Mitochondrial DNA

The Journal of DNA Mapping, Sequencing, and Analysis

ISSN: 1940-1736 (Print) 1940-1744 (Online) Journal homepage: http://www.tandfonline.com/loi/imdn20

Molecular phylogenetics of the glass frog *Hyalinobatrachium orientale* (Anura: Centrolenidae): evidence for Pliocene connections between mainland Venezuela and the island of Tobago

Michael J. Jowers, Richard M. Lehtinen, Roger J. Downie, Andrew P. Georgiadis & John C. Murphy

To cite this article: Michael J. Jowers, Richard M. Lehtinen, Roger J. Downie, Andrew P. Georgiadis & John C. Murphy (2015) Molecular phylogenetics of the glass frog *Hyalinobatrachium orientale* (Anura: Centrolenidae): evidence for Pliocene connections between mainland Venezuela and the island of Tobago, Mitochondrial DNA, 26:4, 613-618, DOI: 10.3109/19401736.2014.880888

To link to this article: https://doi.org/10.3109/19401736.2014.880888

Mitochondrial DNA

http://informahealthcare.com/mdn
ISSN: 1940-1736 (print), 1940-1744 (electronic)

Mitochondrial DNA, 2015; 26(4): 613–618
 DOI: 10.3109/19401736.2014.880888

informa
healthcare

RESEARCH ARTICLE

Molecular phylogenetics of the glass frog *Hyalinobatrachium orientale* (Anura: Centrolenidae): evidence for Pliocene connections between mainland Venezuela and the island of Tobago

Michael J. Jowers[1], Richard M. Lehtinen[2], Roger J. Downie[3], Andrew P. Georgiadis[2], and John C. Murphy[4]

[1]*Departamento de Etologia y Conservacion de la Biodiversidad, Estacion Biologica de Doñana C/Americo Vespucio, CSIC (Consejo Superior de Investigaciones Cientificas), Sevilla, España, Spain,* [2]*Department of Biology, The College of Wooster, Wooster, OH, USA,* [3]*School of Life Sciences, Graham Kerr Building, University of Glasgow, Scotland, UK, and* [4]*Science & Education, Field Museum of Natural History, Chicago, IL, USA*

Abstract

The presence of *Hyalinobatrachium orientale* in Tobago and in northeastern Venezuela is puzzling as this species is unknown from the island of Trinidad, an island often hypothesized to be a stepping-stone for the mainland fauna to colonize Tobago. A period of extended isolation on Tobago could result in the Hyalinobatrachium population becoming distinct from the mainland *H. orientale*. Here, we use 12S and 16S rDNA gene fragments from nine *H. orientale* specimens from Tobago and the mainland to assess their relationship and taxonomy, as well as the tempo and mode of speciation. The results suggest *H. orientale* from Venezuela and Tobago are monophyletic and the two populations diverged about 3 million years ago. This estimate corresponds with the drier climate and lower sea levels of the Pliocene glaciation periods. We hypothesize that lower sea levels resulted in land-bridge formations connecting the mainland and Tobago, with a corridor of habitat allowing *H. orientale* to colonize Tobago to the west of Trinidad.

Keywords

Hyalinobatrachium orientale, Pliocene, Tobago, Trinidad

History

Received 15 October 2013
Revised 3 January 2014
Accepted 5 January 2014
Published online 3 February 2014

Introduction

The southernmost islands of the Lesser Antilles, Trinidad and Tobago offer a unique opportunity to study the processes and patterns of colonization and speciation from nearby mainland Venezuela. Three hypotheses have been put forward to explain the herpetofaunal diversity in these islands: overseas dispersal, land-bridge connections, and vicariance (Boos, 1984a,b; Camargo et al., 2009; Jowers et al., 2008, 2010; Manzanilla et al., 2007; Rodriguez & Lopez, 2003). Furthermore, sea level falls during Pliocene-Pleistocene-Holocene glacial maxima have resulted in prolonged periods of connections between the islands and the mainland (Murphy, 1997), which allow for ancient as well as recent colonization events.

Until recently, *Hyalinobatrachium orientale* (Rivero, 1968) was thought to be distributed throughout Venezuela's Oriental Sector, Cordillera del Litoral and Cordillera del Interior, but work on the morphology, advertisement calls and genetics of the Venezuelan populations confirmed the monophyly of *H. orientale* from the Oriental Sector of northeastern Venezuela (Castroviejo-Fisher et al., 2008). *H. orocostale* (Cordillera del Interior) and *H. fragile* (Cordillera del Litoral) were shown to be sister taxa and the sister clade to *H. orientale*. However, the study lacked genetic work on *H. orientale* from Tobago. Hardy (1984) described the Tobago population as a subspecies (*H. o. tobagense*) of *H. orientale*. Thus, its taxonomic status has never been assessed from a molecular phylogenetic approach.

The distribution of *H. orientale* is puzzling as it is absent from the island of Trinidad (Figure 1). Low sea level throughout the Pliocene and Pleistocene resulted in the connection of the Paria Peninsula (northeastern Venezuela), Trinidad and Tobago and thus, from a phylogeographical perspective it would be reasonable to expect *H. orientale* to have colonized Trinidad from the mainland, but no populations have been located. An alternative scenario would imply the extinction of the Trinidad populations. A third scenario to explain this species' current distribution is that of Pliocene-Pleistocene connecting corridors between north-eastern Venezuela and Tobago that did not involve Trinidad, and a fourth possibility is sea dispersal through rafting when Tobago was proximal to northern Venezuela.

Hyalinobatrachium orientale is highly specialized to its environment and exhibits male parental care (Lehtinen et al., 2012). It is nocturnal, living in close association to *Heliconia* vegetation overhanging fast flowing streams where it lays eggs on the underside of leaves. Upon hatching, tadpoles fall to the water.

The frog's presence in Tobago suggests either marine dispersal through rafting from Northern Venezuela (Boos, 1984a,b) or through a suitable corridor of habitat, including flowing streams from Venezuela, for such a specialist to survive and maintain a viable population. Here, we examine the taxonomic status of the Tobago *H. orientale* from a phylogenetic perspective to determine if the Tobago population is genetically divergent from the mainland populations, and to test hypotheses about the timing and mode of its colonization of Tobago.

Correspondence: Michael J. Jowers, CSIC (Consejo Superior de Investigaciones Cientificas), Departamento de Etologia y Conservacion de la Biodiversidad, Estacion Biologica de Doñana C/ Americo Vespucio, s/n (Isla de la Cartuja), 41092 Sevilla, España, Spain. E-mail: michaeljowers@hotmail.com

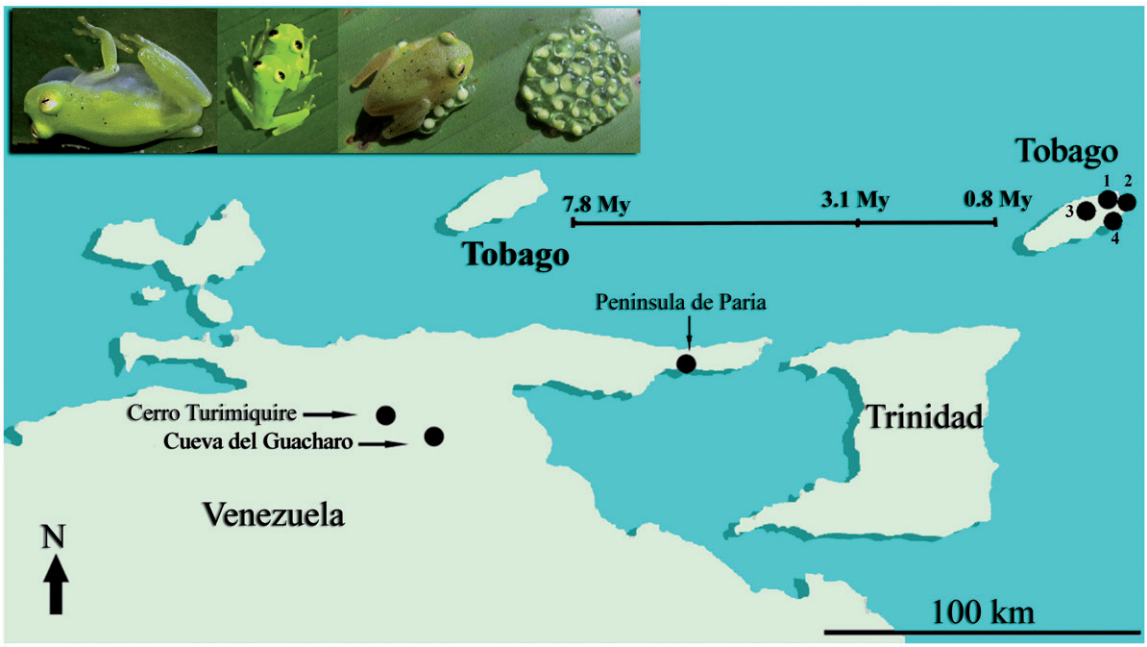

Figure 1. Map of northeastern Venezuela, Trinidad and Tobago. The black dots represent the localities for *H. orientale*. The dots in Venezuela represent the sampling localities for *H. orientale* from Castroviejo-Fisher et al. (2008). Numbered dots on Tobago represent sampling points (1; Hermitage River, 2; Doctors River, 3; Forest Reserve, 4; King's Bay). The black line corresponds to Tobago's movement (at a rate of about 20 mm per year, Pindell & Kennan, 2007, 2009) between 7.8 Mya to 0.8 Mya. Tobago's position at the estimated 3.1 Mya divergence time is indicated in the line. Photographs in the upper left depict some major event in the Tobago glass frogs life history: males call from the underside of a leaf; amplexus; male guards eggs on leaf, sometime multiple clutches (photos JCM).

Table 1. Specimens, museum codes, localities and Genbank accession numbers and haplotypes for the 12S and 16S rDNA gene fragments.

Species	Locality	12S rDNA	16S rDNA	12S rDNA	16S rDNA	combined
H. orientale GLAHM140926.1	Hermitage River, Tobago	KC351744	KC351748	H1	H1	H1
H. orientale GLAHM140926.2	Hermitage River, Tobago	KC351745	KC351749	H1	H2	H2
H. orientale GLAHM140926.3	Hermitage River, Tobago	KC351746	KC351750	H1	H2	H2
H. orientale UWIZM2011.18.8	Hermitage River, Tobago	KC351747	KC351751	H2	H1	H3
H. orientale UWITT.2010.24.1.1	Tributary stream of the Doctor's River, south of Charlotteville, Tobago	KF900139	KF900135	H1	H1	H1
H.orientale UWITT.2010.24.1.2	Forest Reserve, Tobago	KF977149	-	H1	H1	H1
H. orientale UWITT.2010.24.1.3	Forest Reserve, Tobago	KF900140	KF900136	H1	H1	H1
H. orientale UWITT.2010.24.1.4	Forest Reserve, Tobago	KF900141	KF900137	H1	H1	H1
H. orientale Personal Frank Glaw Collection	King's Bay, southwest of Speyside toward Roxborough, Tobago	KF900142	KF900138	H1	H1	H1
H. orientale MHNLS17878	Oriental Sector, Venezuela	EU663413	EU447289	-	-	-
H. orientale MHNLS16443	Oriental Sector, Venezuela	-	EU447289	-	-	-
H. orientale MHNLS17117	Oriental Sector, Venezuela	-	EU447283	-	-	-
H. fragile MHNLS17051	Cordillera del Litoral, Venezuela	-	EU447285	-	-	-
H. fragile MHNLS17161	Cordillera del Litoral, Venezuela	EU663407	EU447286	-	-	-
H. orocostale MHNLS17247	Cordillera del Litoral, Venezuela	EU663414	EU447284	-	-	-
H. orocostale MHNLS17249	Cordillera del Litoral, Venezuela	-	EU447288	-	-	-
H. sp MIZA317	Estado Aragua, Venezuela	EU663417	EU447290	-	-	-
Vitreorana antisthenesi MHNLS17909	Estado Aragua, Venezuela	EU663390	EU447287	-	-	-

Materials and methods

DNA was extracted from nine *H. orientale* from different localities (Table 1). Individual DNA was extracted using the Chelex extraction process (Bio-Rad®, Hercules, 12 CA; Walsh et al., 1991), following slight protocol modifications. Muscle tissue (2 mm^3) was cut thoroughly and incubated for 2 h at 57 °C in 160 µl of 5% Chelex with 40 µl Proteinase K to increase tissue digestion. Following the incubation period, it was heated at 100 °C for 15 min. After a 4 min centrifugation at 12,500 rpm, all supernatant was transferred into a 1.5 ml tube. We aimed to amplify a fraction of the mitochondrial 12S and16S rDNA genes with mitochondrial vertebrate universal primers to compare with *H. orientale* sequences available in Genbank. For each PCR, the 20 µl PCR volume contained approx. 50 ng DNA, 200 µM of each

dNTP, 0.15 μM of each primer, 2 μl 10X Buffer, 0.8 μl $MgCl_2$ and 0.1 unit of taq polymerase (QIAGEN). The thermal cycle profile was as follows: an initial denaturation step of 2 min at 94 °C; 35 cycles of denaturation at 30 s at 94 °C, annealing for 30 s at 52 °C and extension for 45 s at 72 °C; and a final extension for 5 min at 72 °C. Following the PCR, excess primers and dNTPs were removed using enzymatic reaction of *E. coli* Exonuclease I, Antartic phosphatase and Antartic phosphatase buffer (all New England Biolabs). Sequencing was carried out in both directions using the BigDye® Terminator v1.1 cycle sequencing kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Labelled fragments were resolved on an automated A3130*xl* genetic analyzer (Applied Biosystems). The primers for the 12S rDNA: 12SA 5′-AAACTGGGATTAGATACCCCACTAT-3′, 12SB 5′-GAGGG TGACGGGCGGTGTGT-3′ Kocher et al. (1989) and 16S rDNA: 16SL 5′-GCCTGTTTATCAAAAACAT-3′, 16SH 5′-CCGGTCTGAACTCAGATCACGT- 3′ Palumbi (1996) amplified approximately a 400 and 700, respectively, base pair fraction. Templates were sequenced on both strands, and the complementary reads were used to resolve rare, ambiguous base-calls in Sequencher v.4.9. After removing PCR primers and incomplete terminal sequences, 305 and 684 base pairs of the 12S and 16S rDNA gene fraction, respectively, (989 bp total) were available for analyses. Sequences were aligned in Seaview v.4.2.11 (Gouy et al., 2010) under ClustalW2 (Larkin et al., 2007) default settings. Nucleotide differences and uncorrected *p*-distances (%) analyses were calculated using MEGA v5 (Tamura et al., 2011).

In order to assess the phylogenetic relationships of *H. orientale* from Tobago to mainland *H. orientale* and its closely related sister species, we included in the analyses all taxa used in Castroviejo-Fisher et al. (2008) (*H. orientale*, *H. fragile*, *H. orocostale* and *H. sp*) and all available 12S rDNA gene partial sequences for these same taxa for which 16S rDNA were also available (Guayasamin et al., 2008). In addition, to root the phylogenetic tree, we employed the same outgroup as Castroviejo-Fisher et al. (2008; i.e. *Vitreorana antisthenesi*) (Table 1).

We used the program BEAST 1.5.4 (Drummond & Rambaut, 2007) to estimate coalescence times between haplotypes (TMRCAs). We ran the analyses under a birth-death prior using a relaxed (uncorrelated lognormal) molecular clock model. Inspection of trace files indicated that a strict-clock model could not be rejected (the parameter ''Coefficient of Variance'' in relaxed clock analyses abuts zero), and thus, we report results based on this model only. Several possible calibration dates can be estimated for mitochondrial ribosomal genes. Dates of divergence estimated for 16S rDNA corrected distances adopted for other anurans have been estimated to correspond to 0.39–0.40% divergence per million years (Manzanilla et al., 2007; Martinez-Solano et al., 2004). These rates are in accordance with rates assumed for the 16S and 12S rRNA genes in other amphibians such as newts (Caccone et al., 1997) and true salamanders (Veith et al., 1998), ranging between 0.4 and 0.7% per million years and 0.44–0.54% for the 12S rDNA in *Rana* (Sumida et al., 2000). Thus, in the absence of a reliable prior on the substitution rate or of a well-dated calibration point, we specified a normal prior on the clock rate with a mean value of 0.0045 substitutions per lineage per million years for the combined 12S and 16S rDNA gene fragments. Analyses were run for 40,000,000 generations, and posterior distributions of parameter estimates were visually inspected in Tracer v1.5 (Rambaut & Drummond, 2007). Ten percent of the resulting topologies and parameter values were discarded as burn in. The most appropriate substitution model for the Bayesian Inference (BI) analysis was determined by the Bayesian Information Criterion (BIC) in jModeltest v.0.1.1 (Posada, 2008). The tree was constructed using the BI optimality criteria under the best fitting substitution model (TIM2 + I). MrBayes was used with default priors and Markov chain settings, and with random starting trees. Each run consisted of four chains of 10,000,000 generations, sampled each 10,000 generations for a total of 750 trees. A plateau was reached after few generations with 25% (250 trees) of the trees resulting from the analyses discarded as burn in.

Results

All sequences for *H. orientale* from Tobago were identical, with three exceptions; one purine (A–G) transition occurring between H1 and H3 in the 12S rDNA and another (C–T) between H1, H3 and H2 in the partial 16S rDNA gene fragment, respectively (Table 1). The results suggest an old divergence between *H. orientale* from Tobago and the mainland (median value for the TMRCA: 3.1 million years, the 95% HPD interval ranging between 0.8 and 7.8 Mya). However, it is important to keep in mind that these estimates represent divergence times between haplotypes and thus necessarily precede population divergence times (although the magnitude of this offset between haplotype and population divergence is unknown). The topologies resulting from BEAST analyses are fully concordant with those resulting from MrBayes analyses, including support for the same clades (Figure 2). The genetic divergence between localities (mainland versus Tobago) was $1.8\% \pm 0.03$ for the 12S rDNA and $1.3\% \pm 0.05$ for the 16S rDNA (Table 2). The best-fitting model for the BI tree was the TIM2 + I ($-\ln L = -2075.3344$, BIC = 4322.6269). The BI phylogram recovered a well-resolved monophyletic clade (BPP: 1) where *H. orientale* from Tobago is reciprocally monophyletic to *H. orientale* from northeastern Venezuela (BPP 0.99). *H. fragile* is strongly supported (BPP 1.00) as the sister clade to *H. orientale* (Figure 2). Genetic divergence estimates from the 16S rDNA partial gene sequences show contrasting levels of differentiation within localities (Tobago: $0.2\% \pm 0.03$ and Venezuela: $3.8\% \pm 0.01$).

Discussion

Hyalinobatrachium orientale from Tobago has considerable genetic divergence from its presumed ancestral population in northeastern Venezuela, but despite such divergence, both populations are the same species and share recent common ancestry, as inferred from the BI tree (Figure 2). The genetic divergence between localities (mainland versus Tobago) suggests some level of differentiation but not enough for complete lineage sorting. The estimated date (3.1 million years) implies a genetic divergence between the mainland and Tobago populations to when Trinidad was already separated from the mainland in the late Miocene (Audemard & Audemard, 2002; Moretti et al., 2007; Persad, 2009), which may explain the absence of this species in Trinidad. Despite this, we cannot fully discard the possibility of a stepping stone colonization mode by which it may have reached Trinidad previous to the colonization of Tobago (Camargo et al., 2009). This scenario implies the extinction of the Trinidad populations, or more unlikely, that *H. orientale*'s presence in Trinidad is still unrecorded. Trinidad's amphibian fauna has been extensively studied, though new discoveries continue to be made (Heyer, 1994; Murphy, 1997; Smith et al., 2011).

Tobago is located on the front edge of the Caribbean Plate, and has been moving eastward (Pindell & Kennan, 2007, 2009). Both slab break-off and STEP (Subduction-Transform-Edge-Propagator) detachment processes have been proposed at the southeast Caribbean plate boundary (Clark et al., 2008). However, Van Benthem et al. (2013) have supported a STEP margin between the Caribbean Plate and the South American Plate and used this to support the eastern Venezuelan orogeny at 25–10 Ma.

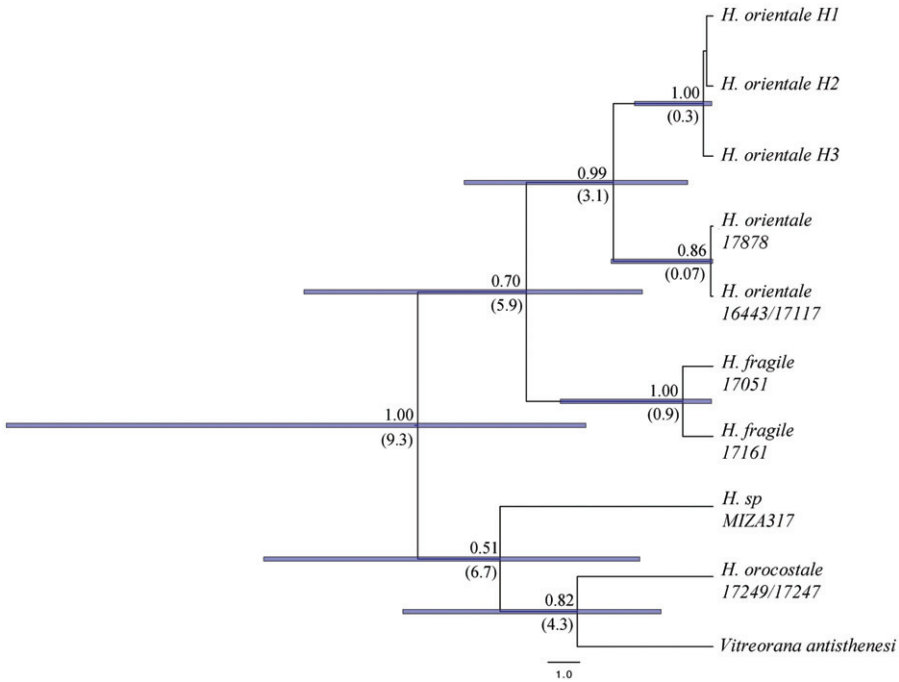

Figure 2. Bayesian Inference phylogram of all Hyalinobatrachium species studied (12S and 16S rDNA partial genes). Values on nodes are the posterior probabilities recovered from the Bayesian Inference analysis and under nodes are the median 95% credibility values per million years. See Table 1 for *H. orientale* locations.

Table 2. 12S rDNA (top) and 16S rDNA (bottom) matrices of *p*-uncorrected distances (below) and nucleotide differences (above) among species of Hyalinobatrachium.

12S rDNA

	H.or H1	H.or H2	H.or H3	H.or.17878	H.fra.17161	H.sp.317	V.anti
H.or H1	–	0	1	7	17	15	24
H.or H2	0.000	–	1	7	17	15	24
H.or H3	0.002	0.002	–	8	18	16	25
H.or.17878	0.017	0.017	0.020	–	13	12	22
H.fra.17161	0.041	0.041	0.044	0.032	–	13	31
H.sp.317	0.037	0.037	0.039	0.029	0.032	–	26
V.anti	0.059	0.059	0.061	0.054	0.076	0.063	–

16S rDNA

	H.or H1	H.or H2	H.or H3	H.or.17878	H.or.16443/17117	H.oro.17249/17247	H.fra.17051	H.fra.17161	H.sp.317	V.anti
H.or H1	–	1	0	7	6	25	28	30	34	49
H.or H2	0.002	–	1	8	7	25	27	29	35	48
H.or H3	0.000	0.002	–	7	6	25	28	30	34	49
H.or.17878	0.013	0.015	0.013	–	1	26	23	25	31	50
H.or.16443/17117	0.011	0.013	0.011	0.002	–	25	22	24	32	49
H.oro.17249/17247	0.047	0.047	0.047	0.049	0.047	–	20	20	26	38
H.fra.17051	0.053	0.051	0.053	0.044	0.042	0.038	–	3	31	46
H.fra.17161	0.057	0.055	0.057	0.047	0.046	0.038	0.006	–	33	47
H.sp.317	0.065	0.066	0.065	0.059	0.061	0.049	0.059	0.063	–	46
V.anti	0.093	0.091	0.093	0.095	0.093	0.072	0.087	0.089	0.087	–

H. or (*Hyalinobatrachium orientale*), H. oro or (*Hyalinobatrachium orocostale*), H. fra (*Hyalinobatrachium fragile*), H. sp (*Hyalinobatrachium sp*), V. anti (*Vitreorana antisthenesi*).

Both models use the rate of plate movement as 20 mm/year. Here, we use the STEP model proposed by Pindell et al. (2005) as the model for tectonic movements in the eastern Caribbean. By 10 Mya, transtenional movements between the two plates were well defined by a strike-slip fault at Coche-North Coast Fault and the Gulf of Paria pull-apart-basin. By 4 Mya, the two plates were strongly coupled, possibly because the deep Antilles arc could not override the northern limit of the Trinidad basement high of the South America lithosphere (Pindell et al., 2005). According to this model, Tobago was at 61.5 W longitude 4 Mya, which places it just north of the Peninsula de Paria and Trinidad was close to its current position, attached to the South American plate. Thus, according to an annual 20 mm eastward movement, Tobago's position 3.1 Mya was approximately 62 km to the west of its current position (Figure 2).

Shallow marine depths (less than 100 m) surrounding the Paria Peninsula (Venezuela) and the vicinity of the coastal areas surrounding Trinidad and Tobago, suggest that sea-level reduction during the Pliocene (Miller et al., 2005) would have resulted in land-bridge connections between the mainland and the islands. At our mean estimated genetic divergence time (3.1 Mya) between *H. orientale* of Tobago and the mainland, the sea level was estimated to be between 40 and 80 m below present (Woodburne, 2010). The connections caused during sea level falls were sustained throughout the Pliocene-Pleistocene period lasting thousands of years (Murphy, 1997). Thus, *H. orientale*

could have colonized the newly available habitat, assuming the ecological requirements were suitable for a stepping stone colonization process through the corridor. The simplest explanation for the absence of *H. orientale* from Trinidad is that conditions for colonization were suitable along the landbridge to Tobago but perhaps not suitable for the glass frog's survival on Trinidad. However, Trinidad's Northern Range, as well as other areas, would seem to meet the habitat needs for *H. orientale*. Based on the geography and connections throughout the Pliocene, it is likely that this species did colonize Trinidad but the populations became locally extinct, possibly as a consequence of small numbers or fluctuations of environmental factors in the area (Jowers et al., 2008). An overwater dispersal scenario would also be consistent with its absence on Trinidad, but the time of divergence makes this scenario less probable.

Congruent with our divergence time estimate is the genetic split of *Gonatodes cecilae* in Trinidad from its sister species *G. ocellatus* from Tobago based on 16S rDNA and nuclear markers (Gamble et al., 2008). The authors estimated a divergence of around 3 million years (Pliocene-Pleistocene) implying that during this period sea level falls were likely to have contributed to the colonization of Tobago. *H. orientale's* tempo and mode of colonization of Tobago contrast with other studies employing anuran mtDNA. For example, *Leptodactylus fuscus* (Camargo et al., 2006, 2009) and *L. validus* (Yanek et al., 2006) are both thought to have colonized Tobago from Trinidad more recently during the Pleistocene by over water dispersal. However, *Mannophryne olmonae* (Jowers et al., 2010) colonized Tobago in the early Miocene, but unlike *H. orientale*, this species does have a closely related species in Trinidad (*M. trinitatis)* suggesting independent colonization of Trinidad and Tobago by different *Mannophryne* lineages. To the best of our knowledge, this is the first study to report on the tempo and mode of speciation between a species present in Tobago and the mainland, with no close sister species inhabiting Trinidad from where it may have colonized Tobago.

Braby et al. (2012) suggest that under the general lineage (unified) species concept, the definition of subspecies be restricted to evolving populations representing partially isolated lineages of species that are allopatric, phenotypically distinct, and have at least one fixed diagnosable character state. Castroviejo-Fisher et al. (2008) examined mainland specimens of *H. orientale* and reported an eye-to-snout distance/eye diameter of 0.55 to 0.77 (mean = 0.64) for males and 0.54 for a single female. Murphy (1997) reported an average eye-to- snout/eye diameter ratio of 0.51 (for both sexes combined) for Tobago specimens. This combined with the genetic differences reported here, the geographic isolation of the Tobago population, and the call differences reported by Castroviejo-Fisher et al. (2008) between the Tobago and mainland populations meet Braby et al.'s (2012) criteria for retaining trinomials. Additional morphological, ecological and behavioral traits will be forthcoming. Therefore, we here revalidate *H. orientale tobagense* (Hardy, 1984).

Acknowledgements

We thank the Wildlife Section of the Trinidad Government for permission to carry out this work. This study was aided by several members of the University of Glasgow Trinidad and Tobago Expeditions 2012. We are grateful to Alejandro Gonzalez-Voyer for his valuable assistance with Beast.

Declaration of interest

This work was supported by the William H. Wilson fund at the College of Wooster. The authors report no conflict of interests. The authors alone are responsible for doing the research and writing the article.

References

Audemard FE, Audemard SE. (2002). Structure of the Mérida Andes, Venezuela: Relations with the South America-Caribbean geodynamic interaction. Tectonophysics 345:299–327.

Boos HEA. (1984a). A consideration of the terrestrial reptile fauna on some offshore islands north west of Trinidad. Living World. J Trinidad Tobago Field Naturalists' Club 1984:19–26.

Boos HEA. (1984b). Reptiles of Soldado Rock, Trinidad. Living World. J Trinidad Tobago Field Naturalists' Club 1984:12.

Braby MF, Eastwood R, Murray N. (2012). The subspecies concept in butterflies: Has its application in taxonomy and conservation biology outlived its usefulness? Biol J Linn Soc 106:699–716.

Caccone A, Milinkovich MC, Sbordoni V, Powell JR. (1997). Mitochondrial DNA rates and biogeography in European newts, genus *Euproctus*. Syst Biol 46:126–44.

Camargo A, De Sa RO, Heyer WR. (2006). Phylogenetic analyses of mtDNA sequences reveal three cryptic lineages in the widespread neotropical frog *Leptodactylus fuscus* (Schneider, 1799) (Anura, Leptodactylidae). Biol J Linn Soc 87:325–41.

Camargo A, Heyer WR, De Sa RO. (2009). Phylogeography of the frog *Leptodactylus validus* (Amphibia: Anura): Patterns and timing of colonization events in the Lesser Antilles. Mol Phyl Evol 53:571–9.

Castroviejo-Fisher S, Senaris JR, Ayarzaguena JA, Vila C. (2008). Resurrection of *Hyalinobatrachium orocostale* and notes on the *Hyalinobatrachium orientale* species complex (Anura: Centrolenidae). Herpetologica 64:472–84.

Clark SA, Sobiesiak M, Zelt CA, Magnani MB, Miller MS, Bezada MJ, Levander A. (2008). Identification and tectonic implications of a tear in the South American plate at the southern end of the Lesser Antilles. Geochem Geophys Geosyst 9:Q11004 (1–10). doi: 10.1029/2008GC002084.

Drummond AJ, Rambaut A. (2007). BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol Biol 7:214.

Gamble T, Simons AM, Colli GR, Vitt LJ. (2008). Tertiary climate change and the diversification of the Amazonian gecko genus *Gonatodes* (Sphaerodactylidae, Squamata). Mol Phyl Evol 46: 269–77.

Gouy M, Guindon S, Gascuel O. (2010). SeaView version 4. A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Mol Biol Evol 27:221–4.

Guayasamin JM, Castroviejo-Fisher S, Ayarzaguena JA, Trueb L, Vila C. (2008). Phylogenetic relationships of glass frogs (Centrolenidae) based on mitochondrial and nuclear genes. Mol Phyl Evol 48:574–95.

Hardy Jr JD. (1984). A new subspecies of *Centrolenella orientalis* (Anura: Centrolenidae) from Tobago, West Indies. Bull Maryland Herpetol Soc 20:165–73.

Heyer WR. (1994). Variation within the *Leptodactylus podicipinus-wagneri* complex of frogs (Amphibia: Leptodactylidae). Smith Cont Zool 546:1–124.

Jowers MJ, Cohen BL, Downie JR. (2008). The cyprinodont fish *Rivulus* (Aplocheiloidei: Rivulidae) in Trinidad and Tobago: Molecular evidence for marine dispersal, genetic isolation and local differentiation. J Zool Syst Evol Res 46:48–55.

Jowers MJ, Martinez-Solano I, Cohen BL, Manzanilla J, Downie RJ. (2010). Genetic differentiation in the Trinidad endemic *Mannophryne trinitatis* (Anura: Aromobatidae): Miocene vicariance, in situ diversification and lack of geographical structuring across the island. J Zool Syst Evol Res 48:133–40.

Kocher TD, Thomas WK, Meyer A, Paabo S, Villablanca F, Wilson AC. (1989). Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. Proc Natl Acad Sci 86:6196–200.

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, et al. (2007). Clustal W and Clustal X version 2.0. Bioinf Appl Note 23:2947–8.

Lehtinen R, Georgiadis AP. (2012). Observations of parental care in the glass frog *Hyalinobatrachium orientale* (Anura: Centrolenidae) from Tobago, with comments on its natural history. Phyllomedusa 11: 75–177.

Manzanilla J, Jowers MJ, La Marca E, Garcia-Paris M. (2007). Taxonomic reassessment of *Mannophryne trinitatis* (Anura: Dendrobatidae) with a description of a new species from Venezuela. J Herp 17:31–42.

Martinez-Solano I, Gongsalves HA, Arntzen JW, Garcia-Paris M. (2004). Phylogenetic relationships and biogeography of midwife toads (Discoglossidae: *Alytes*). J Biogeol 31:603–18.

Miller KG, Kominz MA, Browning JV, Wright JD, Mountain GS, Katz ME, Sugarman PJ, et al. (2005). The Phanerozoic record of global sea-level change. Science 310:1293–8.

Moretti I, Delos V, Letouzey J, Otero A, Calvo J. (2007). The use of surface restoration in foothills exploration: Theory and application to the Sub-Andean Zone of Bolivia. In: Lacome O, Laveal J, Roure F, Vergeal SJ, editors. Thrust belts and foreland basins. From fold kinematics to hydrocarbon systems. Berlin, Germany: Springer, p 149–78.

Murphy JC. (1997). Amphibians and Reptiles of Trinidad and Tobago. Malabar, FL: Krieger Publishing Company.

Palumbi S. (1996). Nucleic acids II: The polymerase chain reaction. In: Hillis DM, Moritz C, Mable BK, editors. Molecular systematics. Sunderland, MA: Sinauer, p 205–48.

Persad KM. (2009). The petroleum geology and prospects of Trinidad and Tobago. In: Trinidad and Tobago: Celebrating a century of commercial oil production. London: Official centenary publication of the Ministry of energy and energy studies, First Publishing. p 178–86.

Pindell J, Kennan L. (2007). Cenozoic kinematics and dynamics of oblique collision between two convergent plate margins: The Caribbean-South America collision in eastern Venezuela, Trinidad, and Barbados. In: Kennan L, Pindell JL, Rosen NC, editors. Transactions of the 27th GCSSEPM Annual Bob F. Perkins Research Conference: The Paleogene of the Gulf of Mexico and Caribbean Basins: Processes, Events and Petroleum Systems. p 458–553.

Pindell J, Kennan L. (2009). Tectonic evolution of the Gulf of Mexico, Caribbean and northern South America in the mantle reference frame: An update. In: James JK, Lorente MA, Pindell J, editors. The geology and evolution of the region between North and South America. London: Geological Society of London, Special Publication. p 1–80.

Pindell J, Kennan L, Maresch WV, Stanek K, Draper G, Higgs R. (2005). Plate-kinematics and crustal dynamics of circum-Caribbean arc-continent interactions: Tectonic controls on basin development in Proto-Caribbean margins. Special papers-Geol Soc Am 394:7.

Posada D. (2008). jModelTest: Phylogenetic model averaging. Mol Biol Evol 25:1253–6.

Rambaut A, Drummond AJ. (2007). Tracer v1.4. Available at: http://beast.bio.ed.ac.uk/Tracer (Accessed 10 July 2013).

Rivero JA. (1968). Los centrolénidos de Venezuela (Amphibia, Salientia). Mem Soc Ciencias Naturales Salle 28:301–34.

Rodriguez G, Lopez B. (2003). Insular species of Neotropical freshwater crabs (Crustacea: Brachyura). J Nat Hist 37:2599–614.

Smith JM, Downie RJ, Dye RFm, Thornham TG, Rutherford MG, Charles SP, Murphy JC. (2011). Amphibia, Anura, Hylidae, *Scarthyla vigilans* (Solano, 1971): Range extension and new country record for Trinidad, West Indies, with notes on tadpoles, habitat, behavior and biogeographical significance. Checklist 7:574–7.

Sumida M, Ogata M, Nishioka M. (2000). Molecular phylogenetic relationships of pond frogs distributed in the Palearctic region inferred from DNA sequences of mitochondrial 12S ribosomal RNA and cytochrome b genes. Mol Phyl Evol 16:278–85.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. (2011). MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28:2731–9.

Van Benthem S, Govers R, Spakman W, Wortel R. (2013). Tectonic evolution and mantle structure of the Caribbean. J Geophys Res Solid Earth 118:3019–36.

Veith M, Steinfartz S, Zardoya R, Seitz A, Meyer A. (1998). A molecular phylogeny of true salamanders (family Salamandridae) and the evolution of terrestriality of reproductive modes. J Zool Syst Evol Res 36:7–16.

Walsh PS, Metzger DA, Higuchi R. (1991). Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. BioTechniques 39:506–13.

Woodburne MO. (2010). The great American biotic interchange: Dispersals, tectonics, climate, sea level and holding pens. J Mammal Evol 17:245–64.

Yanek K, Heyer WR, De Sa RO. (2006). Genetic resolution of the enigmatic Lesser Antillean distribution of the frog *Leptodactylus validus* (Anura, Leptodactylidae). South Am J Herp 1:192–201.