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Phylogeography and population genomics of a lotic water beetle across a complex tropical landscape

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Abstract

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Phylogeography and population genomics of a lotic water beetle across a complex tropical landscape

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Keywords

Population connectivity, lentic, New Guinea, tropical streams, Dytiscidae, ddRAD

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Abstract

The Habitat Template Concept applied to a freshwater system indicates that lotic species, or those which occupy permanent habitats along stream courses, are less dispersive than lentic species, or those that occur in more ephemeral aquatic habitats. Thus, populations of lotic species will be more structured than those of lentic species. Stream courses include both flowing water and small, stagnant microhabitats that can provide refuge when streams are low. Many species occur in these microhabitats but remain poorly studied. Here we present population genetic data for one such species, the tropical diving beetle *Exocelina manokwariensis* (Dytiscidae), sampled from six localities along a ~300 km transect across the Birds Head Peninsula of New Guinea. Molecular data from both mitochondrial (CO1sequences) and nuclear (ddRAD loci) regions document fine-scale population structure across populations that are ~ 45 km apart. Our results are concordant with previous phylogenetic and macroecological studies that applied the Habitat Template Concept to aquatic systems. This study also illustrates that these diverse but mostly overlooked microhabitats are promising study systems in freshwater ecology and evolutionary biology. With the advent of next generation sequencing, fine-scale population genomic studies are feasible for small non-model

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organisms to help illuminate the effect of habitat stability on species' natural history, population structure, and geographic distribution.

1 BACKGROUND

The Habitat Template Concept suggests that the properties and constraints of various habitats drive the evolution of ecological traits and evolutionary strategies of their inhabitants (Southwood 1977; Korfiatis & Stamou 1999). In particular, habitat instability is considered one of the most important factors influencing species' propensity to disperse (Denno *et al.* 1991; Roff 1994; Bohonak 1999). Species inhabiting ephemeral habitats experience greater risk of local extinction, and thus a greater selective pressure for dispersal (e.g., Roff 1986, 1990, 1994; Denno *et al.* 1996; Travis & Dytham 1999; Grantham 2003; Friedenberg 2003; Marten *et al.* 2006). Consequently, populations found in unstable environments are predicted to be less structured as a result of more gene flow than populations in more stable environments (Ribera *et al.* 2001, 2003; Ribera & Vogler 2004).

Freshwater ecosystems are broadly classified into two main habitat types: lotic or running water (e.g. - rivers and streams, including the habitats along their channels) and lentic or standing water (e.g. - lakes and ponds). These two habitat types differ in their stability both spatially and temporally, with lotic habitats considered to be more persistent over geological time (Ribera & Vogler 2000). Although seasonal variation may alter the annual stability of both habitat types, lotic systems are typically connected by a drainage network and/or to other water bodies. This connectivity results in persistence in both geological and ecological time compared to small or medium sized lakes and ponds that, once dried out, are cut off from other aquatic habitats (Hutchinson 1957). In an aquatic system, the Habitat Template Concept predicts that a species' dispersal rate, and therefore its genetic structure, is associated with the flow regime of their habitat; species in lotic habitats are less dispersive, will exhibit greater population subdivision and smaller

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ranges compared to their lentic counterparts (Ribera 2008). Indeed, several studies of species from aquatic systems suggest that habitat type is a good predictor for dispersal capability (e.g. Ribera & Vogler 2000; Ribera *et al.* 2003; Hof *et al.* 2006; Abellán *et al.* 2009; Damm *et al.* 2010), including dispersal-associated traits such as wing size (Arribas *et al.* 2012) and range size (Ribera & Vogler 2000; Hof *et al.* 2006). In studies that have used genetic data, increased population structure has generally been associated with species found in lotic habitats (Marten *et al* 2006 - freshwater invertebrates; Drotz et al 2012 – mayflies; Hjalmarsson et al 2014 - beetles).

Despite growing evidence, the link between habitat instability and dispersal is still not well established in aquatic systems, recent studies suggest that a lentic/lotic dichotomy might be too simplistic and may not reflect the genuine complexity of aquatic ecosystems or lineage-idiosyncratic microhabitat, dispersal capacity, and behavior. Notably, within the lentic/lotic classification, there is also variation in microhabitat use. For example, Short & Caterino (2009) examined CO1 data for three lotic beetle species from three families and found differences in habitat exploitation and genetic structure. Among these three species, only one, *Eubrianax edwardsii*, occupied the streaming water, whereas the other two species, *Anacaena signaticollis* and *Stictotarsus striatellus*, respectively occupied detritus at the stream margin and stream pools. Of the three species, only the one found in the stream (*Eubrianax edwardsii*) exhibited strong genetic structure. In a separate study, Lam *et al.* (2018) found idiosyncratic patterns in population structures in a widespread species of diving beetles, *Philaccolilus ameliae* (Dytiscidae), throughout the island of New Guinea. These examples highlight the need for more research to understand the importance of microhabitat use along and/or across the streambed and its relationship to population structuring. A simplistic view also ignores the organisms' dispersal-associated traits and behaviors (e.g., Alp *et al.* 2012) and how these interact with microhabitat use when explaining distribution patterns of populations and species (Phillipsen *et al.* 2014).

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The rugged and geologically complex New Guinea topography (see Toussaint *et al.* 2014) provides an opportunity to study population connectivity of stream organisms in a tropical environment. The island is rich in running water habitats separated by mountain ranges and are thus relatively discrete. Despite high biodiversity and many threatened species, the phylogeography and population genetics of the islands' aquatic fauna remain largely understudied (but see McGuigan *et al.* 2000).

Here, we examine one species of lotic New Guinea diving beetle (Dytiscidae): *Exocelina manokwariensis* (Shaverdo *et al.* 2016). These beetles, less than 4 mm long, inhabit small stagnant water microhabitats (often less than the size of a tea cup) along low order forest creeks and stream margins. These habitats differ from intermittent stream pools in that they exist near actual streaming water but are highly dynamic due to flooding after rainfall and drying out during dry spells. During drought, the beetles were observed hiding under stones or deep in the moist gravel. There are approximately 150 *Exocelina* species in New Guinea with most being narrow endemics (Shaverdo *et al.* 2012). A few species, including the one studied here, have wider ranges, found across the Birds Head Peninsula. We chose this as our target species because it was abundant in six localities across the Peninsula, allowing us to conduct a peninsular-wide, fine scale population genetic study. At least five other *Exocelina* species overlap with *E. manokwariensis* across the peninsula, but they appear more restricted locally (Shaverdo *et al.* 2012).

We examined the population structure of *E. manokwariensis* over its entire geographical range using DNA sequence data from both nuclear and mitochondrial genomes. We hypothesized that as inhabitants of stable stream margin habitats, the beetles would require little dispersal to cope with environmental variation, resulting in significant population structure. The sampling localities lie along a ~300 km transect, from altitudes of c. 100–1,000 m above sea level as well as from areas with different lithologies and potentially different uplift histories (Table 1, Supplement S1). Specifically, we asked: (1) does significant geographical structuring exist among these lotic

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inhabitants among locations, and (2) if so, to what extent can substructuring of the species be explained by past geological events or topographical features?

2 MATERIALS AND METHODS

2.1 Taxon sampling and DNA extraction

We assembled a dataset for 68 specimens from 6 localities (Figure 1), comprising COI data from 65 specimens and double digest restriction site associated DNA sequencing (ddRAD) data from 63 specimens (Supplement S2, also see Figure 1). The outgroup used for the ddRAD-based phylogenetic reconstruction was *Exocelina*_sp_MB6812 also from the Birds Head Penninsula. Genomic DNA was extracted non-destructively from whole beetles using the DNeasy and NucleoSpin 96 Tissue kits (Qiagen, Hilden; Macherey Nagel, Düren, Germany).

2.2 Mitochondrial DNA sequencing

Mitochondrial DNA sequences were obtained for 65 individuals. The 3' end of the mitochondrial cytochrome oxidase subunit 1 (CO1), which is commonly used for molecular taxonomic work in diving beetles (e.g. Monaghan *et al.* 2006), was amplified using the primers Jerry and Pat (Simon *et al.* 1994). PCR reactions were 20 µl total volume: 12.5µL ddH2O, 0.5µL each of 10µm primers, 2µL 10mm DNTPs, 1.25µL 50mm MgCl2, 5µL reaction buffer, 0.2µL Taq polymerase. PCR was performed with an initial denaturation step of 96°C for 3 mins, 35 cycles of 94°C for 30 s, 48°C for 1 min, 72°C for 1 min and a final extension step of 72°C for 10 mins. Sequences were edited using Sequencher 5.0.1 (GeneCodes Corp., Ann Arbor, MI, USA), aligned in Mesquite 3.04 (Maddison & Maddison 2008) using the ClustalW algorithm (Larkin *et al.* 2007), and subsequently manually checked for stop codons.

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2.3 ddRAD sequencing and output treatment

The library was prepared following a modified protocol from Mastretta-Yanes *et al.* (2014) (Supplement S3). Briefly, DNA extracts were double digested using the restriction enzymes SbfI and MseI. Restricted DNA was then ligated to uniquely barcoded adaptors, and Illumina technical sequences were added by PCR amplification with adaptor-matching Illumina PCR primers. The library profile was analyzed with a Fragment analyzer (Advanced Analytical, Ankeny, USA) and size selected accordingly with a BluePippin 2% cassette (Sage Science, Beverly, MA, USA). The library was sequenced on a single lane on an Illumina 2500 HiSeq platform (Lausanne Genomics Technology Facility, Lausanne, Switzerland) using a 100 bp single-read protocol.

To demultiplex and assemble the raw ddRADseq output data we used pyRAD v.3.0.3 (Eaton 2014). The low quality threshold (Mindepth) for each site was fixed to 6, and the value for Wclust set to 0.88. Additionally, we fixed the maximum sites per read with an error rate > 1% to 4, the minimum number of individuals sharing a locus (MinCov) to 2 and the maximum proportion of shared polymorphic sites in a locus (MaxSH) set to 3. Defaults were used for other mandatory parameters. For optional parameters, the strictness of filtering (option 21) was fixed at 2, enforcing a strict filter for adaptors, barcodes and cut sites. After retrieving the filtered sequences for each individual, we applied a cut-off based on coverage to discard individuals with a weak signal. Only one individual was discarded with this additional filter.

Signals of selection were tested for all polymorphic loci using the Bayesian simulation method of Beaumont & Balding (2004) as implemented in BAYESCAN 2.1 (Foll & Gaggiotti 2008). We used a prior odds value of 10, with 100,000 iterations and a burn-in of 50,000. We identified and removed loci that were significant outliers at a *q*-value (i.e., false discovery rate) of 0.05.

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2.4 Population genetics and phylogenetic inference

2.4.1 CO1 dataset

To examine the distribution of mtDNA sequence diversity in the six populations, haplotype networks were constructed using the TCS algorithm (Clement *et al.* 2002) implemented in the PopART software (Leigh & Bryant 2015).

2.4.2 ddRAD dataset

We analyzed the concatenated dataset (including both informative and uninformative sites) with maximum-likelihood (ML). The partitions and corresponding substitution models were selected using PartitionFinder 2.00 (Lanfear *et al.* 2012) using the 'greedy' algorithm. Character sets were grouped by the number of phylogenetically informative sites under the GTR+G model for RAxML, and the Akaike Information Criterion corrected (AICc) was used to select among models. The ML analyses were performed using RAxML 8.0.19 (Stamatakis 2014), conducting 20 independent tree searches for the best ML tree. We assessed support for the best ML topology by performing nonparametric bootstrapping using the autoMRE option. A calculated bootstrap support (BS) value of ≥ 70 was considered indicative of strong clade support (Hillis & Bull 1993; Erixon *et al.* 2003).

Levels of genetic differentiation between each population pair were estimated by pairwise F_{st} (Weir & Cockerham 1984) using GENETIX (Belkhir *et al.* 2004), evaluated using 1,000 permutations on the ddRAD concatenated dataset. To determine hierarchical levels of genetic structure within and among populations, we conducted a hierarchical analysis of molecular variance (AMOVA; Excoffier *et al.* 1992) using Arlequin 3.5 (Excoffier & Lischer 2010), with significance assessed using 1,023 permutations.

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To identify major genetic clusters in populations of *E. manokwariensis* throughout the Birds Head Peninsula, we used the Bayesian clustering approach implemented in STRUCTURE 2.3.4 (Pritchard *et al.* 2000) using the SNP dataset including only variable sites. We ran 10 replicates, each with a burn-in of 100,000 and a run length of 1,000,000 steps, using the admixture and correlated allele frequencies models without prior population information (geographic sampling location). We varied the number of clusters (*K)* from 1 to 10.

The broad scale number of clusters was initially determined examining both the posterior probabilities of the data for each K and the ΔK estimator described by Evanno *et al.* (2005) as calculated in Structure Harvester (Earl & vonHoldt 2012). However, many recent studies suggest that this method tends to underestimate genetic structure in groups with fine-scale genetic structure and in taxa with more complex evolutionary history (e.g. Waples & Gaggiotti 2006; Coulon *et al.* 2008; Levy *et al.* 2012; Lambert *et al.* 2013; Gowen *et al.* 2014). Because we aimed to study fine-scale population structure within a small geographic range, we subsequently conducted a hierarchical approach using STRUCTURE, and ran analyses on successively smaller clusters as in Gowen *et al.* (2014). For example, when two distinct population clusters were identified in the full dataset, we analyzed each cluster separately for $K = 2$ until no geographical clustering was discernable or only one pre-defined population (based on collecting locality) remained. Results from replicates for the inferred K from each run were analyzed in CLUMPP (Jakobsson & Rosenberg 2007) to produce averaged matrices of individual and population cluster membership coefficients. Finally, we used *distruct* v1.1 (Rosenberg 2004) to produce graphical displays of the resulting barplots.

Principal component analyses (PCA) was conducted on the ddRAD dataset as an alternative to compare the consistency of results obtained using the methods described above. Specifically, we used GenAlEx 6.1 (Peakall & Smouse 2006) to calculate genetic distances 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).

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One possible mechanism promoting structuring of populations across the overall range of a species is isolation-by-distance (IBD), a simple consequence of limited dispersal across space. IBD was evaluated by testing for a statistically significant association between genetic differentiation as measured by $F_{ST}/(1 - F_{ST})$ (Rousset 1997) and log-transformed geographic distance among each of the populations. The significance of the correlation was evaluated with a Mantel test (Mantel 1967) with 10,000 permutations as implemented in GenAlEx 6.1.

Finally, to determine the raw migration rates between populations, we used a Bayesian coalescence-based approach implemented in G-PhoCS v1.3 (Gronau *et al.* 2011). The concatenated data was converted into the software's required input format using a custom script. Our model encompassed all possible migration bands between the 6 different populations. The analysis was run with 1,500,000 iterations with a burn-in of 150,000. The migration rates were set to 0.002 and 0.00001 for alpha and beta, respectively. All fine-tuned parameters were estimated during the burn-in period. To evaluate convergence, we used the software Tracer v1.6 (Rambaut *et al.* 2014) and verified the posterior distribution using the effective sample size (ESS). Because we were not able to upload the entire dataset into Tracer, we sub-sampled the log file every 10 iterations. To visualize the results, we plotted the mean migration rates using the ggplot2 package (Wickham 2009).

2.5 Data accessibilty

The CO1 sequences are deposited in the European Nucleotide Archive (LT615638–LT615714). The ddRAD dataset is deposited in Dryad in fasta format (doi:10.5061/dryad.hq77h24).

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3 RESULTS

3.1 Mitochondrial and genomic data

The trimmed CO1 alignment comprised 729 base pairs from 65 individuals. For the ddRAD SEQ, the Hiseq run produced 258 million reads. After pyRAD filtering, *de novo* assembly and minimum coverage cut off, we recovered 3,196 loci encompassing 2,689 SNPs for 63 individuals. BAYESCAN determined that no loci displayed signals of selection at $q = 0.05$ (Supplement S4), and therefore all SNPs were retained for analyses.

3.2 Population and phylogenetic inference

3.2.1 CO1 dataset

The TCS network shows that the CO1 haplotypes are broadly divided in two groups: (1) a "Western cluster", consisting of populations from BH041 and BH044; and (2) an "Eastern cluster" consisting of populations from BH028, BH033, BH034, and BH039. Individuals from BH033 and BH034 all share a single haplotype. Though interconnected beyond the 95% threshold, the Western and Eastern clades remain distinct (Figure 1D).

3.2.2 ddRAD dataset

The RAxML analyses resulted in a topology with a highly supported backbone (Figure 1C). The phylogeny is split into "Eastern" and "Western" clades corresponding to the two clusters described above. In the Eastern clade, BH028 is sister to the 2 subclades BH039 and BH033/BH034. In the Western clade, BH044 and BH041 are sister to each other.

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The AMOVA revealed a moderate amount of variation among populations from the Birds Head Peninsula (54.07%, $P < 0.0001$) (Table 2). These patterns were congruent with those from pairwise F_{ST} estimates (Table 3), with moderate to large values (Hartl & Clark 1997) found between all populations except BH033 and BH034, which are geographically extremely close (less than 1 km apart). The F_{ST} values among populations in the Eastern and Western clades are generally large (F_{ST} = 0.18–0.26). Values within each clade are generally moderate (F_{ST} = 0.05–0.15) with the exception of BH033 and BH034 (F_{ST} = 0.02).

The first three components of the PCA explained a majority of the total variance (62.22% cumulative). Overall, the PCA showed similar broadscale patterns to the other analyses: two separate clusters representing clades 1 and 2 described above. Each population, except BH033 and BH034, remains somewhat distinct (Figure 2).

In the initial STRUCTURE run of K = 1–10 using the full dataset, the ΔK test (Evanno *et al.* 2005) depicted K = 2 as the best model for the data (see Supplement S5 for ΔK table and graph). Congruent with the other analyses, the two major and most distinct clades are: The Eastern clade, including BH028, BH033, BH034, BH039, and the Western clade, including BH041 and BH044 (Figure 1B i). However, subsequent hierarchical analyses revealed strong structure within these clades associated with geography. Indeed, STRUCTURE runs on successively smaller clusters revealed five distinct geographic clusters that were largely uniform in their population assignment: The Western clade was split into two distinct populations with no significant admixture, i.e. (1) BH041 and (2) BH044 (Figure 1B ii). In the Western clade, (3) BH028 first split off from the rest of the group and seems to be distinct (Figure 1B ii); (4) BH039 also differs from BH033 & BH034, (Figure 1B iii), but STRUCTURE results suggest a moderate level of admixture between the two groups; finally, (5) BH033 and BH034 form a single population (Figure 1B iv). The Mantel test showed no significant association between genetic and geographic distance (R^2 = 0.161, P = 0.087) (Figure 3).

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The migration rates estimated with G-PhoCS revealed comparatively high gene flow between populations BH033 and BH034, for which the mean unidirectional migration rates were 0.2293 and 0.2609, respectively (Figure 4, see also Table S6). The analysis also depicted high gene flow from BH033 to BH039 (mean relative migration rate of 0.0273). Surprisingly, the opposite migration band has a much lower rate (0.0012). Low migration rates were also found from BH034 to BH039 (0.0023) and from BH044 to BH028 (0.0023). All other migration bands show almost no migration.

4 DISCUSSION

Our results document significant fine scale population structure in a widespread lotic diving beetle across the geologically complex Birds Head Peninsula of New Guinea. Though lotic species are predicted to exhibit more genetic structuring than lentic species, *E. manokwariensis* is adapted to microhabitats of stagnant water along the course of forest creeks, often immediately proximal to the flowing water (edge of backflows, water filled holes in bedrock, tiny waterholes on gravelly stream edges). The complexity of the aquatic habitat used by *E. manokwariensis* and features of the geology of the Birds Head Peninsula both contribute to the surprising degree of population structure found over relatively small distances.

E. manokwariensis, as morphologically defined (Shaverdo *et al.* 2016), has a large geographic range compared to other *Exocelina* species, with significant genetic variation among populations. Excluding the localities BH033 and BH034 (which are less than 1 km from each other on the same mountain slope), the average pairwise distance between differentiated populations is only 45 km. We found comparatively high levels of migration for three bands or sets of populations (Figure 4). The two highest migration rates were found between BH033 and BH034 (noted above), which harbored similar unidirectional gene flow levels, and a third band, which also showed migration but

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on a much lower level, was from population BH033 to BH039. All other bands show very low levels of gene flow. The maternally-inherited mitochondrial and nuclear genomic markers illustrate the same pattern in *E. manokwariensis*, suggests there is no apparent sex-biased dispersal in this species, which is expected given the lack of sexual dimorphism in body size and wing structure. The specific dispersal behavior of these beetles remains to be quantified, such as with flight interception traps that could elucidate the relative roles of stream courses, forest edges, and mountain ridges on movement. The significant genetic structure in *E. manokwariensis* without evidence of isolation by distance suggests that gene flow among population is low, even between streams in close proximity and that random genetic drift is an important mechanism driving population divergence (Hutchinson 1957; Phillipsen *et al.* 2014). That each population can evolve somewhat independently increases the likelihood of microendemism and may help to explain the small geographic ranges of other *Exocelina* species. The occasional appearance of broadly distributed (lotic) species producing narrow endemics was described by Ribera *et al.* (2011) for Iberian *Hydraena* species. This was attributed to either successive bouts of dispersal equivalent to island hopping or stepping stone dispersal (MacArthur & Wilson, 1967), fragmentation of an existing larger range (vicariance) or "…speciation by increased reduction of gene flow between favourable patches" (refuge speciation cf. Moritz *et al.* (2000) (Ribera *et al.* 2011).

Population structure at this small scale as well as low levels of dispersal among localities is generally predicted by the habitat template concept for lotic species and is consistent with results of previous studies finding correlations between habitat type and dispersal in aquatic insects. For example, in a study of Southern California water beetles, Short & Caterino (2009) found that levels of genetic structure can vary widely among species, even for lotic species found in the same geographic range. Among the three species studied, strong genetic structure was only observed only in a species adapted to flowing water, whereas species on stream margins and stream pools lacked pronounced structure. The authors attributed these differences to differences in habitat preferences among species, as well as variation in other natural history traits.

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The geomorphology of the Birds Head Peninsula observed today began to develop only in the past few million years (~ < 3 Mya) (e.g. Haq & Al-Qahtani 2005; Snedden & Liu 2010). However, the extent of land during the past million years remains unclear as does the origins of *Exocelina* speices in the gegion. Gold *et al.* (2017) present models suggesting a landmass larger than the present-day Birds Head in the Oligo-Miocene. However, much of the region has been subaerial since the Late Pliocene possibly associated with a larger landmass possibly again by marine intrusions in the Pleistocene. A phylogeny of New Guinea *Exocelina* (Toussaint *et al.* 2014) placed *E. manokwariensis* in a shallow clade including other Birds Head species, dated at ~ < 3 Mya. Thus, the colonization of the peninsula by *Exocelina* might be relatively recent with only rare dispersal across the existing complex landscape, as indicated by limited current gene flow. Such a pattern would be expected to occur prior to the formation of microendemic species, consistent with the extraordinarily richness of the New Guinea fauna. Alternatively, a reduction of gene flow could be due to occasional vicariance events, such as marine intrusions, ongoing orogeny or regional fluctuations of microclimates. A third hypothesis is that colonization of the Birds Head area might have occurred when the region consisted of several islands (e.g. in the Pleistocene, Gold *et al.* (2017), with populations adapting to local environmental factors.

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In conclusion, our study find significant geographical structuring over small special scales in the diving beetle, *E. manokwariensis*, is consistent with predictions of the habitat template concept as applied to aquatic habitats. In addition, both past geological events and current topographical

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features help explain patterns of genetic variation at small spatial scales that likely contribute to the evolution of microendemism in *Exocelina* beetles.

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Data Accessibility

DNA sequences: European Nucleotide Archive (LT615638–LT615714).

ddRAD dataset was deposited in Dryad in fasta format:

doi:10.5061/dryad.hq77h24

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Author Contributions

AL & MB conceived the study. MB conducted field work. MB & AL performed laboratory work for Sanger sequencing. MG & NA performed ddRAD laboratory work and sequencing bioinformatics. AL performed population genetic and phylogenetic analyses. MVD assist with R analyses. HS carried morphological investigations and sample preparations. RP organized fieldwork with the University of Papua and conducted fieldwork with MB. LTW provided geological information. AL & MB led the writing and MVD, MG, GR, NA, LW contributed to the manuscript. AL, CK, MB & GR led the rewriting of the reviewed manuscript.

Locality	Latitude	Longitude	Geological Formation	Geological age and description	Estimated time of uplift
BH028	-0.88385	132.73706	Kemum Formation	Paleozoic metasedimentary rocks overlain by Miocene (16-15 Ma) limestone and Oligocene sandstone	Uplift sometime between ~15 Ma and 0 Ma
BH033	-0.78320	133.07214	Quaternary Alluvium	Quaternary alluvium deposited on top of the Triassic Netoni granitoids	Uplift sometime between 3 Ma and 0 Ma
BH034	-0.77452	133.06993	Quaternary Alluvium	Quaternary alluvium deposited on top of the Triassic Netoni granitoids	Uplift sometime between 3 Ma and 0 Ma
BH039	-0.90758	133.92147	Maruni Limestone /Quaternary Alluvium	Boundary between the Lower-Middle Miocene Maruni Limestone and Quaternary alluvium	Uplift sometime between ~15 Ma and 0 Ma
BH041	-0.76297	131.61770	Limestone within the Sorong Fault Zone	Miocene limestones within the Sorong Fault Zone	Uplift sometime between ~23 Ma and 0 Ma
BH044	-0.69750	132.07225	Tamrau Formation	Middle Jurassic to Cretaceous metasedimentary rocks unconformably overlain by Pliocene melange and Quaternary alluvial deposits	Uplift sometime between ~66 Ma and 0 Ma

TABLE 1 Geological information and likely uplift history that corresponds with each of the sampling sites according to existing geological maps (Pieters *et al*., 1989; Amri *et al.*, 1990; Robinson *et al.*, 1990) as well as inferences drawn from recently collected, but unpublished field data.

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Source of variation	df	Sum of squares	Variance components	Percentage of variation	Fixation index
Among populations	5	856.448	15.34367	54.0703	FST=0.54070, P=0.000000
Within population	62	656.572	13.03359	45.9297	
Total		1513.02	28.37726		

TABLE 2 Analyses of molecular variance (AMOVA) of the six populations from the Birds Head Peninsula using ddRAD data. df - degrees of freedom.

Location	BH028	BH033	BH034	BH039	BH041	BH044
BH028	-	**0.15***	**0.14***	**0.13***	**0.18***	**0.15***
BH033		-	**0.02**	**0.05***	**0.17***	**0.26***
BH034			-	**0.05***	**0.15***	**0.20***
BH039				-	**0.15***	**0.18***
BH041					-	**0.13***

TABLE 3 Pairwise F_{ST} estimates based on ddRAD data. Values with * were significant (P<0.05). Pairwise values for among clade (Eastern and Western clades) comparisons are shaded.

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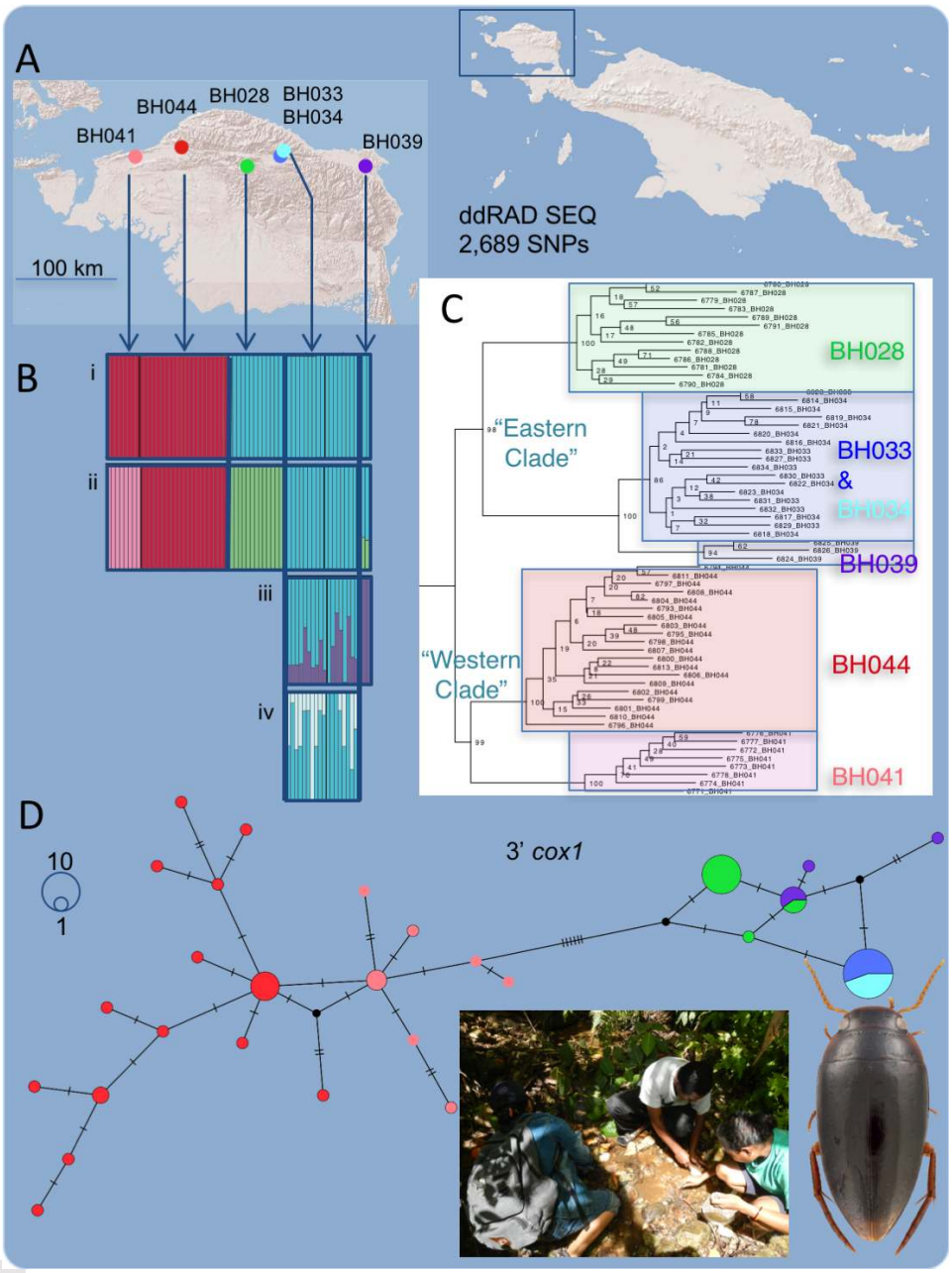

FIGURE 1 Population structure of *Exocelina manokwariensis.* **A** Birds Head Peninsula with collecting localities of the six populations studied; location of peninsula indicated on map of New Guinea in upper right (locality colors correspond to colors in Figures C and D). **B** Bayesian clustering analyses of ddRAD SNPs data in STRUCTURE. Barplots from STRUCTURE runs for successively smaller genetic clusters (*K=1-10* for the first run, *K* = 2 for each successive run) (marked at the barplot with "i") The first run split the individuals broadly into an Eastern clade and Western clade. (ii) The Western clade split into two distinct groups corresponding to the two collecting localities BH041 and BH044. (iii) Population BH028 is split from the rest of the Eastern clade. (iv) There is some evidence for differential assignment between BH039 and BH033 + BH034. (Bv) Individuals form BH033 and BH034 have mixed assignments and showed no geographic structure. **C** Maximum Likelihood (RAxML) tree based on the ddRAD dataset with bootstrap support values. Numbers and colors correspond to collecting locations. **D** TCS network based on CO1 sequences. Colors correspond to locality colors on the map. Lower right, fieldwork in typical habitat (photo: M. Balke) and habitus of *Exocelina manokwariensis* (photo: Harald Schillhammer, Vienna).

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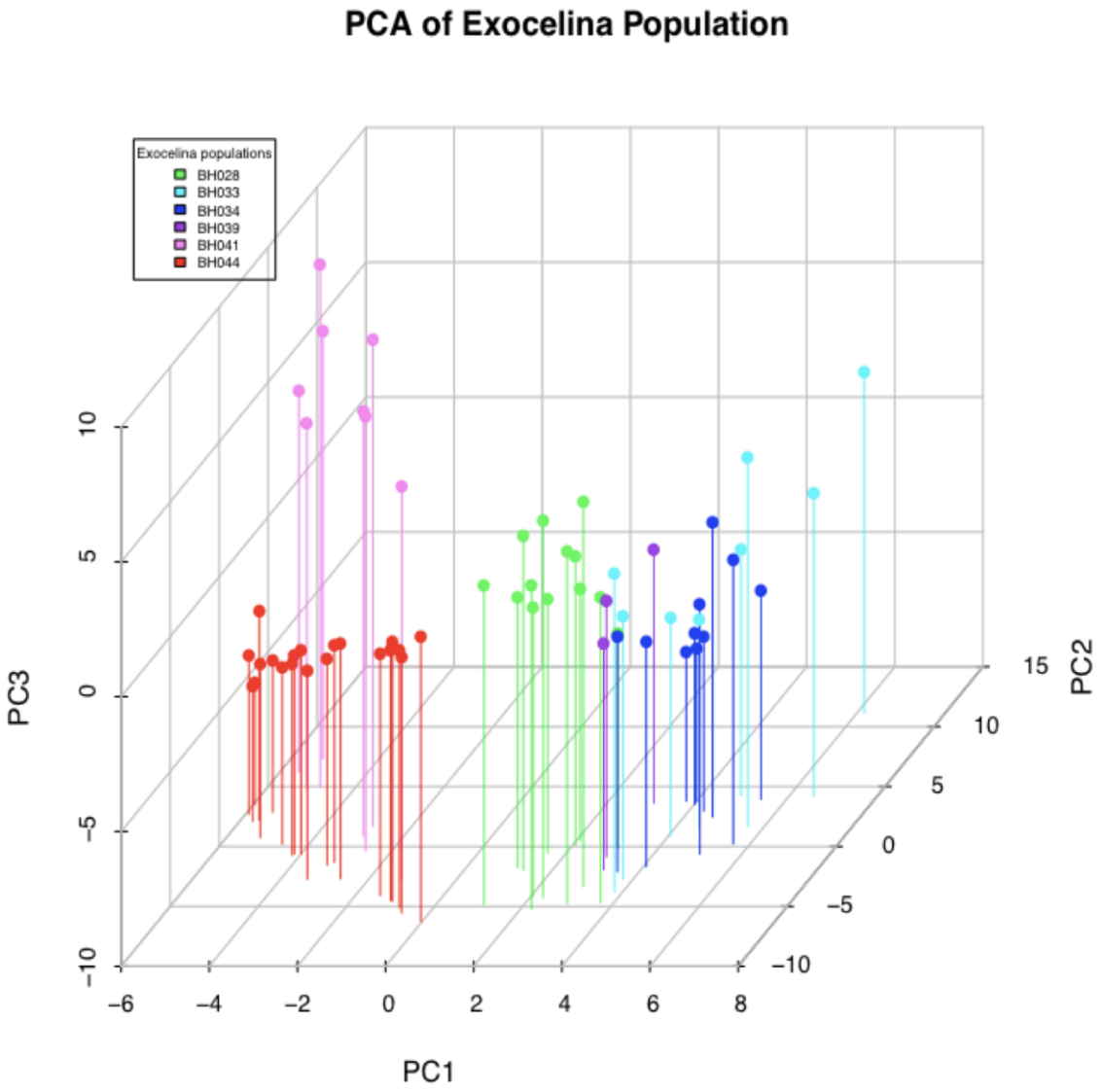

FIGURE 2 Three-dimensional plot of a Principal Coordinates Analysis based on individual ddRAD genotypes. Individuals are color-coded according to collection locality. Axis PC1 explains 28.40%, axis PC2 17.65% and axis PC3 16.17% of variation.

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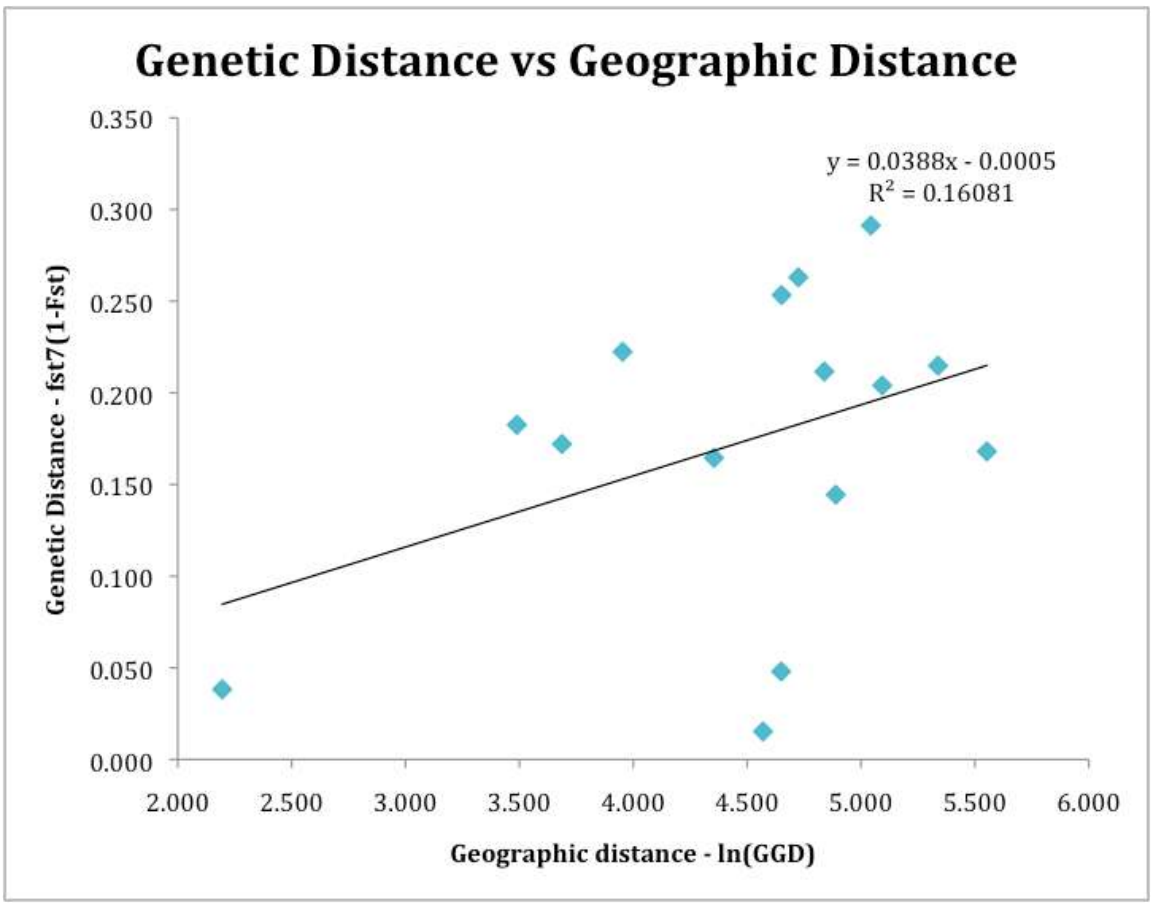

FIGURE 3 Relationships between genetic and geographic distances between pairs of populations. Genetic distances were based on the ddRAD sequences calculated from linearized F_{ST}. Geographic distances are log transformed Euclidean distances in km.

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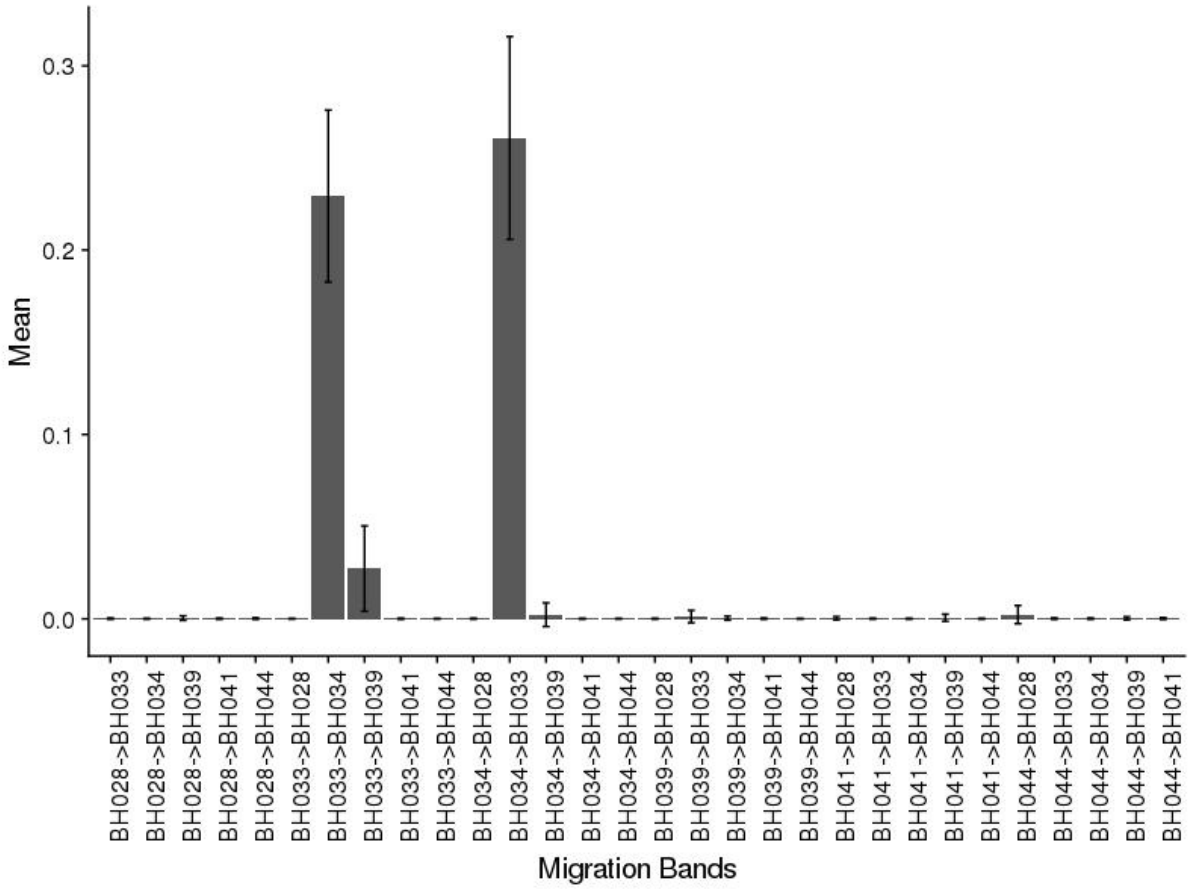

FIGURE 4 Relative mean migration rates between the different *Exocelina* populations calculated with G-PhoCS using ddRAD data. The migration rates were set to 0.002 and 0.00001 for alpha and beta, respectively, and all fine-tuned parameters were estimated during the burn-in period.

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