimum, 500 independent tree searches were performed in IQ-TREE.

We used SVDquartets (Chifman & Kubatko, 2014) as implemented in PAUP* 4.0a (Swofford, 2003) to analyse the entire specimen-level dataset in a coalescent framework. SVDquartets has the advantage of not requiring a priori inferred gene topologies as in several summary methods, but rather directly relies on the DNA sequences (Chifman & Kubatko, 2014; Chou et al., 2015). Therefore, SVDquartets allows the consideration of both mutational and coalescent variance in the estimation of phylogenetic relationships (Chifman & Kubatko, 2014). SVDquartets has also been shown to perform better than other species tree inference programs in presence of gene flow (Long & Kubatko, 2018). SVDquartets assumes that each site has its own genealogy drawn from the coalescent model (Chifman & Kubatko, 2014). The method uses the coalescent model to infer the quartet trees for all subsets of four lineages and then combines the set of quartet trees into a species tree. To assemble the resulting quartets, a quartet tree agglomeration method is necessary. In PAUP*, the quartets were assembled using the Quartet FM heuristic approach (Reaz, Bayzid, & Rahman, 2014). We ran SVDquartets in PAUP* to infer a species tree and using 10,000 randomly generated quartets and with 100 nonparametric bootstrap replicates to assess nodal support across the phylogeny.

2.4 | Population genetics analyses

We assessed overall population structuring using a Bayesian clustering analysis in STRUCTURE 2.3.4 (Pritchard, Stephens, & Donnelly, 2000). Run length was set to 100,000 MCMC replicates after a burn-in period of 1,000,000 using correlated allele frequencies under a straight admixture model. We varied the number of clusters (K) from 1 to 8, with 10 replicates for each value of K. We used the admixture model with correlated allelic frequencies. The broadscale number of clusters was initially determined examining both the posterior probabilities of the data for each K and the ΔK estimator described by Evanno, Regnaut, and Goudet (2005) as calculated in Structure Harvester (Earl & vonHoldt, 2012). Results for the identified optimal values of K were summarized using CLUMPP ver. 1.1 (Jakobsson & Rosenberg, 2007) using 1,000 permutations and the LargeKGreedy algorithm. The result was then plotted using DISTRUCT ver. 1.1 (Rosenberg, 2004). Initial results suggested divergent clusters within *P. ameliae* (described in detail in the Results section below). To detect population subdivision that may be overlooked within each cluster (potential cryptic species), we subsequently conducted STRUCTURE analyses for each subclade, as in Gowen et al. (2014).

In addition, structuring was examined using principal component analyses (PCAs), a multivariate approach. Specifically, we used the Excel-based program GENALEX 6.1 (Peakall & Smouse, 2006) to calculate a genetic distance and to convert this into a covariance matrix with data standardization for the PCA. The first three principal components were plotted in the R package scatterplot3d (Ligges & Martin, 2003). Each cluster was analysed separately.

Levels of genetic differentiation among groups of each of the three clusters were estimated by pairwise F_{ST} (Weir & Cockerham, 1984) as implemented in GENETIX (Belkhir, Borsa, Chikhi, Raufaste, & Bonhomme, 2004) using 1,000 permutations. For the interpretation of pairwise F_{ST} values, we follow the very general classification genetic differentiation of Hartl and Clark (1997): $F_{ST} < 0.05$ very little, 0.05–0.15 moderate, 0.15–0.25 great and >0.25 very great. To determine the extent to which genetic variation was partitioned across samples within each clade, we conducted a hierarchical analysis of molecular variance (AMOVA, Excoffier, Smouse, & Quattro, 1992) using ARLEQUIN 3.5 (Excoffier & Lischer, 2010) with significance assessed using 1,000 permutations.

3 | RESULTS

3.1 | Molecular phylogenetics

The result of the IQ-TREE and SVDquartets phylogenetic analyses are presented in Figure 2. GTR+G was chosen for the best model for IQ-TREE. IQ-TREE recovered a well-resolved topology with robust nodal support at every node along the backbone. *P. bacchusi* is found as sister to *P. ameliae* with strong nodal support (UFBoot = 100/SH-aLRT = 100). *P. ameliae* is recovered as paraphyletic with respect to *P. mas* which is recovered as sister to clade A with strong nodal support (UFBoot = 100/SH-aLRT = 100). Populations of *P. ameliae* are divided into three clades (Figure 2). In this article, we will refer to the clade comprising all *P. ameliae*-like beetles (including *P. mas*) as the "*P. ameliae* complex." The three main *P. ameliae* clades consist of individuals from (A) the Birds Head peninsula (Arfak, Kebar, Testega), (B) Foja mountains and Sandaun province and (C) Ok Sibil and the Weyland Mts. (Figures 1–3). Within clade (C), the two localities also appear structured, forming two subclades.

At the species-level, SVDquartets recovers an identical phylogenetic hypothesis for the genus *Philaccolilus* as the one inferred in IQ-TREE based on the full specimen data set (Figure 1). Nodal support values across the topology are generally high (nonparametric bootstrap > 70); however, the most derived nodes received lower nodal support.

3.2 | Population genetics

Initial STRUCTURE analyses including all individuals of the *P. ameliae* complex recovered the same clusters described above (Figure 3). As these clusters seem to act as independent evolutionary units (putative cryptic species), we analysed each cluster separately in subsequent population genetic analyses. The single *P. mas* individual makes up a unique lineage that is clearly distinct from all other samples included; therefore, it was not included in further population genetic analyses. Analysing each group separately, the ΔK method identified two genetic populations in each group (Figure 3). However, using the method of Evanno et al. (2005) for estimating the optimal number of cluster(s) based on ΔK, it would not be possible to select a scenario of $K = 1$. Moreover, Latch, Dharmarajan, Glaubitz, and Rhodes (2006) showed that STRUCTURE could not distinguish clusters and delivered inconsistent results when populations have an F_{ST} of 0.03 or below. Therefore, we distinguish between scenarios of two discrete populations and one panmictic population by observing the level of admixture.

Both clades (A) and (B) have very weak structure and unclear assignments of individuals into clusters, suggesting that each group forms a single population (Figure 3). In the Birds Head group (A), the single individual from "Arfak" is distinct from individuals of the "Kebar" populations, but there is high level of mixed assignment between the "Kebar" and "Testega" populations. In the "Foja" + "Sandaun" group (B), there is high level of admixture between the Foja and Sandaun populations. In clade (C), the "Ok Sibil" and "Weyland 2" populations remain distinct with no observable admixture.

The AMOVA revealed a low amount of variation in clade (A; 9.98%, $F_{ST} = 0.1$; Table 1). Pairwise F_{ST} estimations showed congruent results with AMOVA and STRUCTURE, suggesting insignificant differentiation between the three populations in the Birds Head peninsula (pairwise $F_{ST} = 0.02–0.03$, Table 2). The PCA showed that the first three components explained only a minor portion of the total variance (20.82% cumulative, Table 3). Overall, our PCAs are congruent in showing no distinct clustering between the Kebar and Testega populations (Figure 4). Similarly, the AMOVA and pairwise F_{ST} analyses revealed a low amount of variation between the two populations of *P. ameliae* found in clade (B), Foja and Sandaun (variation among group = 26.74%, $p = 0.045$; $F_{ST} = 0.27$; Table 1). Between the two localities, there is little differentiation (pairwise $F_{ST} = 0.03$, Table 2). PCA showed a similar pattern with high level of admixture, the first three principal components explaining 44% of the total variation (Figure 4).

In contrast, the AMOVA and pairwise F_{ST} analyses confirmed the high level of variation between the two populations of *P. ameliae* found in Ok Sibil and the Weyland mountains (clade C; variation among group = 51.66%, $p = 0.00587$; $F_{ST} = 0.51$; Table 1). Differentiation between the two localities was significant (pairwise $F_{ST} = 0.34$, Table 2). PCA also showed two distinct clusters (Figure 4).

4 | DISCUSSION

4.1 | Taxonomy of the *Philaccolilus ameliae* species complex

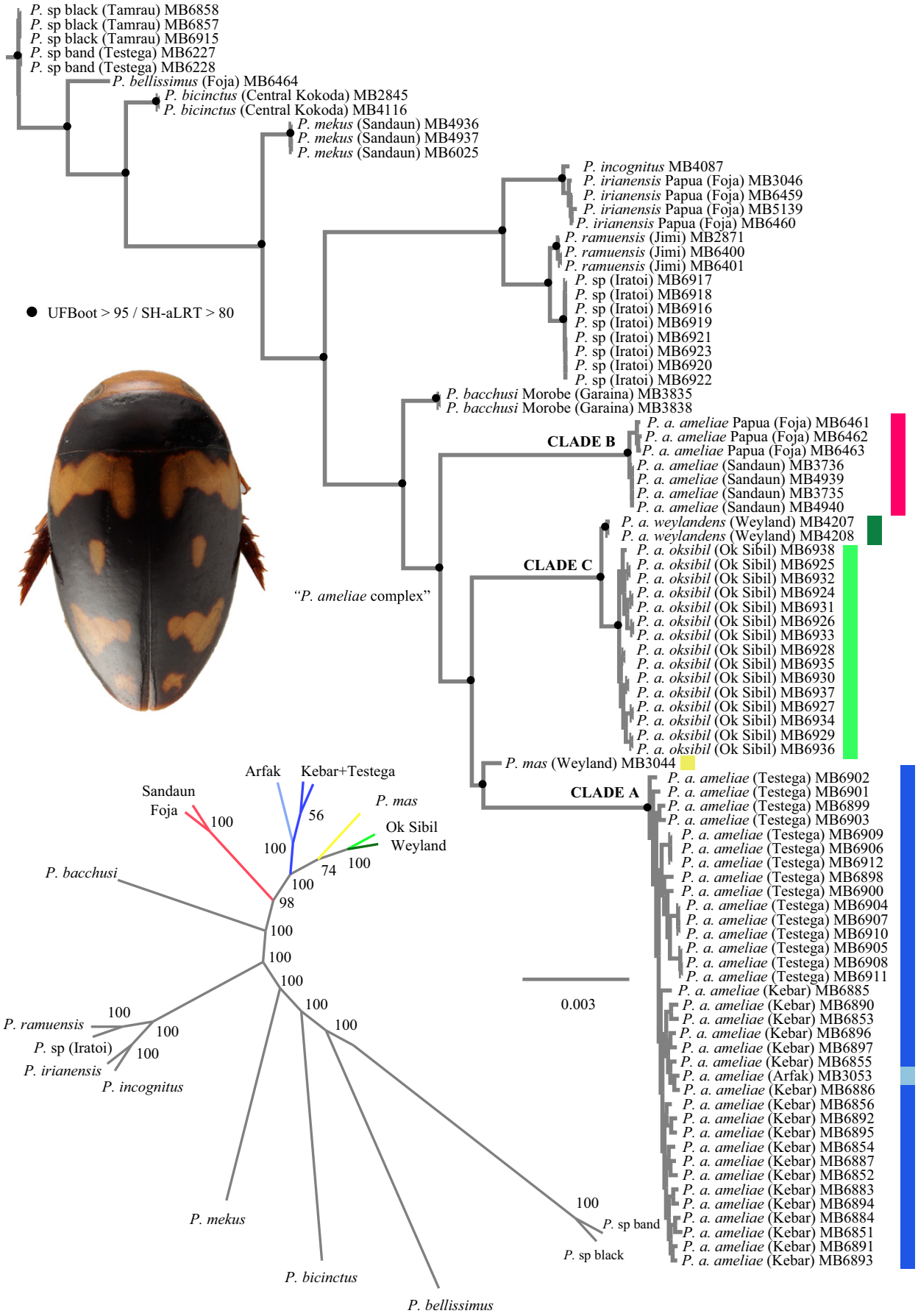
Based on both gene tree (concatenated results of IQ-TREE) and species tree methods (SVDquartets), *P. ameliae* is recovered as paraphyletic with strong support. *P. mas*, which is nested within the *P. ameliae* clade, is morphologically distinct from *P. ameliae* in terms of male genital morphology and structure of male protarsal setation. They are sympatric, possibly Syntopic, in the Weyland Mountains area (Balke et al., 2000).

Within what is currently considered a single species *P. ameliae*, the data presented here suggest the presence of not one widespread species, but a complex of three or four cryptic species, corresponding to clades A, B and C, and possibly two species within clade C. These appear in a geographical sequence (Figure 3), with one species occurring on the Birds Head peninsula, one in the West New Guinea mainland, and one more towards the east. The latter represents the nominal species *P. ameliae*, which was described from Papua New Guinea (Figure 1, white dots) but for which we had no genomic data because only old museum specimens exist. We did, however, obtain a short fragment (600 bp) from the 3' mtDNA cox1 gene from four PNG specimens collected close to the type locality of *P. ameliae* and these specimens group with Foja/Sandaun specimens found in clade B (98.9% identical, data not shown here as a formal integrative taxonomic review will be presented in a separate paper).

4.2 | Population genomics of *Philaccolilus ameliae*

New Guinea is a geologically complex, mostly tropical environment, with a large central mountain range that divides the island in the middle, as well as numerous other mountain ranges (e.g., along the north coast), with uncounted watersheds and stream systems.

FIGURE 2 Phylogenetic relationships as recovered in the IQ-TREE and SVDquartets analyses of the concatenated nextRAD data set. The maximum likelihood phylogeny inferred in IQ-TREE based on the concatenated data set (1,726 loci across 89 *Philaccolilus* specimens; 172,600 aligned base pairs) is presented with colour-coding for the main geographical regions. Nodal support values are given according to the inserted caption (black dots indicate robust nodal support; UFBoot >95 and SH-aLRT >80). On the bottom left corner is presented the SVDquartets species tree based on the analyses performed in PAUP*. Nodal support in the form of nonparametric bootstrap values is given for each node of the species tree. A picture of *Philaccolilus ameliae* is also shown (photo: M. Balke) [Colour figure can be viewed at wileyonlinelibrary.com]


P. sp black (Tamrau) MB6858
P. sp black (Tamrau) MB6857
P. sp black (Tamrau) MB6915
P. sp band (Testega) MB6227
P. sp band (Testega) MB6228
P. bellissimus (Foja) MB6464
P. bicinctus (Central Kokoda) MB2845
P. bicinctus (Central Kokoda) MB4116
P. mekus (Sandaun) MB4936
P. mekus (Sandaun) MB4937
P. mekus (Sandaun) MB6025
P. incognitus MB4087
P. irianensis Papua (Foja) MB3046
P. irianensis Papua (Foja) MB6459
P. irianensis Papua (Foja) MB5139
P. irianensis Papua (Foja) MB6460
P. ramuensis (Jimi) MB2871
P. ramuensis (Jimi) MB6400
P. ramuensis (Jimi) MB6401
P. sp (Iratoi) MB6917
P. sp (Iratoi) MB6918
P. sp (Iratoi) MB6916
P. sp (Iratoi) MB6919
P. sp (Iratoi) MB6921
P. sp (Iratoi) MB6923
P. sp (Iratoi) MB6920
P. sp (Iratoi) MB6922
UFBoot > 95 / SH-aLRT > 80
P. bacchusi Morobe (Garaina) MB3835
P. bacchusi Morobe (Garaina) MB3838
CLADE B
P. a. ameliae Papua (Foja) MB6461
P. a. ameliae Papua (Foja) MB6462
P. a. ameliae Papua (Foja) MB6463
P. a. ameliae (Sandaun) MB3736
P. a. ameliae (Sandaun) MB4939
P. a. ameliae (Sandaun) MB3735
P. a. ameliae (Sandaun) MB4940
P. a. weylandens (Weyland) MB4207
P. a. weylandens (Weyland) MB4208
CLADE C
P. a. oksibil (Ok Sibil) MB6938
P. a. oksibil (Ok Sibil) MB6925
P. a. oksibil (Ok Sibil) MB6932
P. a. oksibil (Ok Sibil) MB6924
P. a. oksibil (Ok Sibil) MB6931
P. a. oksibil (Ok Sibil) MB6926
P. a. oksibil (Ok Sibil) MB6933
P. a. oksibil (Ok Sibil) MB6928
P. a. oksibil (Ok Sibil) MB6935
P. a. oksibil (Ok Sibil) MB6930
P. a. oksibil (Ok Sibil) MB6937
P. a. oksibil (Ok Sibil) MB6927
P. a. oksibil (Ok Sibil) MB6934
P. a. oksibil (Ok Sibil) MB6929
P. a. oksibil (Ok Sibil) MB6936
"P. ameliae complex"
P. mas (Weyland) MB3044
CLADE A
P. a. ameliae (Testega) MB6902
P. a. ameliae (Testega) MB6901
P. a. ameliae (Testega) MB6899
P. a. ameliae (Testega) MB6903
P. a. ameliae (Testega) MB6909
P. a. ameliae (Testega) MB6906
P. a. ameliae (Testega) MB6912
P. a. ameliae (Testega) MB6898
P. a. ameliae (Testega) MB6900
P. a. ameliae (Testega) MB6904
P. a. ameliae (Testega) MB6907
P. a. ameliae (Testega) MB6910
P. a. ameliae (Testega) MB6905
P. a. ameliae (Testega) MB6908
P. a. ameliae (Testega) MB6911
P. a. ameliae (Kebar) MB6885
P. a. ameliae (Kebar) MB6890
P. a. ameliae (Kebar) MB6853
P. a. ameliae (Kebar) MB6896
P. a. ameliae (Kebar) MB6897
P. a. ameliae (Kebar) MB6855
P. a. ameliae (Arfak) MB3053
P. a. ameliae (Kebar) MB6886
P. a. ameliae (Kebar) MB6856
P. a. ameliae (Kebar) MB6892
P. a. ameliae (Kebar) MB6895
P. a. ameliae (Kebar) MB6854
P. a. ameliae (Kebar) MB6887
P. a. ameliae (Kebar) MB6852
P. a. ameliae (Kebar) MB6883
P. a. ameliae (Kebar) MB6894
P. a. ameliae (Kebar) MB6884
P. a. ameliae (Kebar) MB6851
P. a. ameliae (Kebar) MB6891
P. a. ameliae (Kebar) MB6893
0.003
Arfak
Kebar+Testega
Sandaun
Foja
P. mas
Ok Sibil
Weyland
P. bacchusi
P. ramuensis
P. sp (Iratoi)
P. irianensis
P. incognitus
P. mekus
P. bicinctus
P. bellissimus
P. sp band
P. sp black
100
56
74
98

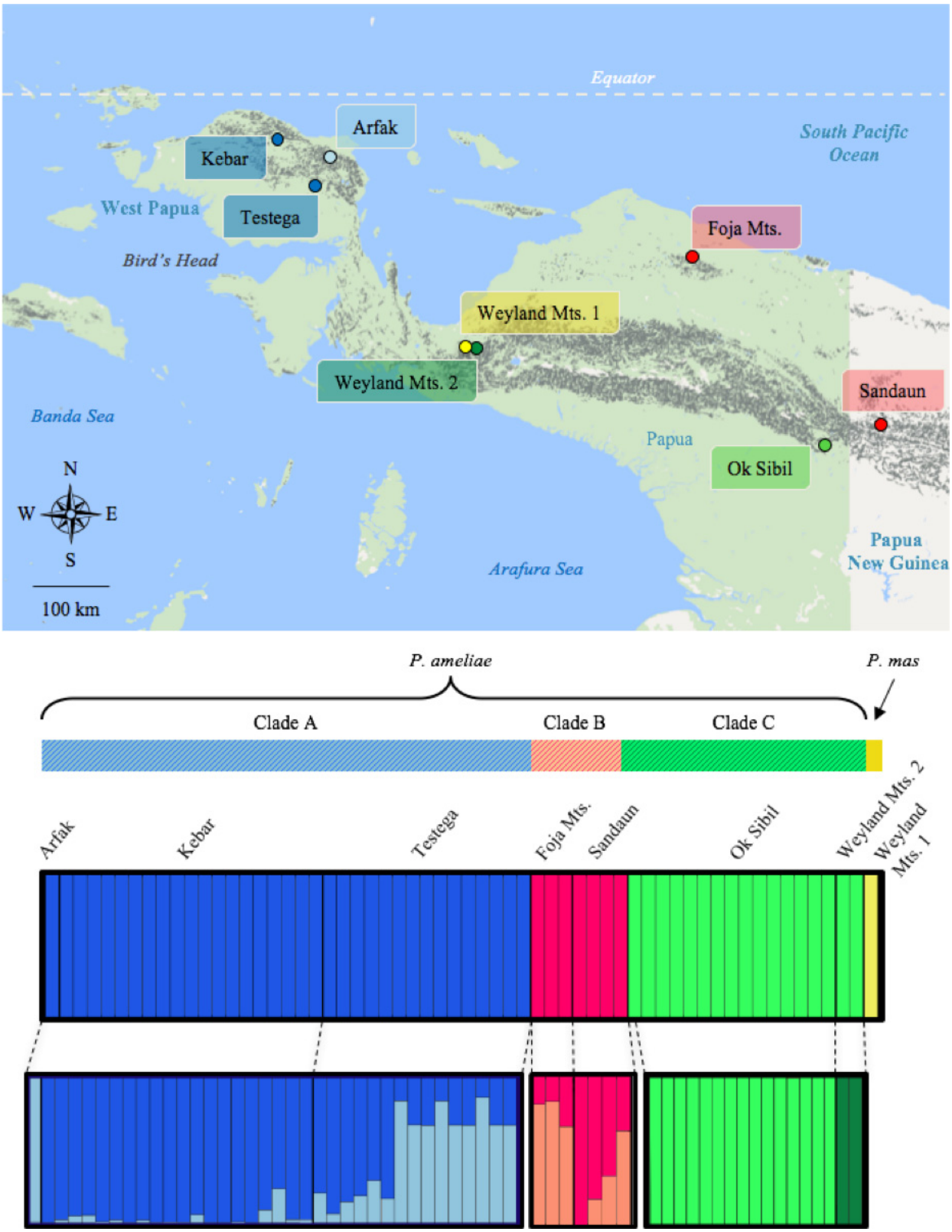


FIGURE 3 Population genetics of the *Philaccolilus ameliae* species complex. (a) New Guinea with collecting localities of the eight populations studied, locality colours correspond to colours in STRUCTURE barplots; (b) Bayesian clustering analyses of nextRAD SNPs data in STRUCTURE. Top: barplot for the *P. ameliae* species complex (K = 1–10) shows that the complex is split into four distinct clusters with no genetic admixture. Bottom: Barplots of clusters A, B and C of *P. ameliae* separately (K = 2 for each successive run) reveal a high level of admixture within clusters A and B. The two populations in cluster C remain discrete. The base map was generated in the Google Maps API's StylingWizard (https://mapstyle.withgoogle.com/) and edited in a graphic design software [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Analyses of molecular variance (AMOVA) of population pairs within clades A, B and C of *P. ameliae*

Source of variation	*df*	Sum of squares	Percentage of variation	Fixation index
Clade A				
Among group	2	453.22	9.98	F_{ST} = 0.10, p = 0.00098
Within populations	69	4475.33	90.02	
Clade B				
Among group	1	130.81	26.74	F_{ST} = 0.27, p = 0.045
Within populations	26	566.33	73.26	
Clade C				
Among group	1	241.55	51.66	F_{ST} = 0.51, p = 0.00587
Within populations	32	904.8	48.34	

TABLE 2 Pairwise F_{ST} estimates calculated with GENETIX. Values with * were significant ($p < 0.05$)

F_{ST} of *P. ameliae* clade A.		
Arfak	**Kebar**	**Testega**
Arfak	0.03	0.02
Kebar		0.02
F_{ST} of *P. ameliae* clade B		
		Foja
Sandaun		0.03
F_{ST} of *P. ameliae* clade C		
		Weyland mountains
Ok Sibil		0.34*

TABLE 3 Principal coordinates analysis based on individual nextRAD genotypes: percentage of variation explained by the first three axes

		Axis 1	Axis 2	Axis 3
Clade A	%	8.23	7.47	5.12
	Cumulative %	8.23	15.70	20.82
Clade B	%	21.69	16.55	5.91
	Cumulative %	21.69	38.23	44.14
Clade C	%	40.58	19.20	5.33
	Cumulative %	40.58	59.78	65.12

Despite the unique biota and paleogeological history of the island, evolutionary studies are rare, principally due to the difficulty to collect samples (but see, e.g., Deiner et al., 2011; Georges et al., 2011; Lam et al., 2018; Unmack, Allen, & Johnson, 2013; Toussaint et al., 2014; Oliver, Iannella, Richards, & Lee, 2017; Van Dam et al., 2017). This lack of information is especially true at the intraspecific level, with only a handful of phylogeographic works in the New Guinean region (e.g., Bruxaux et al., 2018; Janda et al., 2016; Skale, Tänzler, Hendrich, & Balke, 2012; Toussaint et al., 2013; Tallowin et al., 2018). As a result, the micro- and macroevolutionary processes leading to the astonishing biodiversity of the island remain mostly unknown. Following the habitat constraints hypotheses (Ribera, 2008), one can predict that New Guinean lotic lineages should exhibit high levels of local endemism and population structure between watersheds and geological terranes on the island because of low dispersal in stable environments. Such a pattern has been previously suggested by several studies involving large time-calibrated molecular phylogenies, including lotic *Exocelina* diving beetles (Toussaint, Hendrich, Shaverdo, & Balke, 2015; Toussaint et al., 2014), rainbowfishes (Unmack et al., 2013) and the New Guinea snapping turtle *Elseya novaeguineae* (Georges et al., 2011). However, there are no studies to date that focus on fine-scale phylogeographic patterns among populations of aquatic lineages across the island (but see Lam et al., 2018; Georges et al., 2011 for Miocenic divergence of regional populations).

Morphological systematic work has described *P. ameliae* as a single widespread species ranging across the entire island, with subtle male genitalia differences for the "Weyland 2" samples (Figure 2). However, the genomic nexRAD data used in this study reveal geographical subdivision. Yet, within two of the three genetic clusters, we find comparatively little differentiation across complex landscapes and at different elevations (e.g., Foja localities are 150 m high, while Sandaun localities are 700 m high).

Clade (A) includes samples from different watersheds of different mountain ranges of the Birds Head (i.e., Arfak Mountain localities "Arfak" and "Testega" and Tamrau Mts.: "Kebar", maximum 100 km apart), yet our data suggest insignificant differentiation across the entire peninsula (Figure 3; pairwise F_{ST} = 0.02–0.03, Table 2). Geologically, the localities are all situated on uplifted Gondwanan rock (Testega), although in a few cases, it is unclear whether localities are on uplifted Gondwanan rock or the attached transition towards younger rocks that overlie accreted Pacific material. The altitudes reach from 320 m (Arfak) to 1,010 m (Testega), which means that clade (A) is distributed from just above tropical lowland up to the cooler mid-montane altitudes. While not specifically quantified in the field, it is obvious that the streams exhibit different abiotic features, ranging from slow-flowing, shallow, small streams on valley floor with an abundance of sand banks; to deep and sandy streams (Kebar valley floor, Figure 1 and Testega); to fast-flowing streams on mountain slope with rocky/gravelly substrate (Kebar, Figure 1, and Arfak).

Populations of clade B originate from different catchments and are even more geographically and geologically distinct from each other, yet we observed the same pattern of little differentiation across complex landscapes (Figure 3), also with insignificant differentiation (pairwise F_{ST} = 0.03, Table 2). The "Sandaun" localities in Papua New Guinea are situated around the central orogeny that are of Gondwanan origin or directly attached transition towards younger rocks that overlie accreted Pacific material, while the "Foja Mts." locality in Papua is at the northern foot of an isolated mountain range of Pacific origin (430 km apart; Gautier Terrane, see Hill & Hall, 2003; Toussaint et al., 2014). The Foja locality (120 m) is a

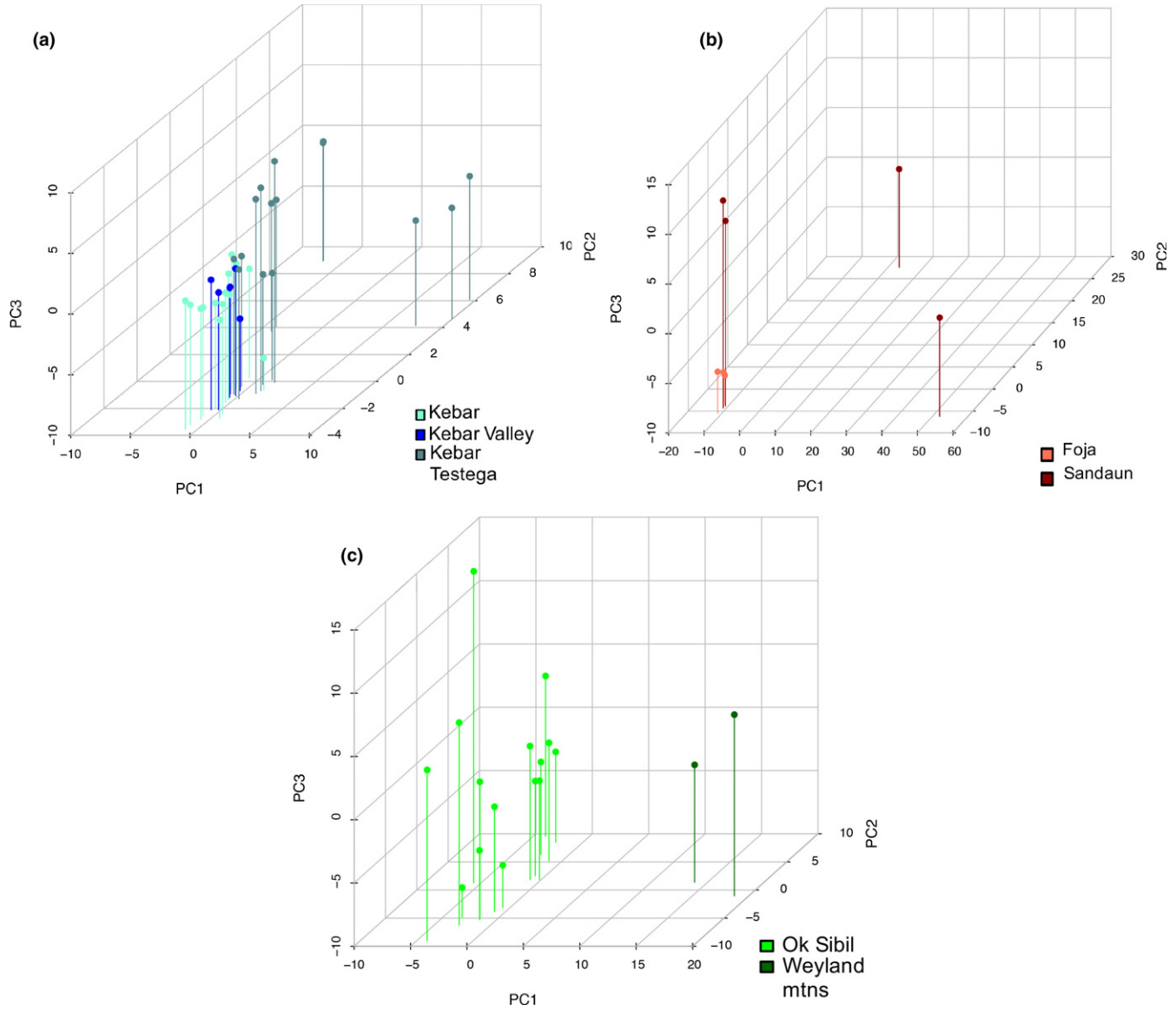


FIGURE 4 Three-dimensional plots of a principal component analysis based on individual nextRAD genotypes. Individuals are colour-coded according to collection locality (see Figure 3), Figures (a–c) according to the clades A–C. Table 3 indicates percentage of variation explained by the first three axes [Colour figure can be viewed at wileyonlinelibrary.com]

wider lowland stream with fine grey substrate and few sandy stretches. The stream in Sandaun is a mountain stream (localities 600–700 m) although the substrate is not reported.

Thus, our results suggest genetic exchange between highly disjunct localities within each of these two clades, or putative species. The limited abiotic data available here do not allow robust conclusions in how temperature and/or geology contribute to the structuring of populations, or, more specifically, isolation between the clades A and B. However, the lack of significant differentiation within clades A and B across high mountain ranges and different stream types indicates a high level of gene flow. Such a pattern has also been documented for the diving beetle *Boreonectes aequinoctialis* from Arizona, with little increase in differentiation by distance among different sky islands (Phillipsen et al., 2014).

In contrast, we find evidence for a high degree of differentiation (F_{ST} = 0.90173) between the two populations "Ok Sibil" (in clade C) and "Sandaun" (in clade B) although these two localities are only about 100 km apart and are both located in the central orogeny (different watershed, Sandaun north and Ok Sibil south of the central divide; Figure 3). "Ok Sibil" lies on 290 m altitude, on uplifted Gondwanan rock. Importantly, samples from "Ok Sibil" and the "Weyland Mts. 2" far west and north of the central divide make up the separate clade C in all the analyses, distinct from the other two clades. Within clade C, "Ok Sibil" and the "Weyland Mts. 2" individuals (540 km apart) appear to be isolated from each other (pairwise F_{ST} = 0.34, Table 2), but it will be necessary to obtain samples from the area in-between to draw further conclusions.

For comparison, Hjalmarsson et al. (2014) compared the degree of population differentiation in Malagasy water beetles. The authors

found F_{ST} <0.15 for their lentic species and >0.43 for the lotic ones. In two of the three clades in our study (clades A and B), we found F_{ST} Values ≤0.03. Only clade C exhibits level of F_{ST} comparable to those of lotic Malagasy beetles. It is interesting that such diversity of connectivity is found within one complex of closely related river dwelling species. Similar degrees of variation of F_{ST} values in lotic beetles were otherwise only found by Short and Caterino (2009); however, the comparison was made on three species from three different families, and with very different life histories and habitats.

We thus find lineage-idiosyncratic patterns within the *P. ameliae* species complex; from insignificant differentiation between populations across a broad geographical range (Figure 3, clades A and B), to significant differentiation between populations within clade C. These differences might be due in part to a range of temporal processes underlying population divergence, in which some populations are already isolated, while others still show signatures of recent dispersal across major landforms (e.g., Foja/Sandaun clade B and in the Birds Head clade A).

4.3 | *P. ameliae* and the habitat constraint hypotheses

The widespread diving beetle *Philaccolilus ameliae* does indeed show significant genetic structuring characteristic for lotic species; however, the different lineages are not necessarily narrow endemics and some do occur across more than one mountain range and stream system. In fact, what we interpret as *P. ameliae* sensu stricto might have a range spanning half of the width of New Guinea (Supporting Information Figure S1). This geographical distribution is only partially congruent with generalized geological history, in that the Birds Head peninsula features one endemic lineage (clade A), which, however, spreads across different mountain ranges (Arfak and Tamrau). Clade B is spread across different geological formations (central orogen of uplifted Gondwanan rock and/or transition zone, and uplift of Pacific oceanic origin). Assuming clade C represents one species, "Ok Sibil" and "Weyland 2", both represent uplifted Gondwanan rock or transition zone towards younger Pacific rocks, but different mountain ranges. These distribution patterns might be explained by an expansion phase across a complex landscape with subsequent separation, for example, by marine intrusions or dry corridors during glacial cycles, leading to various degrees of genetic isolation (high between clades A–C, high to low within the three clades). This mechanism of species formation in lotic insects has been suggested for European minute moss loving stream beetles in the genus *Hydraena* by Ribera et al. (2011) and should be further tested in our tropical study system. All data suggest the morphologically delineated, widespread diving beetle *P. ameliae* species actually consists of three or four species (Supporting Information Figure S1).

Our results for *P. ameliae* fully support predictions of the habitat constraints hypotheses, with a general pattern of significant genetic structure in species of lotic habitats. However, many evolutionary processes in this system remain poorly understood, especially the extent to which geological events may facilitate range expansion followed by speciation, as others have suggested (e.g., Ribera et al., 2011; Short & Caterino, 2009; Wilson, 1959, 1961). For instance, the widespread clade B of *P. ameliae* exhibits little differentiation between populations separated by ~300 km, while there are two sympatric species in the *P. ameliae* complex found in the Weyland Mts. The lack of differentiation between some distant populations may represent an example of a recent range expansion across the New Guinea landscape (see Toussaint et al., 2013 for an example in a lentic species). Clearly, knowledge of the temporal dimension coupled with fine-scale ecological data will be crucial to gain new insights into the evolutionary history of population and metapopulation structuring across complex geographical landscapes, such as New Guinea.

ACKNOWLEDGEMENTS

Field research was conducted during field courses with the State University of Papua (UNIPA). We thank the Conservation Division of the West Papuan Department of Forestry (BKSDA Manokwari) as well as local landowners for their permissions. Transfer of selected samples to West Java was granted by BKSDA Manokwari, and export was granted via a loan by Museum Zoologicum Bogoriense. These efforts are most gratefully acknowledged. This research was regulated with a MoU and MTA between ZSM and UNIPA. Financial support came from grants DFG BA2152/11-1, 11-2, 17-1, 19-1 and 19-2. The visit and project work of Rawati Panjaitan to and at ZSM was supported by DFG BA2152/17-1. Athena Lam was a NSF Graduate Research Fellow and received additional support from the Department of Environmental Science Policy and Management, UC Berkeley. Matt Van Dam was supported by NSF DBI #1402102.

DATA ACCESSIBILITY

Raw nextRAD sequences are deposited in Dryad, https://doi.org/10.5061/dryad.hq77h24

AUTHOR CONTRIBUTIONS

A.L. and M.B. conceived the study. M.B. conducted fieldwork. M.B. and A.L. performed laboratory work for Sanger sequencing. A.L. performed population analyses, M.V.D. constructed the alignment, E.T. conducted the phylogenetic analyses for the IQ-TREE, and M.H.V.D. conducted the SVDquartets analyses. A.L. and M.V.D. conducted the R population genetic analyses. R.P. organized fieldwork with the University of Papua and conducted fieldwork. A.L., M.B. and E.T. led the writing, and all authors contributed to the manuscript. C.K., M.B. and G.R. led the manuscript revision after review and rewrote the manuscript significantly with the help of E.T.

ORCID

Athena Lam http://orcid.org/0000-0002-7782-6041
Matthew H. Van Dam http://orcid.org/0000-0002-7473-9727

REFERENCES

Arribas, P., Velasco, J., Abellán, P., Sánchez-Fernández, D., Andújar, C., Calosi, P., … Bilton, D. T. (2012). Dispersal ability rather than ecological tolerance drives differences in range size between lentic and lotic water beetles (Coleoptera: Hydrophilidae). *Journal of Biogeography*, *39*, 984–994. https://doi.org/10.1111/j.1365-2699.2011.02641.x

Balke, M., Jäch, M. A., & Hendrich, L. (2004). Coleoptera. In C. M. Yule, & H. S. Yong (Eds.), *Freshwater invertebrates of the Malaysian region* (pp. 555–609). Kuala Lumpur, Malaysia: Akademi Sains Malaysia.

Balke, M., Larson, D., Hendrich, L., & Konyorah, E. (2000). A revision of the New Guinea water beetle genus *Philaccolilus* Guignot, stat.n. (Coleoptera, Dytiscidae). *Deutsche Entomologische Zeitschrift*, *47*, 29–50.

Balke, M., Ribera, I., Hendrich, L., Miller, M. A., Sagata, K., Posman, A., … Meier, R. (2009). New Guinea highland origin of a widespread arthropod supertramp. *Proceedings of the Royal Society of London B: Biological Sciences*, *276*, 2359–2367. https://doi.org/10.1098/rspb.2009.0015

Beaumont, M. A., & Balding, D. J. (2004). Identifying adaptive genetic divergence among populations from genome scans. *Molecular Ecology*, *13*, 969–980. https://doi.org/10.1111/j.1365-294X.2004.02125.x

Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N., & Bonhomme, F. (2004). *GENETIX 4.05, Population genetics software for Windows TM*.

Bilton, D. T., Freeland, J. R., & Okamura, B. (2001). Dispersal in Freshwater Invertebrates. *Annual Review of Ecology and Systematics*, *32*, 159–181. https://doi.org/10.1146/annurev.ecolsys.32.081501.114016

Bohonak, A. (1999). Dispersal, gene flow, and population structure. *Quarterly Review of Biology*, *74*(1), 21–45. https://doi.org/10.1086/392950

Bohonak, A., & Jenkins, D. (2003). Ecological and evolutionary significance of dispersal by freshwater invertebrates. *Ecology Letters*, *6*, 783–796. https://doi.org/10.1046/j.1461-0248.2003.00486.x

Bruxaux, J., Gabrielli, M., Ashari, H., Prŷs-Jones, R., Joseph, L., Milá, B., … Thébaud, C. (2018). Recovering the evolutionary history of crowned pigeons (Columbidae: Goura): Implications for the biogeography and conservation of New Guinean lowland birds. *Molecular Phylogenetics and Evolution*, *120*, 248–258. https://doi.org/10.1016/j.ympev.2017.11.022

Bushnell, B. (2016). *University of California, Berkeley, California*. Retrieved from http://sourceforge.net/projects/bbmap

Chifman, J., & Kubatko, L. (2014). Quartet inference from SNP data under the coalescent model. *Bioinformatics*, *30*, 3317–3324. https://doi.org/10.1093/bioinformatics/btu530

Chou, J., Gupta, A., Yaduvanshi, S., Davidson, R., Nute, M., Mirarab, S., & Warnow, T. (2015). A comparative study of SVDquartets and other coalescent-based species tree estimation methods. *BMC Genomics*, *16* (Suppl 10), S2. https://doi.org/10.1186/1471-2164-16-S10-S2

Deiner, K., Lemmon, A. R., Mack, A. L., Fleischer, R. C., & Dumbacher, J. P. (2011). A passerine bird's evolution corroborates the geologic history of the island of New Guinea (B Schierwater, Ed,). *PLoS ONE*, *6*, e19479. https://doi.org/10.1371/journal.pone.0019479

Denno, R. F., Roderick, G. K., Olmstead, K., & Döbel, H. G. (1991). Density-related migration in planthoppers: The role of habitat persistence. *American Naturalist*, *138*, 1513–1541. https://doi.org/10.1086/285298

Désamoré, A., Laenen, B., Miller, K. B., & Bergsten, J. (2018). Early burst in body size evolution is uncoupled from species diversification in diving beetles (Dytiscidae). *Molecular Ecology*, *27*, 979–993. https://doi.org/10.1111/mec.14492

Dijkstra, K.-D. B., Monaghan, M. T., & Pauls, S. U. (2014). Freshwater biodiversity and aquatic insect diversification. *Annual Review of Entomology*, *59*, 143–163. https://doi.org/10.1146/annurev-ento-011613-161958

Dobson, M., & Frid, C. (1998). *Ecology of aquatic ecosystems*. Essex, UK: Addison Wesley Longman Limited.

Earl, D. A., & vonHoldt, B. M. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, *4*, 359–361. https://doi.org/10.1007/s12686-011-9548-7

Economo, E. P., & Sarnat, E. M. (2012). Revisiting the ants of Melanesia and the taxon cycle: Historical and human-mediated invasions of a tropical archipelago. *The American Naturalist*, *180*(1), E1–E16. https://doi.org/10.1086/665996

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software structure: A simulation study. *Molecular Ecology*, *14*, 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Excoffier, L., & Lischer, H. E. L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, *10*, 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Excoffier, L., Smouse, P. E., & Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplo- types: Application to human mitochondrial DNA restriction data. *Genetics*, *131*, 479–491.

Foll, M., & Gaggiotti, O. (2008). A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: A Bayesian perspective. *Genetics*, *180*, 977–993. https://doi.org/10.1534/genetics.108.092221

Gaston, K. J. (2000). Global patterns in biodiversity. *Nature*, *405*, 220–227. https://doi.org/10.1038/35012228

Georges, A., Spencer, R., Welsh, M., Shaffer, H. B., Walsh, R., & Zhang, X. (2011). Application of the precautionary principle to taxa of uncertain status: The case of the Bellinger River turtle. *Endangered Species Research*, *14*, 127–134. https://doi.org/10.3354/esr00350

Gowen, F. C., Maley, J. M., Cicero, C., Townsend Peterson, A., Faircloth, B. C., Caleb Warr, T., & McCormack, J. E. (2014). Speciation in Western Scrub-Jays, Haldane's rule, and genetic clines in secondary contact. *BMC Evolutionary Biology*, *14*, 135. https://doi.org/10.1186/1471-2148-14-135

Grewe, Y., Hof, C., Dehling, D. M., Brandl, R., & Brändle, M. (2013). Recent range shifts of European dragonflies provide support for an inverse relationship between habitat predictability and dispersal. *Global Ecology and Biogeography*, *22*, 403–409. https://doi.org/10.1111/geb.12004

Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W., & Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: Assessing the performance of PhyML 3.0. *Systematic Biology*, *59*, 307–321. https://doi.org/10.1093/sysbio/syq010

Hartl, D. L., & Clark, A. G. (1997). *Principles of population genetics* (Vol. 116). Sunderland, MA: Sinauer Associates.

Hill, K. C., & Hall, R. (2003). Mesozoic-Cainozoic evolution of Australia's New Guinea margin in a West Pacific context. In R. Hillis & R. D. Muller (Eds.), *Defining Australia: The Australian plate*. Planet Earth. Geol. Soc. Australia Spec. Pub., 22 and GSA Spec. Paper, 372, 259–283.

Hjalmarsson, A., Bergsten, J., & Monaghan, M. (2014). Dispersal is linked to habitat use in 59 species of water beetles (Coleoptera: Adephaga) on Madagascar. *Ecography*, *38*(7), 732–739.

Hof, C., Brändle, M., & Brandl, R. (2006). Lentic odonates have larger and more northern ranges than lotic species. *Journal of Biogeography*, *33*, 63–70. https://doi.org/10.1111/j.1365-2699.2005.01358.x

Hughes, J. M. (2007). Constraints on recovery: Using molecular methods to study connectivity of aquatic biota in rivers and streams. *Freshwater Biology*, *52*(4), 616–631. https://doi.org/10.1111/j.1365-2427.2006.01722.x

Jakobsson, M., & Rosenberg, N. A. (2007). CLUMPP: A cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics*, *23*, 1801–1806. https://doi.org/10.1093/bioinformatics/btm233

Janda, M., Matos-Marav, P., Borovanska, M., Zima, J., Youngerman, E., & Pierce, N. E. (2016). Phylogeny and population genetic structure of the ant genus Acropyga (Hymenoptera : Formicidae) in Papua New Guinea. *Invertebrate Systematics*, *30*, 28. https://doi.org/10.1071/IS14050

Korfiatis, K. J., & Stamou, G. P. (1999). Habitat templets and the changing worldview of ecology. *Biology and Philosophy*, *14*, 375–393. https://doi.org/10.1023/A:1006543127454

Lam, A. W., Gueuing, M., Kindler, C., Van Dam, M. H., Alvarez, N., Panjaitan, R., … Balke, M. (2018). Phylogeography and population genomics of a lotic water beetle across a complex tropical landscape. *Molecular Ecology*, http://doi.org/10.1111/mec.14796

Latch, E. K., Dharmarajan, G., Glaubitz, J. C., & Rhodes, O. E. (2006). Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. *Conservation Genetics*, *7*, 295–302. https://doi.org/10.1007/s10592-005-9098-1

Letsch, H., Gottsberger, B., & Ware, J. L. (2016). Not going with the flow: A comprehensive time-calibrated phylogeny of dragonflies (Anisoptera: Odonata: Insecta) provides evidence for the role of lentic habitats on diversification. *Molecular Ecology*, *25*, 1340–1353. https://doi.org/10.1111/mec.13562

Ligges, U., & Martin, M. (2003). Scatterplot3d – an R package for Visualizing Multivariate Data. *Cran*, *8*, 1–36.

Long, C., & Kubatko, L. (2018). The effect of gene flow on coalescent-based species-tree inference. *Systematic Biology*, https://doi.org/10.1093/sysbio/syy020

Marten, A., Brandle, M., & Brandle, R. (2006). Habitat type predicts genetic population differentiation in freshwater invertebrates. *Molecular Ecology*, *15*, 2643–2651. https://doi.org/10.1111/j.1365-294X.2006.02940.x

Minh, B. Q., Nguyen, M. A. T., & von Haeseler, A. (2013). Ultrafast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution*, *30*, 1188–1195. https://doi.org/10.1093/molbev/mst024

Monaghan, M. T., Gattolliat, J.-L., Sartori, M., Elouard, J.-M., James, H., Derleth, P., … Vogler, A. P. (2005). Trans-oceanic and endemic origins of the small minnow mayflies (Ephemeroptera, Baetidae) of Madagascar. *Proceedings of the Royal Society of London B: Biological Sciences*, *272*, 1829–1836. https://doi.org/10.1098/rspb.2005.3139

Múrria, C., Bonada, N., Arnedo, M. A., Prat, N., & Vogler, A. P. (2013). Higher β- and γ-diversity at species and genetic levels in headwaters than in mid-order streams in Hydropsyche (Trichoptera). *Freshwater Biology*, *58*, 2226–2236.

Nguyen, L.-T., Schmidt, H. A., von Haeseler, A., & Minh, B. Q. (2015). IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. *Molecular Biology and Evolution*, *32*, 268–274. https://doi.org/10.1093/molbev/msu300

Nilsson, A. N. (2016). *A world catalogue of the family dytiscidae, or the diving beetles (Coleoptera, Adephaga)*. Version 1, 300 pp. Retrieved from http://www.norrent.se and www.waterbeetles.eu

Oliver, P. M., Iannella, A., Richards, S. J., & Lee, M. S. (2017). Mountain colonisation, miniaturisation and ecological evolution in a radiation of direct-developing New Guinea Frogs (*Choerophryne*, Microhylidae). *PeerJ*, *5*, e3077. https://doi.org/10.7717/peerj.3077

Papadopoulou, A., Anastasiou, I., Keskin, B., & Vogler, A. (2009). Comparative phylogeography of tenebrionid beetles in the Aegean archipelago: The effect of dispersal ability and habitat preference. *Molecular Ecology*, *18*, 2503–2517. https://doi.org/10.1111/j.1365-294X.2009.04207.x

Papadopoulou, A., Bergsten, J., Fujisawa, T., Monaghan, M. T., Barraclough, T. G., & Vogler, A. P. (2008). Speciation and DNA barcodes: Testing the effects of dispersal on the formation of discrete sequence clusters. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *363*, 2987–2996. https://doi.org/10.1098/rstb.2008.0066

Peakall, R., & Smouse, P. E. (2006). GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, *6*, 288–295. https://doi.org/10.1111/j.1471-8286.2005.01155.x

Phillipsen, I. C., Kirk, E. H., Bogan, M. T., Mims, M. C., Olden, J. D., & Lytle, D. A. (2014). Dispersal ability and habitat requirements determine landscape-level genetic patterns in desert aquatic insects. *Molecular Ecology*, *24*, 54–69.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*, 945–959.

Reaz, R., Bayzid, M. S., & Rahman, M. S. (2014). Accurate phylogenetic tree reconstruction from quartets: A heuristic approach. *PLoS ONE*, *9*, e104008. https://doi.org/10.1371/journal.pone.0104008

Ribera, I. (2008). Habitat constraints and the generation of diversity in freshwater macroinvertebrates. In J. Lancaster, & R. A. Briers (Eds.), *Aquatic insects: Challenges to populations* (pp. 289–311). Wallingford, UK: CABI. https://doi.org/10.1079/9781845933968.0000

Ribera, I., Castro, A., Díaz, J. A., Garrido, J., Izquierdo, A., Jäch, M. A., & Valladares, L. F. (2011). The geography of speciation in narrow-range endemics of the 'Haenydra' lineage (Coleoptera, Hydraenidae, Hydraena). *Journal of Biogeography*, *38*(3), 502–516. https://doi.org/10.1111/j.1365-2699.2010.02417.x

Ribera, I., Dolédec, S., Downie, I., & Foster, G. (2001). Effect of land disturbance and stress on species traits of ground beetle assemblages. *Ecology*, *82*, 1112–1129. https://doi.org/10.1890/0012-9658(2001)082[1112:EOLDAS]2.0.CO;2

Ribera, I., Foster, G., & Vogler, A. (2003). Does habitat use explain large scale species richness patterns of aquatic beetles in Europe? *Ecography*, *26*, 145–152. https://doi.org/10.1034/j.1600-0587.2003.03271.x

Ribera, I., & Vogler, P. (2000). Habitat type as a determinant of species range sizes: The example of lotic-lentic differences in aquatic Coleoptera. *Biological Journal of the Linnean Society*, *71*, 33–52.

Ribera, I., & Vogler, A. (2004). Speciation of Iberian diving beetles in Pleistocene refugia (Coleoptera, Dytiscidae). *Molecular Ecology*, *13*, 179–193. https://doi.org/10.1046/j.1365-294X.2003.02035.x

Roff, D. (1986). The evolution of wing dimorphism in insects. *Evolution*, *40*, 1009–1020. https://doi.org/10.1111/j.1558-5646.1986.tb00568.x

Roff, D. (1994). Habitat persistence and the evolution of wing dimorphism in insects. *American Naturalist*, *144*, 772–798. https://doi.org/10.1086/285706

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D. L., Darling, A., Höhna, S., … Huelsenbeck, J. P. (2012). Mrbayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, *61*, 539–542. https://doi.org/10.1093/sysbio/sys029

Rosenberg, N. A. (2004). DISTRUCT: A program for the graphical display of population structure. *Molecular Ecology Notes*, *4*, 137–138.

Russello, M. A., Waterhouse, M. D., Etter, P. D., & Johnson, E. A. (2015). From promise to practice: Pairing non-invasive sampling with genomics in conservation. *PeerJ*, *3*, e1106. https://doi.org/10.7717/peerj.1106

Shaverdo, H. V., Surbakti, S., Hendrich, L., & Balke, M. (2012). Introduction of the Exocelina ekari-group with descriptions of 22 new species from New Guinea (Coleoptera, Dytiscidae, Copelatinae). *ZooKeys*, *250*, 1–76. https://doi.org/10.3897/zookeys.250.3715

Short, A. E. Z., & Caterino, M. S. (2009). On the validity of habitat as a predictor of genetic structure in aquatic systems: A comparative study using California water beetles. *Molecular Ecology*, *18*, 403–414. https://doi.org/10.1111/j.1365-294X.2008.04039.x

Skale, A., Tänzler, R., Hendrich, L., & Balke, M. (2012). High mitochondrial DNA sequence divergence in New Guinea Carabdytes stream beetles and the taxonomist's dilemma when other evidence is kind of subtle…(and collecting localities are far far away). *ZooKeys*, *247*, 31–43.

Slatkin, M. (1985). Gene flow in natural populations. *Annual Review of Ecology and Systematics*, *16*, 393–430. https://doi.org/10.1146/annurev.es.16.110185.002141

Soubrier, J., Steel, M., Lee, M. S., Der Sarkissian, C., Guindon, S., Ho, S. Y., & Cooper, A. (2012). The influence of rate heterogeneity among sites on the time dependence of molecular rates. *Molecular Biology and Evolution*, *29*, 3345–3358. https://doi.org/10.1093/molbev/mss140

Southwood, T. R. E. (1977). Habitat, the templet for ecological strategies? *The Journal of Animal Ecology*, *64*, 337–365.

Southwood, T. R. E. (1988). Tactics, strategies and templets. *Oikos*, *52*, 3–18. https://doi.org/10.2307/3565974

Swofford, D. L. (2003). *PAUP*. Phylogenetic analysis using parsimony and other methods. Version 4*. Sunderland, MA: Sinauer Associates.

Tallowin, O. J. S., Tamara, K., Meiri, S., Allison, A., Kraus, F., Richards, S. J., & Oliver, P. M. (2018). Early insularity and subsequent mountain uplift were complementary drivers of diversification in a Melanesian lizard radiation (Gekkonidae: Cyrtodactylus). *Molecular Phylogenetics and Evolution*, *125*, 29–39.

Toussaint, E. F. A., Hall, R., Monaghan, M. T., Ibalim, S., Shaverdo, H. V., Vogler, A. P., … Balke, M. (2014). The towering orogeny of New Guinea as a trigger for arthropod megadiversity. *Nature Communications*, *5*, 4001. https://doi.org/10.1038/ncomms5001

Toussaint, E. F. A., Hendrich, L., Shaverdo, H., & Balke, M. (2015). Mosaic patterns of diversification dynamics following the colonization of Melanesian islands. *Scientific Reports*, *5*, 16016. https://doi.org/10.1038/srep16016

Toussaint, E. F. A., Sagata, K., Surbakti, S., Hendrich, L., & Balke, M. (2013). Australasian sky islands act as a diversity pump facilitating peripheral speciation and complex reversal from narrow endemic to widespread ecological supertramp. *Ecology and Evolution*, *3*, 1031–1049. https://doi.org/10.1002/ece3.517

Unmack, P. J., Allen, G. R., & Johnson, J. B. (2013). Phylogeny and biogeography of rainbowfishes (Melanotaeniidae) from Australia and New Guinea. *Molecular Phylogenetics and Evolution*, *67*, 15–27. https://doi.org/10.1016/j.ympev.2012.12.019

Van Dam, M. H., Lam, A. W., Sagata, K., Gewa, B., Laufa, R., Balke, M., … Riedel, A. (2017). Ultraconserved elements (UCEs) resolve the phylogeny of Australasian smurf-weevils. *PLoS ONE*, *12*, e0188044. https://doi.org/10.1371/journal.pone.0188044

Weir, B. S., & Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution*, *38*, 1358–1370.

Wilson, E. O. (1959). Adaptive shift and dispersal in a tropical ant fauna. *Evolution*, *13*, 122–144. https://doi.org/10.1111/j.1558-5646.1959.tb02996.x

Wilson, E. O. (1961). The nature of the taxon cycle in the Melanesian ant fauna. *American Naturalist*, *95*, 169–193. https://doi.org/10.1086/282174

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Lam A, Toussaint EFA, Kindler C, et al. Stream flow alone does not predict population structure of diving beetles across complex tropical landscapes. *Mol Ecol*. 2018;27:3541–3554. https://doi.org/10.1111/mec.14807