Colonizing the Caribbean: biogeography and evolution of livebearing fishes of the genus *Limia* (Poeciliidae)

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**ABSTRACT**

**Aim** We investigate the origin and colonization of the West Indian endemic freshwater fish group *Limia*. We evaluate the leading hypotheses for the origins of West Indian life, including trans-oceanic dispersal, late Cretaceous vicariance, and the GAARlandia land bridge at the Eocene/Oligocene boundary.

**Location** Greater Antilles, with extensive sampling in the Dominican Republic.

**Methods** We obtained DNA from wild sampling and the aquarium trade. We sequenced three mitochondrial (12S, ND2 and Cytb) and two nuclear genes (Rh, MYH6) for a combined molecular phylogenetic analysis to evaluate species relationships and the timing of divergence events between islands and the mainland. We used Bayesian and likelihood approaches to build phylogenies, a BEAST analysis to establish the timing of colonization, and R package BioGeoBEARS to perform a historical biogeographical reconstruction.

**Results** Relaxed molecular clock results show that the ancestor to the West Indian clade, which includes the *Limia* and Hispaniolan *Poecilia*, diverged from a South American ancestor at the Eocene/Oligocene boundary. The basal Jamaican species, *L. melanogaster*, split from the rest of *Limia* at the Oligocene/Miocene boundary. Cuban and Cayman taxa are sister to a diverse species group from Hispaniola. Historical biogeographical reconstruction supported the GAARlandia DEC+ model as the best fitting model for colonization.

**Main conclusions** Our results support a colonization model for *Limia* that is concordant with the timing of GAARlandia and climate change during the Eocene/Oligocene boundary. *Limia* colonization was most likely a result of facilitated dispersal during a period of lower sea levels and shorter passage along the Aves Ridge. These results are also consistent with other recent molecular clock studies of dispersal limited cichlids, toads and frogs, indicating a growing body of support for the significance of Eocene/Oligocene climate change for the historical biogeography of West Indian life.

**Keywords** colonization, GAARlandia, Hispaniola, historical biogeographical reconstruction, *Limia*, relaxed molecular clocks, vicariance, West Indian biogeography

**INTRODUCTION**

After nearly two centuries of debate, biogeographical reconstruction in the West Indies continues to be a contentious issue, with wide disagreement over the relative importance of dispersal–vicariance. The islands of the West Indies exhibit exceptional biodiversity and are ranked in the top five of the world’s most important biodiversity hotspots, with endemism reaching over 90% in some groups (Hedges, 1996; Myers et al., 2000). However, the island ecosystems are relatively depauperate and some major mainland groups, including marsupials, carnivores, lagomorphs, salamanders and most families of frogs, turtles, and snakes are missing (Hedges, 1996, 2001). These patterns are typical of isolated oceanic islands, in which a few successful dispersers radiate in situ. Molecular clock analyses across taxa, including some reptiles, amphibians, and
mammals, show a pattern of random and recent divergences from mainland sources (mostly from South America), concordant with a dispersal model of colonization (see Hedges, 1996, 2001 for figure showcasing random colonization patterns). The primary mechanism of dispersal for these groups is thought to be a combination of large flooding events in South America, floating mats of debris, and the prevailing north-westerly oceanic currents (King, 1962; Vonhof et al., 1998; Hedges, 2001; Glor et al., 2005). Dispersal continues in the present day, as evidenced by a colony of iguanas (Iguana iguana) arriving to the island of Anguilla from nearby Guadeloupe in the wake of Hurricane Luis in 1995 (Lawrence, 1998).

Alternatively, the vicariance argument highlights the presence of organisms with poor dispersal abilities and relies on the correlation between evolutionary patterns and geological reconstructions. Advocates of West Indian vicariance biogeography envision entire ecosystems, trapped on drifting island fragments, splitting from a proto-Central America in the late Cretaceous (65 Ma) through plate tectonics (Rosen, 1975, 1985; Nelson & Platnick, 1981; Guyer & Savage, 1986; Page & Lydeard, 1994). The presence of freshwater fish on the islands of the West Indies has sparked much interest over the years because of their diversity and endemism, as well as their presumed limited dispersal abilities (Rosen & Bailey, 1963; Rosen, 1975; Rivas, 1986; Pooser, 2003), and they were instrumental in the development of vicariance biogeography as a discipline (Rosen, 1975). Previous taxonomic studies of West Indian freshwater fish show that species on Hispaniola and Cuba are polyphyletic; northern Hispaniolan species are more closely related to eastern Cuban and Cayman island species than they are to southern Hispaniolan species (Rauchenberger, 1988; Burgess & Franz, 1989; Rodriguez, 1997; Hamilton, 2001). These patterns seemed concordant with proposed geological models of island formation and a late Cretaceous connection with the mainland (Pitman et al., 1993; Pindell, 1994). However, the late Cretaceous vicariance model has received much criticism, mainly because of a lack of molecular clock support and the unlikely survival of life in this region during and shortly after the late Cretaceous bolide impact near the Yucatan peninsula (Hildebrand & Boynton, 1990).

An alternative geological reconstruction that shows a more recent land connection between the mainland and the West Indies, is the GAARandia model (Greater Antilles Aves Ridge) (Itruralde-Vinent & MacPhee, 1999; Itruralde-Vinent, 2006). In this reconstruction, any late Cretaceous land formations in the Caribbean would have been inundated by subsequent sea level change, and permanent land was only present after the Middle Eocene (<40 Ma). During the Eocene/Oligocene transition (30–35 Ma), a rapidly cooling planet caused global declines in sea level upwards of 60 m (Haq et al., 1987; Miller et al., 2008). Coupled with general tectonic uplift in the region, this may have allowed for potential overland colonization of the West Indies from South America across the Aves Ridge (Itruralde-Vinent & MacPhee, 1999; Itruralde-Vinent, 2006).

One of the groups for which colonization routes is still unknown is Limia (family Poeciliidae), a genus of livebearing freshwater fishes endemic to the islands of the West Indies (Rauchenberger, 1988; Burgess & Franz, 1989; Rodriguez, 1997; Hamilton, 2001). With 17 described endemic species on Hispaniola and one endemic species on each of the islands of Cuba, Jamaica and Grand Cayman, Limia is the dominant freshwater fish group in the Greater Antilles (Burgess & Franz, 1989). However, the historical biogeography of Limia has not been critically assessed using molecular techniques and the timing and mechanism of colonization for Limia remains unresolved. Our hope is that by reconstructing the colonization of Limia, we will gain a vital piece in the ever-evolving debate on the origins of West Indian life and gain insight into other organisms with limited long-distance dispersal abilities.

Freshwater fish in the Limia genus provide an ideal opportunity to evaluate colonization hypotheses because of their diversity and presence on multiple islands. Using Limia species from Hispaniola (Dominican Republic and Haiti), Jamaica, Cuba, as well as poeciliid species from the mainland, evaluation of multiple divergence events is possible: West Indies from the mainland (all Limia), Jamaica from Cuba/Hispaniola (L. melanogaster split) and Cuba from Hispaniola (L. vittata split). We test the above colonization scenarios using a combined gene molecular phylogenetic approach with a robust sampling of poeciliids from the islands and the mainland, together with a relaxed molecular clock analysis and historical biogeographical reconstruction.

The goal for this study was to test biogeographical hypotheses of the West Indies through an updated multigene phylogeny of Limia. We examine three alternative scenarios, with regard to Limia colonization. First, long-distance transoceanic dispersal models, which predict colonization across the Caribbean sea by salt tolerant taxa from diverse mainland sources, with subsequent evolution of the fresh-water clades (Myers, 1966; Briggs, 1984, 1987; Hedges, 1996, 2001, 2006). Second, late Cretaceous vicariance models, which predict colonization during the late Cretaceous (65–85 Ma) and persistence of biota on incipient island fragments (Rosen, 1975, 1985; Nelson & Platnick, 1981; Guyer & Savage, 1986; Rauchenberger, 1988; Page & Lydeard, 1994). Finally, the GAARlandia model, which postulates inundation of the incipient island fragments after the late Cretaceous and until the Middle Eocene (<40 Ma), with range expansion of South American groups along a short-lived, exposed Aves Ridge, which was created by sea level decline and Caribbean plate uplift during the Eocene/Oligocene boundary (33–35 Ma) (Itruralde-Vinent & MacPhee, 1999; Itruralde-Vinent, 2006).

**METHODS**

**Sampling**

For an analysis of Limia diversity within the West Indies, we sampled poeciliids from across the region, including wild
collections and species we could obtain through the aquarium trade. Wild collections were focused on the island of Hispaniola to sample from the greatest diversity of poeciliids and to ensure that each of the major clades of *Limia* was included. A total of 30 Dominican populations were sampled using seine and dip nets during trips in 2000, 2003, 2004, 2010, 2012 and 2013 (Fig. 1, see Appendix S1 in Supporting Information). All appropriate permits for collection and transport were obtained through local wildlife agencies. In addition, we included the Haitian species (*L. nigrofasciata*), the Jamaican species (*L. melanogaster*), the Cuban species (*L. vittata*) and the Grand Cayman species (*L. caymanensis*), which were obtained through aquarium stock at the University of Colorado (see Appendix S1). All specimens were preserved in 80% ethanol prior to DNA extraction, in accordance with IACUC approval at the University of La Verne. DNA sequences from additional outgroup taxa from the mainland genera of *Pamphorichthys*, *Micropoecilia*, *Poecilia* and *Xiphophorus* were obtained through Genbank (see Appendix S1).

**Molecular methods**

Genomic DNA was extracted from ethanol-preserved caudal peduncle muscle tissue following the manufacturer’s protocol for the DNeasy Kit (Qiagen Inc., Valencia, CA, USA). To provide resolution at both the shallower and deeper divergence nodes, we amplified both mitochondrial and nuclear loci (Hrbek et al., 2007). Five gene fragments, three mitochondrial (12S, ND2 and Cytb) and two nuclear (Rh, MYH6), were amplified and sequenced for the 68 ingroup individuals. Primer sequences for polymerase chain reaction (PCR) are listed in Appendix S1.

Amplifications were performed in an Eppendorf Mastercycler (Eppendorf, Westbury, NY, USA) under the thermal conditions listed in Appendix S1. PCR products were purified using ExoSAP enzymes (USB Corp., Cleveland, OH, USA). Purified PCR products were cycle sequenced using Big Dye chemistry v.3.1 (Applied Biosystems Inc., Foster City, CA, USA) and visualized on an ABI 377XL (Life Technologies, Grand Island, NY, USA). DNA sequences were edited with Geneious Pro 5.6.6 and alignments were performed with Clustal W (Thompson et al., 1997).

**Phylogenetic analyses**

Phylogenetic analyses were performed using both Bayesian analyses (BA) and maximum likelihood (ML) methods. Substantial discussion has revolved around the contrasting approaches of multigene concatenated data sets versus consensus gene trees (see Gadagkar et al., 2005; Degnan & Rosenberg, 2009). To evaluate the utility of combining the
nuclear and mitochondrial data sets, we ran preliminary analyses on the mitochondrial and nuclear data sets independently using Mr. Bayes 3.1.2 (Ronquist & Huelsenbeck, 2003). Doubts about the utility of incongruence length difference tests (Barker & Lutzoni, 2002; Darlu & Lecointre, 2002; Alonso et al., 2012) lead us to visually inspect the mitochondrial and nuclear trees for congruence. The mitochondrial and nuclear trees were overall similar in their topologies, with the mitochondrial tree showing better resolution near the tips and the nuclear tree showing better resolution at deeper nodes (see Appendix S2).

We used PartitionFinder (Lanfear et al., 2012) to evaluate the appropriate nucleotide substitution models and partition scheme on our combined data set. PartitionFinder allowed us to evaluate various partition schemes, including the fragment of 12S rDNA and each codon position independently in all of the protein coding genes (ND2, Cytb, Rh and MYH6). The optimal partition scheme for the data, as outlined by PartitionFinder, was a 7-way partition strategy with the following models: subset 1 = K80+I for 12S and MYH6 pos 2, subset 2 = GTR+I+G for Cytb pos 1, ND2 pos 1, subset 3 = HKY+G for Cytb pos 2 and ND2 pos 2, subset 4 = HKY + I for Cytb pos 3, ND2 pos 3 and Rh pos 1, subset 5 = JC+I for Rh pos 2, subset 6 = F81 for Rh pos 3, subset 7 = HKY+I for MYH6 pos 1 and MYH6 pos 3.

Bayesian analyses were then performed on the combined data set, using the seven partition scheme provided by PartitionFinder. The Markov chain Monte Carlo (MCMC) simulation was performed utilizing two independent runs, using eight chains and 10 million generations, sampling every 1000 generations. Convergence and stability of the runs was detected using Tracer 1.5. (Rambaut & Drummond, 2007). The first 25% of each run was established as burn-in and discarded. The remaining trees were used to compute a 50% majority rule consensus tree. Maximum likelihood trees were built using PhyML (Guindon & Gascuel, 2003) as implemented in Geneious Pro 5.6.6 and paup 4.0 (Swofford, 2003). Support for nodes for both methods was established with 1000 bootstrap replicates. For the Phylm analysis, we employed a general GTR+I+G substitution model, optimizing topology, length and rate, and used the BEST topology search strategy. In the Paup analysis, we used the seven partition scheme as outlined by PartitionFinder. Tree searching was performed with a branch and bound strategy, using the FURTHEST addition method and bootstrapping was performed using a random seed and branch and bound search type. Trees were rooted with the Central American livebearing species Xiphophorus hellerii after Hrbek et al. (2007).

**Divergence time estimation**

We chose single representatives from each of the main lineages (34 in total) to maximize coverage of available gene sequences and calculated divergence time estimates using a relaxed-clock method implemented in BEAST 1.7.4 (Drummond & Rambaut, 2007). The data set was partitioned with the same nucleotide substitution model as the seven partition scheme we used for the MrBayes analysis, except that base frequencies were estimated for all partitions. We used the uncorrelated lognormal distribution model on the partitioned data set to account for lineage specific rate heterogeneity. We used the Yule speciation process as the tree prior. Because no fossil records were available for our taxa, we calibrated the tree based on the opening of the Windward Passage, which split Cuba from Hispaniola (L. vittata from the Hispaniolan Limia clade) 14–17 Ma (Iturralde-Vinent, 2006). Additional analyses were run using an alternative uniform prior distribution of 20–25 Ma for the Windward Passage (Pitman et al., 1993) to evaluate the influence of this parameter (see Appendix S2). The estimation of 14–17 Ma is supported by the geological reconstruction of Iturralde-Vinent (2006) as well as independent molecular clock estimates for toads (Alonso et al., 2012) and cichlids (Chakrabarty, 2006; Pérez et al., 2007; Ricán et al., 2012). This calibration point has also been used successfully in examination of the Cuban Girardinus group (Doadrio et al., 2009), as well as the Central American Poecilia sphenops (Alda et al., 2013). The analyses were performed with two independent runs for 30 million generations, with trees selected every 10,000 estimations. We checked for convergence diagnostics with Tracer 1.5 (Rambaut & Drummond, 2007) and combined runs using Log-Combiner 1.7 (part of the BEAST package). The data were summarized using TreeAnnotator 1.7.4 (part of the BEAST package) and visualized using FigTree 1.4.2. (Rambaut, 2009).

**Biogeographical analyses**

To investigate the historical biogeography of Limia, ancestral ranges were estimated using the R package BioGeoBEARS (Matzke, 2013); this allowed direct tests of the fit of dispersal and GAARlandia models. For all of the hypotheses listed, we applied the Dispersal-Extinction-Cladogenesis (DEC; Rec & Smith, 2008) model and its modified version, DEC+j, which allows for founder event speciation. Additionally, analyses included a time-stratified approach that represents different dispersal rates across time slices (see Appendix S3). Finally, the relative likelihood of all resulting models were compared using Akaike’s information criterion (AIC; Burnham & Anderson, 2002). The best model was then used to estimate the biogeographical history of Limia.

On the basis of the dated chronogram for Limia, we pruned the tree to remove outgroups, but kept the sister group comprised of the Hispaniolan Poecilia species, as well as the closest mainland relative Pamphorichthys. We defined five geographical areas occupied by Limia and its closest relatives, including South America (SA), Hispaniola (HI), Jamaica (JA), Cuba (CU) and Cayman Islands (CA) (Table 1). We evaluated the likelihood of a dispersal model versus a GAARlandia model of colonization, using time slices that correspond with the proposed colonization pathway applied to each model (see Appendix S3). We did not test the late Cretaceous vicariance hypothesis, as the timing was
Table 1 Current species distributions used to reconstruct the biogeographical history of Limia in the West Indies. The species included in this analysis were: Pamphorichthys hollandi, from the South America Biogeographic region, Limia dominicensis, Limia perugiae, Limia nigrofasciata, Limia zonata, Limia versicolor, Poecilia elegans, Poecilia dominicensis and Poecilia hispaniolana from Hispaniola, Limia melanogaster from Jamaica, Limia vittata from Cuba and Limia caymanensis from Grand Cayman.

<table>
<thead>
<tr>
<th>Biogeographical region</th>
<th>Species occurrences</th>
</tr>
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<tbody>
<tr>
<td>South America (SA)</td>
<td>Pamphorichthys hollandi</td>
</tr>
<tr>
<td>Hispaniola (HI)</td>
<td>L. dominicensis, L. perugiae, L. nigrofasciata, L. zonata, L. versicolor, Poecilia elegans, Poecilia dominicensis, Poecilia hispaniolana</td>
</tr>
<tr>
<td>Jamaica (JA)</td>
<td>L. melanogaster</td>
</tr>
<tr>
<td>Cuba (CU)</td>
<td>L. vittata</td>
</tr>
<tr>
<td>Grand Cayman (CA)</td>
<td>L. caymanensis</td>
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not supported by the divergence time analysis. For the dispersal model, our probability matrices reflect no land connection between the Greater Antilles and South America and the emergence of Jamaica during the mid-Miocene (after Robinson, 1994). For the GAARlandia model, time slices reflect the reconstructions of Iturralde-Vinent & MacPhee (1999) and Iturralde-Vinent (2006) and include facilitated dispersal via a land connection between South America and the landmass that included the proto-island fragments of Cuba, Hispaniola and the Jamaican Blue Mountain terranes from 35 to 30 Ma, followed by flooding and separation of the Greater Antilles from the mainland from 30 Ma to the present. Both models assume the opening of the Windward Passage separating Eastern Cuba from Hispaniola at 15 Ma (Iturralde-Vinent, 2006), as well as the formation of the Cayman Islands during late Miocene uplift, with a short-lived connection to Cuba from 5 to 3 Ma (Jones, 1994).

RESULTS

Phylogenetic analysis

Our combined and concatenated mitochondrial (12S, ND2, Cytb) and nuclear (Rh, MYH6) data sets yielded 2661 characters (1133 mitochondrial, 1528 nuclear). The resulting gene sequences are available in GenBank, accession #s KX023907-KX024245. The 7-way partitioned data set, which included both nuclear and mitochondrial data, provided good resolution at both the shallowest and deeper nodes of our ingroup taxa with Bayesian posterior probabilities (PP) of 0.90–1.0 and maximum likelihood bootstrap (MLB) support of 80–100% (Fig. 2, Appendix S2). Overall tree topologies of the Bayesian, as well as ML reconstructions from both PhyML and PAUP were similar at all nodes. Support for nodes was very similar across ML methods. A discussion of Limia evolution and species relationships within the genus are included in Appendix S2.

Divergence time estimation

The West Indies clade of Limia and Poecilia is shown to split from the South American Pamphorichthys group during the Eocene/Oligocene transition approximately 32.9 Ma (95% highest posterior densities (HPD) = 21.6–48.8) (Fig. 3, node A). The Jamaican species L. melanogaster split from other Limia species near the Oligocene/Miocene transition 22.8 Ma (95% HPD = 17.2–32.0) (Fig. 3, node B). The tree was calibrated by the splitting of Cuban L. vittata and Grand Cayman L. caymanensis from the Hispaniolan Limia clade (Fig. 3, node C), which corresponds with the opening of the Windward Passage and separation of the proto-island landmasses of eastern Cuba and northern Hispaniola 14–17 Ma. The Grand Cayman species, L. caymanensis split from its sister taxon, the Cuban L. vittata at 3.64 Ma (95% HPD = 1.28–7.85) (Fig. 3, node D). The large clade of Hispaniolan lineages, which include several putative L. perugiae populations (see Appendix S2 for further discussion), as well as a sympatric population of L. sulphurophila from La Zurza, and several unidentified groups, is shown to be a recent diversification event at 1.76 Ma (95% HPD = 0.69–3.10) (Fig. 3, node E). The correlation of our molecular clock analyses with the GAARlandia model is shown in Fig. 4. The timing of the West Indies clade divergence (A, Fig. 4), the isolation of Blue Mountains terrane of Jamaica from southern Hispaniola (B, Fig. 4) and the opening of the Windward Passage separating eastern Cuba from Hispaniola (C, Fig. 4) correlate with estimates from the Beast analysis.

Biogeographical analyses

The AIC model comparison (Table 2) supported the GAARlandia DEC-j model as the best fitting model. This model was 4.8 AIC units lower than the second best model, no-GAARlandia DEC. This result shows that a model that includes a land connection between South America and the proto-island fragments during 35 to 30 Ma is better fitted for the clade comprised of Limia, Poecilia and Pamphorichthys than a model where the colonization of the Caribbean islands happened only through dispersal. Moreover, the model with the founder event parameter (j) shows a significant lower likelihood score than its simpler version, which suggests that range expansions alone are not sufficient to explain movements to new areas. For example, the results show that one jump dispersal (or, founder event) is estimated to explain the current range of L. caymanensis (Fig. 5).

DISCUSSION

Biogeography of Limia

West Indian fauna likely colonized the islands during many events and through multiple mechanisms. Here, we show evidence that an ancestor to the West Indies clade of live-bearing freshwater fishes (including all Limia and the
Figure 2 Phylogenetic reconstruction based on a Bayesian analysis of a combined dataset for 88 individuals and five genes (three mitochondrial: 12S, ND2, Cytb and two nuclear: MYH6, Rh) with seven data partitions. Numbers above nodes represent Bayesian Posterior Probabilities and numbers below nodes represent maximum likelihood bootstrap estimates from ML analysis. A version of this phylogeny with labelled terminal nodes is available in Appendix S2.
Hispaniolan *Poecilia* diverged from mainland taxa at the Eocene/Oligocene boundary (32.9 Ma) (Fig. 3). The timing of the split between the West Indian clade and the mainland precludes a late Cretaceous vicariant origin or recent transoceanic dispersal. In addition, our historical biogeographical reconstruction showed the most likely model for colonization is a GAARlandia model, including the colonization of Jamaican and Cuban *Limia* species.

The GAARlandia model predicts a short-lived (~3 Myr) subaerial land connection between South America and the incipient landmasses of the West Indies across the Aves Ridge during the Eocene/Oligocene transition (30–35 Ma) (Iturralde-Vinent & MacPhee, 1999; Iturralde-Vinent, 2006). This time frame marks an important transitional period in the evolution of life on Earth, characterized by global climate cooling and related changes in sea level. Transitioning from the warm climate of the Eocene, the Earth experienced rapid cooling of over 5 °C from the Eocene optimum to the start of the Oligocene, accompanied by the formation of polar ice caps and substantial sea level drops on the order of 60 m (Haq et al., 1987; Miller et al., 2008). Supplemented by tectonic uplift of the Caribbean plate, these conditions may have resulted in either shallow banks or a land bridge connection between proto-Antillean islands and the South American mainland (Iturralde-Vinent & MacPhee, 1999; Iturralde-Vinent, 2006). During this time frame, much of the Greater Antilles existed as a single landmass, with connections between eastern Cuba, north and south Hispaniola, the Blue Mountains of Jamaica, Puerto Rico, and the Aves Ridge south to mainland South America. The paleogeographical model of Iturralde-Vinent & MacPhee (1999) and Iturralde-Vinent (2006) also predicts that after the lowest sea levels of the Oligocene, the Aves Ridge land span was subsequently inundated by rising sea levels before the Miocene (23.5 Ma), severing the mainland connection and isolating several island fragments, including the Blue Mountains of Jamaica (Fig. 4). Further movement of Greater Antillean island fragments acted to separate eastern Cuba from northern Hispaniola.

Figure 3 Chronogram of *Limia* and related groups derived from a relaxed molecular clock analysis of a combined data set (three mitochondrial: 12S, ND2, Cyrb, and two nuclear: MYH6, Rh). Bars indicate the 95% highest posterior densities (HPD). Branch lengths represent time since divergence and major nodes are indicated by lettered circles. Nodes A, B, and C represent significant splitting events during the colonization of the West Indian livebearers and correspond with the geological timeline as illustrated in Fig. 4.
opening the Windward Passage in the mid-Miocene (14–17 Ma).

The results of our analyses follow rather closely several events predicted by the GAARlandia model. Our best model of colonization supports the split of the West Indian/South American clade during the Eocene/Oligocene (32.9 Ma). We also demonstrate support for the separation of Jamaica near the early Miocene (22.8 Ma). Divergence of the Jamaican L. melanogaster corresponds well with the proposed timing of rising sea levels and the separation of the Blue Mountain block of Jamaica from southern Hispaniola (Iturralde-Vinent & MacPhee, 1999; Fig 4). This result is interesting in itself, as many palaeogeographical models debate the independence of the eastern Blue Mountain block from a large western block (part of the Nicaraguan rise) (see Lewis et al., 1990; Robinson, 1994). In addition, orthodox views of Jamaican geology find little evidence for persistence of any aerial Jamaican terranes prior to the mid-Miocene (Robinson, 1965, 1994). Other studies evaluating the relationships between Jamaican and Hispaniolan taxa may help to solidify this pattern and better explain the unique geological history of the Jamaican terranes.

**Implications and future directions**

While our divergence timing results are concordant with the predictions of the GAARlandia model, we do not believe there is sufficient evidence to rule out alternative methods of colonization, such as a combination of dispersal and vicariant events, or short distance, stepping stone dispersal. As discussed by Ali (2012), there is little geological evidence to support the notion of a contiguous land bridge from South America into the West Indies during the Eocene/Oligocene transition. Furthermore, rather than crossing a land bridge, any of the taxa used for support of the GAARlandia model could have also used short distance dispersal to colonize the West Indies. In the case of West Indian freshwater fish fauna, the ability of poeciliids and cichlids to tolerate, and even thrive, in saltwater has been widely demonstrated both on the islands and in mainland taxa (Myers, 1938; Burgess & Franz, 1989). Other taxa that may have used the GAARlandia land span for colonization, including megalonychid sloths and other mammals, were also physically capable of crossing over a saltwater barrier, especially with the potentially shorter distances between landmasses that would have occurred during the Eocene/Oligocene transition. Coupled with the absence of less tolerant groups on the islands, including primary division freshwater fish and caecilians (Hedges, 1996, 2001), it is difficult to rule out ancient dispersal, as argued by Heinicke et al. (2007) and their divergence time estimate for West Indian Eleutherodactylus lineage at 29–47 Ma. The alternative, in which fish were confined to the freshwater connections of a GAARlandia land span seems less likely and would not have restricted the colonization of primary division freshwater fishes.

Whether GAARlandia was a true land bridge or not, the predictions of the GAARlandia model as a corridor for dispersal should not be discounted. The main historical critique of the GAARlandia model was the absence of a clustering of divergence time estimates around 30 to 35 Ma. Until very recently, DNA sequencing and molecular clock techniques did not allow for fine scale resolution of divergence time.
estimates. A poor fossil record and lack of reliable calibration points lead to further inconsistency. As such, those estimates that were available were often crude approximations relying on percentage sequence divergence and seemed to show a scattering of divergence events through time (Hedges, 2001). However, many of these interpretations have changed with recent phylogenetic work utilizing a combined mitochondrial and nuclear DNA approach, in combination with broader sampling, more accurate calibration and relaxed molecular clock methods (see Hedges, 1996 and the updated study, Heinicke et al., 2007).

Many recent re-evaluations of West Indian taxa give estimates for colonization that are now consistent with Eocene/Oligocene colonization, including cichlids (Hulsey et al., 2011; Rícan et al., 2012), bufonids of the Peltophryne genus (Alonso et al., 2012), some frogs, for example, Syrrhophus (Crawford & Smith, 2005) and Osteopilus (Moen & Wiens, 2009), Polistinae wasps (Silva & Noll, 2015), and dispersal limited spiders (Crews & Gillespie, 2010) and butterflies (Wahlberg, 2006). Additional earlier work using fossil and phylogenetic evidence also support GAARlandia colonization, including extinct primates (Horovitz & MacPhee, 1999; Davalos, 2004) and megalonychid sloths (MacPhee & Iturralde-Vinent, 2000; White & MacPhee, 2001; Davalos, 2004), several bat groups (Davalos, 2004), as well as hystrixognath rodents (Woods, 2001; MacPhee et al., 2003; Davalos, 2004; but see Fabre et al. (2014) advocating mid-Miocene dispersal). Even without concrete evidence for a subaerial land span, it seems clear that the time frame of the Eocene/Oligocene transition and its associated climate and
paleogeography have played a major part in West Indian colonization and in shaping the remarkable present-day biodiversity.

**A note on the taxonomy of Limia**

As expected, we find support for the monophyly of *Limia* as a group. Interestingly, however, we also show the closest sister group of *Limia* to be the clade represented by the three Hispaniolan *Poecilia* species. Earlier studies have demonstrated the sister group relationship between *Limia* and the *Pamphorichthys* group (Hamilton, 2001; Hrbek et al., 2007; Meredith et al., 2010, 2011) but did not include the Hispaniolan *Poecilia* species (*P. elegans*, *P. dominicensis* and *P. hispaniola*) in their analyses. One consequence of this new phylogenetic arrangement is to further add to the confusion in the taxonomic designations of *Poecilia*. Several studies have advocated generic rankings for the taxa *Poecilia*, *Limia*, *Mollienesia*, *Pamphorichthys*, *Acanthophacelus* and *Micropoecilia* (Rodriguez, 1997; Hamilton, 2001; Poerer, 2003), while others use them as subgenera within the genus *Poecilia* (Meredith et al., 2010, 2011). In either case, the designation of the Hispaniolan *Poecilia* (*P. elegans*, *P. dominicensis* and *P. hispaniola*) and South American *Poecilia* (*P. vivipara*) as a group does not accurately reflect evolutionary history and needs revision.

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**SUPPORTING INFORMATION**

Additional Supporting Information may be found in the online version of this article:

**Appendix S1** Additional details regarding localities and protocols.

**Appendix S2** Phylogenetic reconstructions and calibration schemes.

**Appendix S3** Matrices used in the historical biogeographical reconstructions.

**BIOSKETCH**

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The main interests of all authors include evolution, phylogenetics and biogeography, with a focus on island systems.

Author contributions: P.W. and A.C. conceived of the study. P.W., A.C., and S.J. collected specimens from the field, and along with K.W., P.W. and S.J. conducted the gene sequencing and phylogenetic analyses. P.W. and S.J. led the writing and provided the artwork. J.D. conducted the biogeographical analyses. A.C. and K.W. provided laboratory space and financial assistance. All authors contributed to the writing of the manuscript.

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