

Colour and pattern variation and Pleistocene phylogeographic origin of the strawberry poison frog, *Oophaga pumilio*, in Nicaragua

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Abstract. The strawberry poison frog (*Oophaga pumilio*), occurring from Panama to northern Nicaragua, is characterized by an extraordinary variation of aposematic colouration and pattern in some Panamanian populations and has become a model system for studying evolutionary and ecological processes. Phylogeographic analyses of this species so far included populations from Panama and Costa Rica only. Based on newly collected samples from representative sites across Nicaragua, we here extend an existing data set of mitochondrial DNA sequences of the cytochrome b gene to analyse the phylogeographic history of *O. pumilio*. The 62 sequenced samples from seven localities are more closely related to the northern clade of *O. pumilio*, four haplotypes are shared amongst populations from Costa Rica and southern Nicaragua, and one haplotype is shared amongst Costa Rica and northern Nicaragua. An analysis of colour patterns reveals slight differences within and amongst Nicaraguan populations, but all of them conform to the general pattern found also in Costa Rica, with a reddish dorsum and blue coloured limbs. Phylogeographic analysis suggests that the Nicaraguan populations originated by a Late Pleistocene range expansion northwards, and reconstructs the origin of the species in southeastern Costa Rica.

Key words. Amphibia, Anura, Dendrobatidae, *Oophaga pumilio*, Nicaragua, phylogeography, colouration.

Introduction

The Central American strawberry poison frog *Oophaga pumilio* has become an important model system for studies in ecology and evolutionary biology due to its complex territorial and courtship behaviour (e.g., DONNELLY 1989, PRÖHL & HÖDL 1999, PRÖHL & OSTROWSKI 2011, SAPORITO et al. 2007, STYNOSKI 2009), and its intriguing variation in aposematic colour pattern (e.g., DALY & MYERS 1967, SUMMERS et al. 1997, WANG & SHAFFER 2008, BATISTA & KÖHLER 2008, MAAN & CUMMINGS 2009). Populations of this species from the Panamanian Bocas del Toro archipelago are extremely polymorphic in colouration and pattern, amongst and in some cases also within populations (DALY & MYERS 1967). In contrast, most mainland populations are relatively uniformly coloured, with a mainly red-orange dorsum and at least partly blue limbs (HAGEMANN & PRÖHL 2007).

The phylogeny and phylogeography of *O. pumilio* are convoluted as well. The genus *Oophaga* contains 9 species (*O. arborea*, *O. granulifera*, *O. histrionica*, *O. lehmanni*, *O. occultator*, *O. pumilio*, *O. speciosa*, *O. sylvatica*, and *O. vi-*

cente (MYERS et al. 1984, GRANT et al. 2006)), and ranges from Colombia to Nicaragua. Molecular phylogenies suggest that *O. pumilio* does not form a monophyletic group from a mitochondrial perspective (HAGEMANN & PRÖHL 2007, HAUSWALDT et al. 2011). The mitochondrial trees typically separate two main lineages within *O. pumilio*, with uncorrected pairwise divergences among cytochrome b haplotypes of up to 7%. Furthermore, congeneric species (*O. speciosa*, *O. arborea*, *O. vicentei*) are phylogenetically grouped with one of these lineages (HAGEMANN & PRÖHL 2007, WANG & SHAFFER 2008, HAUSWALDT et al. 2011). These two lineages are roughly distributed in either the northern or the southern part of the range, and micro-satellite data (HAUSWALDT et al. 2011) also give a weak signal of a northern (northeastern Costa Rica) and a southern (southeastern Costa Rica and Panama) cluster of populations. However, mitochondrial haplotypes belonging to both groups are also found co-occurring across a wide zone of Panama and Costa Rica. Altogether, this complex phylogeographic pattern suggests an intricate series of various past dispersal and vicariance processes, with allopatric divergence and subsequent admixture, and possi-

ble introgression among *O. pumilio* and other species of *Oophaga* (HAGEMANN & PRÖHL 2007, WANG & SHAFFER 2008, HAUSWALDT et al. 2011).

All recent studies on *Oophaga pumilio* focused on populations from Costa Rica and Panama, omitting populations from the largest portion of the distribution range of the species which extends across much of Nicaragua (GALINDO URIBE et al. 2013). Only a few individuals from southernmost Nicaragua (Río San Juan area) were included in general phylogenetic assessments (GRANT et al. 2006, SANTOS & CANATELLA 2011). At present, knowledge about the Nicaraguan populations is scarce. As far as is known, they have a monomorphic reddish and blue colour pattern (COPE 1874, NOBLE 1918, VILLA 1972, SAVAGE 2002). Given this lack of data, several questions about the intraspecific variation of *O. pumilio* have remained unanswered: (i) Do all Nicaraguan populations belong to the “northern” mitochondrial lineage, or are additional, strongly different haplotypes found in this part of the range? (ii) Did the Nicaraguan populations originate from a relatively recent northward range expansion? (iii) Do the Nicaraguan populations differ in colour pattern from the populations in northern Costa Rica?

In this study, we aim at addressing these questions by studying newly collected samples of *O. pumilio* from seven localities across its range in Nicaragua. We analyse the genetic differentiation and phylogeography of these populations based on DNA sequences of the mitochondrial cytochrome b gene and describe their colouration in greater detail.

Material and methods

Background: Previous knowledge on *Oophaga pumilio* from Nicaragua dates back to the early 1870s (BELT 1874, COPE 1874). COPE (1874) described *Dendrobates ignitus* (currently a synonym of *O. pumilio*) based on specimens collected in April 1873 at “Machuco (=Machuca) on the Río San Juan, Departamento Río San Juan, Nicaragua” (SAVAGE 1973). BELT (1874) recorded this species as being very abundant in the damp forests around Santo Domingo (Chontales Department). He experimented with feeding a young duck with one *O. pumilio*, who instead of swallowing it, instantly threw it up (BELT 1874). The only other contribution on toxins of *O. pumilio* from Nicaraguan populations is that of MEBS et al. (2008), who studied alkaloids in populations from Cerro Musún in the central part, and along the Río San Juan and Río Indio in the southeastern portion of the country. The Musún population exhibited a largely different alkaloid profile compared with that of Panamanian specimens, the latter sharing the common alkaloids with those from Río San Juan/Río Indio (MEBS et al. 2008). However, these profiles are of no direct phylogenetic relevance since alkaloids in dendrobatids are of dietary origin (MEBS et al. 2008). Only a few other data are available on the ecology and biology of the species in Nicaragua: NOBLE (1918) studied the stomach contents of several *O. pumilio* collected around Cuckra (today in the Atlántico Sur De-

partment), finding mostly small red ants, a single pill bug, and a spider, and WONG et al. (2009) studied heterospecific acoustic interference effects on the calling of adult males *O. pumilio* at Bartola, Río San Juan Department.

Most of the historical distribution records of this species from Nicaragua still are represented by extant populations, although these are often reduced in size. For example, the populations mentioned by BELT (1874) today persist in a small forest patch close to the Reserva Privada Las Brumas, but are threatened by deforestation. Also the populations referred to by COPE (1874) are still found throughout the southeastern portion of the country. NOBLE (1918) mentioned populations that are now located in a small forest patch in the Reserva Natural Kahka Creek that was spared the devastating effects of hurricane Felix in 2007. On the other hand, the historical populations around the city of Matagalpa (Matagalpa Department) have not been confirmed since the mid 1960s, and there are other uncertainties regarding the historical distribution of *O. pumilio* in Nicaragua. As an example, GAIGE et al. (1937) did not report *O. pumilio* from an extensive three-month herpetological collection carried out in 1935 in the area around the Río Escondido and its tributaries in central-eastern Nicaragua (today in the Atlántico Sur Department), an apparently suitable area, while the species is known to occur to the north, south, east and west of this region. Additionally, there have been several questionable records of *O. pumilio* in Nicaragua from the surroundings of Siuna in the Atlántico Norte Department (VILLA 1972) as well as from Managua and Chinandega Departments. It is possible that the voucher specimens for the Atlántico Norte records, as well as several other sites shown in the distribution map of VILLA (1972), were lost during the catastrophic earthquake in Managua in 1972, which destroyed all of the alcoholic herpetological specimens housed in the Museo Nacional de Nicaragua (VILLA 1981).

Currently, the distribution of *O. pumilio* in Nicaragua is considerable compared to its entire range. In Nicaragua, *O. pumilio* occurs across a continuous area in the southeastern corner of the country (Departamento Río San Juan; KÖHLER 2001, SUNYER et al. 2009) and other remaining lowland forest patches of the Departamento Atlántico Sur. Furthermore, the species is found in a few isolated and threatened forest localities at mid-altitudes in the Departamentos Chontales, Boaco, and Jinotega (KÖHLER 2001). In Nicaragua, this species is found in association with running waters and forests with different degrees of anthropogenic alteration. Individuals sometimes can be found in high densities, even in grasslands where no substrates for refuge and oviposition are obvious. However, it is unknown how these populations respond to increased habitat degradation and transformation.

Within Nicaragua, *O. pumilio* is considered a species of medium vulnerability and known to occur in four forest formations and several bioclimatic regions, with a widespread distribution in Lowland Wet and a peripheral distribution in Lowland Moist, Premontane Wet, and Premontane Moist formations, and can be found from sea level to 960 m a.s.l. (SUNYER & KÖHLER 2010). According to the IUCN cri-

teria, this species is regarded as a species of Least Concern, although populations are decreasing (SOLÍS et al. 2013), and according to the Convention on the International Trade of Endangered Species (CITES), it has been considered an Appendix II species since 1987 (CITES 2013). *Oophaga pumilio* is one of the most profitable amphibian species for the pet trade in Nicaragua, and between 1991 and 1996, 95% of the exports of this species were shipped to the USA (SOLÍS et al. 2013). Even though Cerro Kilambé is not included in the IUCN distribution map of the species, this locality was well known to the pet traders in the early 1990s who offered locals one US dollar per live specimen (pers. comm. by local people to J. SUNYER): a large amount in the aftermath of an impoverishing civil war. Nicaragua then fixed export quotas to 1,100 specimens taken from the wild in 1997, 1,200

ranchered specimens in 1998, 10,000 ranchered specimens in 1999, and 3,450 ranchered and captive-bred specimens in 2000 and 2001, respectively (CITES 2013).

Sampling localities and procedures

Specimens of *Oophaga pumilio* were collected from seven localities in Nicaragua (Fig. 1. Tab. 1) between May and August 2012. In the northernmost distribution area, we sampled two highland populations from the Reservas Naturales Cerro Kilambé (Jinotega region) and Cerro Musún (Matagalpa region), located at higher altitudes in Nicaragua (770 and 572 m a.s.l., respectively). The Kilambé population also represents an extension of the known range and currently

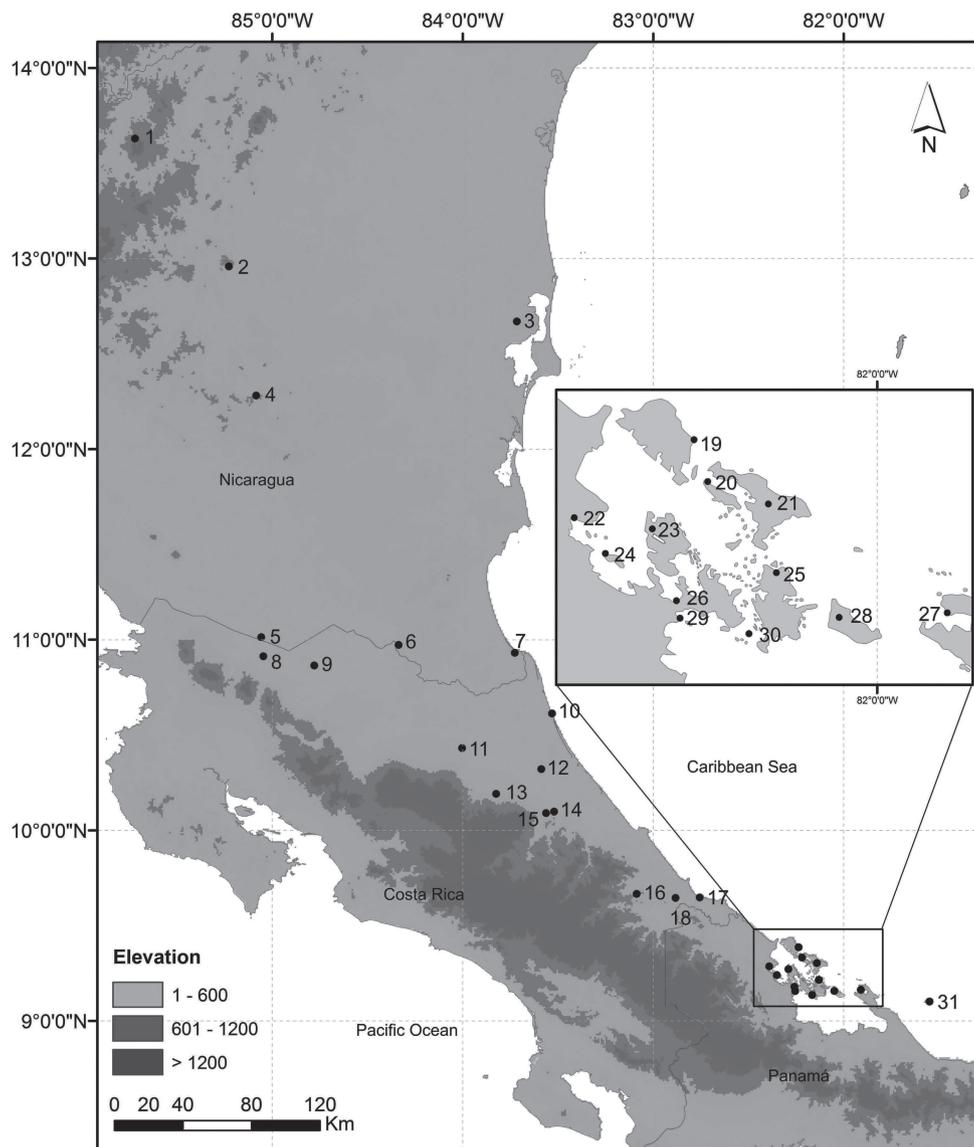


Figure 1. Location of the Nicaraguan populations of *O. pumilio* included in this study, and of the Costa Rican and Panamanian populations previously studied by HAUSWALDT et al. (2011). Numbers correspond to localities listed in Table 1.

Table 1. Locality numbers and names, coordinates, altitudes, and numbers of individuals of *O. pumilio* sequenced for the cytochrome b fragment, from Nicaraguan (NIC) populations (this study), and from Costa Rican (CR) and Panamanian (P) populations previously studied by HAUSWALDT et al. (2011). Populations and numbers of individuals (in parenthesis) assigned to the 93 haplotypes (H) found in 321 individuals of *O. pumilio*, two of *Oophaga vicentei* (O.v.), and one of *Oophaga granulifera* (O.g.).

Locality number	Locality	Province	Coordinates [lat. (°N), long. (°W)]		Cyt b (N)	Haplotypes
1	NIC: Kilambé	Jinotega	13.63	-85.72	8	H5 (7), H6 (1)
2	NIC: Musún	Matagalpa	12.96	-85.23	8	H1 (7), H5 (1)
3	NIC: Kahka Creek	Atlántico Sur	12.67	-83.72	7	H5 (7)
4	NIC: Las Brumas	Chontales	12.28	-85.08	9	H6 (9)
5	NIC: Guatuzos	Río San Juan	11.01	-85.06	10	H9 (10)
6	NIC: Bartola	Río San Juan	10.97	-84.34	10	H1 (1), H2 (2), H7 (2), H8 (5)
7	NIC: Río Indio Lodge	Río San Juan	10.93	-83.73	10	H1 (1), H2 (3), H3 (1), H4 (5)
8	CR: Upala	Alajuela	10.91	-85.05	7	H9 (4), H19 (3)
9	CR: Caño Negro	Alajuela	10.87	-84.78	11	H20 (5), H21 (1), H22 (2), H23 (3)
10	CR: Tortuguero	Limón	10.61	-83.53	9	H1 (4), H8 (2), H10 (3)
11	CR: La Selva	Heredia	10.43	-84.00	11	H2 (2), H8 (1), H33 (1), H34 (2), H35 (3), H36 (1), H37 (1)
12	CR: Pueblo Nuevo		10.32	-83.59	11	H8 (2), H24 (7), H25 (1), H26 (1)
13	CR: Guápiles	Limón	10.19	-83.82	11	H15 (1), H27 (2), H28 (8)
14	CR: Siquirres	Limón	10.10	-83.52	12	H14 (1), H15 (1), H16 (6), H17 (2), H18 (2)
15	CR: Río Reventazón	Cartago	10.09	-83.56	9	H16 (4), H27 (1), H38 (2), H39 (1), H40 (1)
16	CR: Hitoy Cerere	Limón	9.67	-83.09	14	H8 (1), H27 (1), H58 (6), H89 (4), H90 (2)
17	CR: Puerto Viejo de Talamanca	Limón	9.65	-82.76	12	H11 (5), H12 (5), H13 (2)
18	CR: Bribri	Limón	9.65	-82.88	9	H29 (3), H30 (3), H31 (1), H32 (2)
19	P: Colón	Bocas del Toro	9.39	-82.24	12	H41 (8), H42 (1), H43 (1), H44 (1), H45 (1)
20	P: Solarte	Bocas del Toro	9.33	-82.22	12	H42 (1), H50 (11)
21	P: Bastimentos	Bocas del Toro	9.30	-82.14	10	H41 (3), H42 (1), H48 (5), H49 (1)
22	P: Almirante	Bocas del Toro	9.29	-82.39	9	H50 (3), H51 (2), H52 (3), H53 (1)
23	P: San Cristobal	Bocas del Toro	9.27	-82.29	10	H8 (1), H42 (2), H83 (4), H84 (3)
24	P: Pastores	Bocas del Toro	9.24	-82.35	10	H78 (2), H79 (4), H80 (2), H81 (1), H82 (1)
25	P: Popa	Bocas del Toro	9.22	-82.13	19	H46 (1), H50 (1), H69 (7), H70 (1), H71 (4), H72 (1), H73 (1), H74 (1), H75 (1), H76 (1)
26	P: Tierra Oscura	Bocas del Toro	9.18	-82.26	8	H54 (5), H56 (2), H77 (1)
27	P: Punta Alegre	Bocas del Toro	9.16	-81.91	8	H85 (3), H86 (3), H87 (1), H88 (1)
28	P: Cayo de Agua	Bocas del Toro	9.16	-82.05	12	H50 (7), H59 (1), H60 (1), H61 (1), H62 (1), H63 (1)
29	P: Cauchero	Bocas del Toro	9.16	-82.25	12	H53 (3), H54 (2), H55 (1), H56 (4), H57 (1), H58 (1)
30	P: Loma Partida	Bocas del Toro	9.14	-82.17	10	H46 (9), H47 (1)
31	P: Escudo de Veraguas	Escudo de Veraguas	9.10	-81.55	11	H64 (7), H65 (1), H66 (1), H67 (1), H68 (1)
					321	
	O.v.1	Veraguas	8.51	-81.08	1	H91 (1)
	O.v.2	Coclé	8.63	-80.58	1	H92 (1)
	O.g.				1	H93 (1)

is the northernmost known locality of the species. In central Nicaragua, we sampled frogs from the private Reserva Natural Las Brumas at 562 m a.s.l., and from Kahka Creek, a lowland (24 m a.s.l.) population near Laguna de Perlas on the Atlantic coast. In the southern part of Nicaragua, we sampled three populations close to the border with Costa Rica: the Refugio de Vida Silvestre Los Guatuzos close to the Papaturro River, the Reserva Silvestre Privada Refugio

Bartola, and the Río Indio Lodge near the Atlantic coast, at 54, 74 and 14 m a.s.l., respectively. Geographic coordinates were obtained with a Garmin Oregon 550 GPS.

Ventral and dorsal photographs were taken with a digital camera of ten specimens per site. To facilitate body measurements, every frog was placed on a millimetre grid. Colour descriptions were made considering mainly the terminology used by SAVAGE (1968, 2002). Snout-vent length

(SVL) measurements were taken based on the photographs using ImageJ 1.46r (ABRAMOFF et al. 2004). Tissue samples were taken by toe-clipping from up to ten specimens per locality and preserved in 96% ethanol.

DNA sequencing

Genomic DNA was extracted from toe clippings using the QIAGEN DNeasy Blood and Tissue kit. Samples and extractions were stored at -20°C. We amplified a fragment of

the mitochondrial cytochrome b gene using the primers MVZ 15 (MORITZ et al. 1992), and CytbDen1-H (SANTOS & CANNATELLA 2011). PCR conditions consisted of an initial 2 min. at 95°C, 35 cycles of 30 sec. each at 95°C, 40 sec. at 42°C, and 60 sec. at 72°C, and a final extension step of 6 min. at 72°C. Amplified fragments were sequenced with the forward primer MVZ15, using dye-terminator chemistry. DNA sequences (784 bp) were subsequently aligned with those from HAUSWALDT et al. (2011). All newly determined sequences were deposited in GenBank (accession numbers KF645289–KF645350).

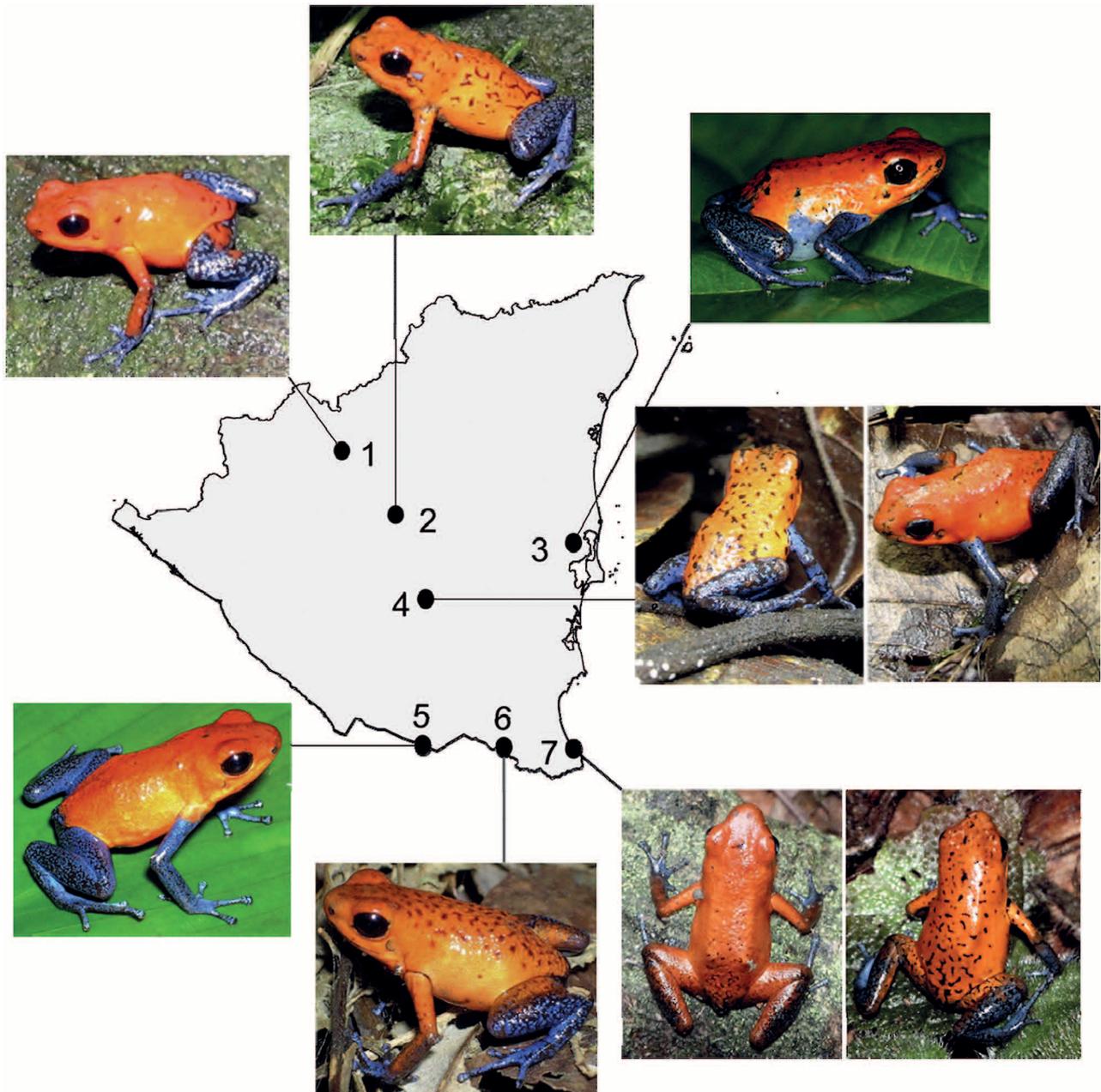


Figure 2. *Oophaga pumilio* colour patterns observed at Nicaraguan sites. 1 – Kilambé; 2 – Musún; 3 – Kahka Creek (Photo taken from www.monarchzman.deviantart.com); 4 – Las Brumas; 5 – Guatuzos; 6 – Bartola; 7 – Río Indio Lodge.

Table 2. Dorsal and ventral colours and patterns described for *Oophaga pumilio* populations in Nicaragua. Colour pattern descriptions and snout–vent length (SVL) measurements (\pm standard deviation) were taken for 10 individuals.

Population	Dorsal body colour	Dorsal limb colour	Dorsal body pattern	Dorsal leg pattern	Ventral body colour	Ventral limb colour	Ventral body and limb pattern	SVL
Kilambé	Red	Blue with or without red markings on one thigh	None or very few scattered small spots or wavy flecks	Reticulated markings	Mainly red with light blue	Blue	Diffused flecks	22.4 \pm 0.6
Musún	Red-orange	Blue, with or without red markings on one thigh	Small spots or wavy flecks	Reticulated markings or diffused spots	Mainly orange-red with light blue	Blue	Diffused small spots or flecks	22.7 \pm 1.1
Kahka Creek	Red and blue near cloaca	Blue	Small spots or wavy flecks	Diffused reticulated markings or small spots	Mainly light blue with red in the flanks	Blue	Diffused flecks	22.2 \pm 0.8
Las Brumas	Red or orange red	Blue, with or without red markings on thighs	Small spots or wavy flecks	Mainly well-defined reticulated markings	Mainly orange-red with light blue	Blue	Diffused flecks	20.9 \pm 0.8
Guatuzos	Red	Blue	None or very few scattered small spots or wavy flecks	Small spots	Mainly orange-red with light blue near cloaca and chin	Blue	None or a few diffused flecks	23.4 \pm 0.7
Bartola	Dull red	Grey-blue with or without red markings on thighs	Very few scattered small spots	Diffused spots	Orange-red with light blue or red with light blue near cloaca	Light blue	Diffused flecks	19.7 \pm 0.8
Río Indio Lodge	Red-orange or orange-red	Red-orange, with or without black or blue at tips	None or small spots or wavy flecks	Small spots or stripes	Orange red	Red, black, and blue	Diffused flecks	21.8 \pm 1.1

Phylogeography

The Bayesian Information Criterion (BIC) incorporated in jModeltest vs. 2.1.1 (DARRIBA et al. 2012) was used to determine the best fitting model of nucleotide substitution for two datasets of the cytochrome b sequences. The HKY + Γ model was selected for dataset 1 composed of all haplotypes of *O. pumilio* as well as of the outgroup species (*O. granulifera* and *O. vicentei*), and the TrN + Γ model was selected for the dataset 2 containing sequences of all individuals of *O. pumilio*.

Dataset 1 was used to reconstruct a phylogenetic tree of haplotypes using Bayesian inference as implemented in Mr. Bayes vs. 3.1.2. (RONQUIST & HUELSENBECK 2003) using an unpartitioned approach. Using the default settings of the software, we ran two chains for 10 million generations each, sampling every 100th tree, and checked for convergence. The first 25% of the sampled trees were discarded as burn-in. Bayesian posterior probabilities are based on a majority-rule consensus tree.

Dataset 2 was used with the software Beast 1.7. (DRUMMOND et al. 2012) to simultaneously estimate tree topology and geographic locality of nodes, using the geographic coordinates of the samples as traits and combined this with the phylogeographic method by LEMEY et al. (2010). Specifically, we used the Relaxed Random Walk model to estimate the geographic origin of the *O. pumilio* clade

and possible dispersal routes throughout Panama, Costa Rica and Nicaragua. We set the coalescent prior to constant size and used an uncorrelated relaxed clock model (DRUMMOND et al. 2006), the TrN + Γ model of substitution, codon partitioning (1+2, 3), and set the mutation rate to 0.01. We ran a MCMC chain 3×10^8 long, sampling every 30,000th. We ensured that effective sample sizes (ESS) were higher than 200 for all parameters with Tracer 1.5. The first 20% of the trees were discarded as burn-ins. The resulting tree was summarized with Tree Annotator 1.7.x. To generate the KML file that could be plotted with Google Earth (www.googleearth.com), we used the software SPREAD (BIELEJEC et al. 2011) and a posterior probability setting of 0.05.

Results

Morphology and colour pattern

No substantial variation in colour pattern and body size was detected among Nicaraguan *O. pumilio* populations. All share the “blue jeans” dorsal colour pattern seen in Costa Rican frogs, with blue or black limbs (Figs 2–3, Tab. 2). The colour of the body differs slightly amongst populations and ranges from dull red to red-orange. The colour of the limbs varies from blue to grey-blue or black. The presence and amount of black on the dorsum can differ as well and

take on the shape of small spots, wavy lines, or reticulated markings. The venter is orange or red, with or without light blue (Fig. 3). Specimens from Kilambé and Guatuzos have a particularly bright red body colour and blue limbs with reduced black pattern on the dorsum, and those from Guatuzos have very small light blue markings on the venter. Individuals from Musún have a red–orange dorsum with a black pattern. Specimens from Kahka Creek have a red dorsum with some blue on the cloaca and limbs, and a reduced amount of red on the venter and flanks. Specimens from Bartola have a dull red-coloured dorsum with light blue limbs, and are mainly red on the ventral side. At Las Brumas, the frogs are either red or red-orange with blue legs, and always exhibit a black dorsal pattern. At Río Indio Lodge, two main morphs can be distinguished: red-orange and blue-legged frogs with a black dorsal pattern, and orange-red frogs with or without black legs and a very re-

duced or even absent dorsal pattern (Fig. 2, Tab. 1). Populations varied in mean body size between 19.7 and 22.7 mm, with the largest frogs occurring in the Río San Juan populations from Guatuzos, and the smallest frogs being found in Bartola (Tab. 2).

Phylogeography

A total of 62 cytochrome b sequences were obtained from Nicaraguan *O. pumilio* and compared with previously determined sequences from Costa Rica and Panama (HAUSWALDT et al. 2011) to yield a total alignment of sequences from 321 individuals. Because the Costa Rica and Panama sequences were shorter than the newly determined ones from Nicaragua, the alignment used for analysis was only 558 bp in length. 88 haplotypes could be distin-

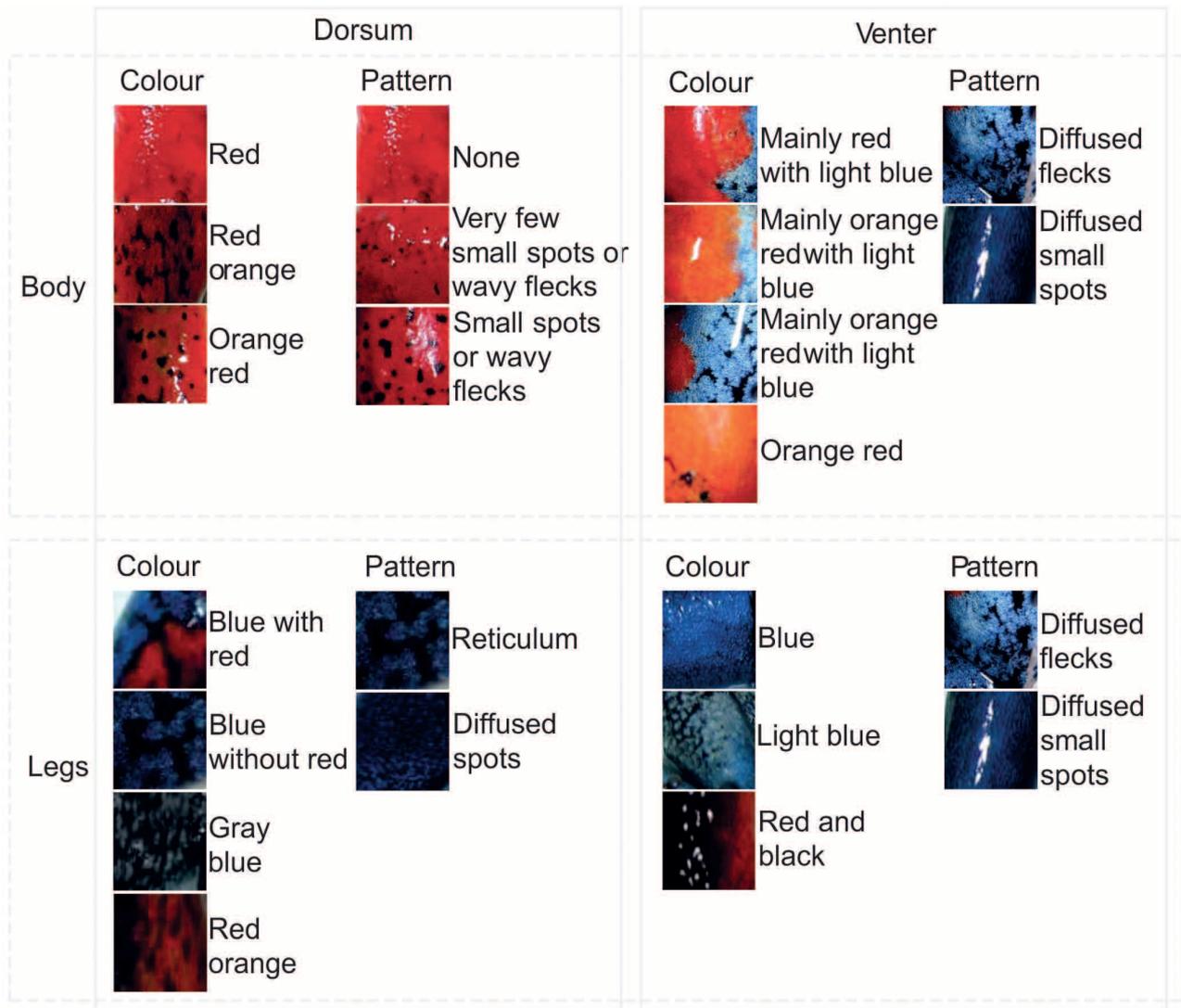


Figure 3. Representation of the terminology used for describing the dorsal and ventral colours and patterns of *Oophaga pumilio* populations from Nicaragua.

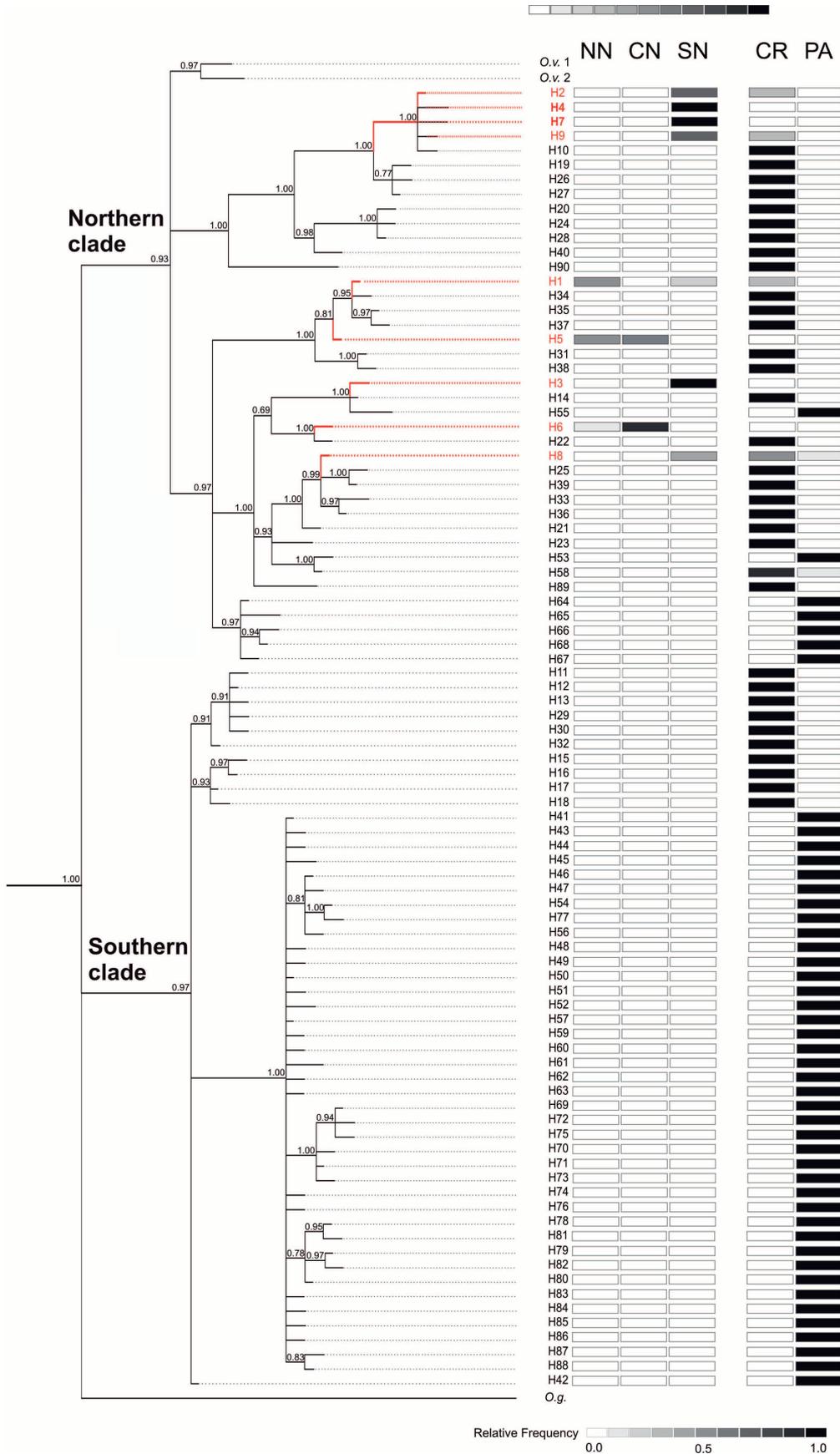


Figure 4. MrBayes tree based on an unpartitioned dataset of 529 bp *cyt-b* sequences (all haplotypes are of equal length), using the HKY+G model and *O. granulifera* as an outgroup. Red branches and numbers indicate haplotypes found in Nicaragua. Grey scales in boxes represent the relative frequency of individuals assigned to each haplotype, from the region of North Nicaragua (NN), Central Nicaragua (CN), South Nicaragua (SN), Costa Rica (CR), and Panama (PA).

guished (Fig. 4). The phylogenetic analysis (Fig. 4) agrees with previous findings in separating the haplotypes into a northern and a southern clade, and in placing sequences of *O. vicentei* with the northern clade of *O. pumilio*. A total of nine haplotypes (numbers as in Fig. 4), all grouped in the northern clade, were found among the Nicaraguan populations. Five of these (H3–H7) had not been observed before and were exclusive to Nicaraguan populations. The other four haplotypes (H1, H2, H8, H9) were shared among Nicaraguan and Costa Rican populations. Haplotype sharing with Costa Rican populations was not limited to the populations from Río San Juan (geographically closest to Costa Rica), but was observed in northern Nicaraguan populations as well: e.g., haplotype H1, was found at Musún, Bartola, Río Indio Lodge, and in the Costa Rican population of Tortuguero (Tab. 1).

The phylogeographic analyses strongly support a centre of origin of *O. pumilio* in southeastern Costa Rica from where the species spread north- and southwards. Dispersal to the island of Escudo de Veraguas likely co-occurred with the spread into Nicaragua (Fig. 5). The origin of the populations in Nicaragua is reconstructed as a recent Pleistocene dispersal at approximately 200,000 years before present (Fig. 5).

Discussion

Understanding the phylogeographic history of *O. pumilio* is crucial for making inferences on the evolution of the extraordinary polychromatism of the species. However, given the lack of molecular data from Nicaragua, i.e., from more than half of the species' range, it used to be impossible to reliably test hypotheses on the geographic origin and directions of range expansion of this species. The data presented herein partly fill this geographical sampling gap, and confirm that the Nicaraguan populations are not strongly differentiated, but rather the result of a recent range expansion from Costa Rica. Our explicit phylogeographic analyses place the origin of the species in southern Costa Rica, and support a radial expansion of this species northwards into Nicaragua and southwards towards the Bocas del Toro archipelago during the Late Pleistocene. However, an important limitation of this analysis is its sole reliance on mitochondrial DNA. If either the northern or southern lineage of *O. pumilio* were the result of an ancient hybridisation and introgression process with another species of *Oophaga*, then some of the detailed reconstructions herein might not fully reflect the true evolutionary history of the species. Despite this limitation, given that the north-

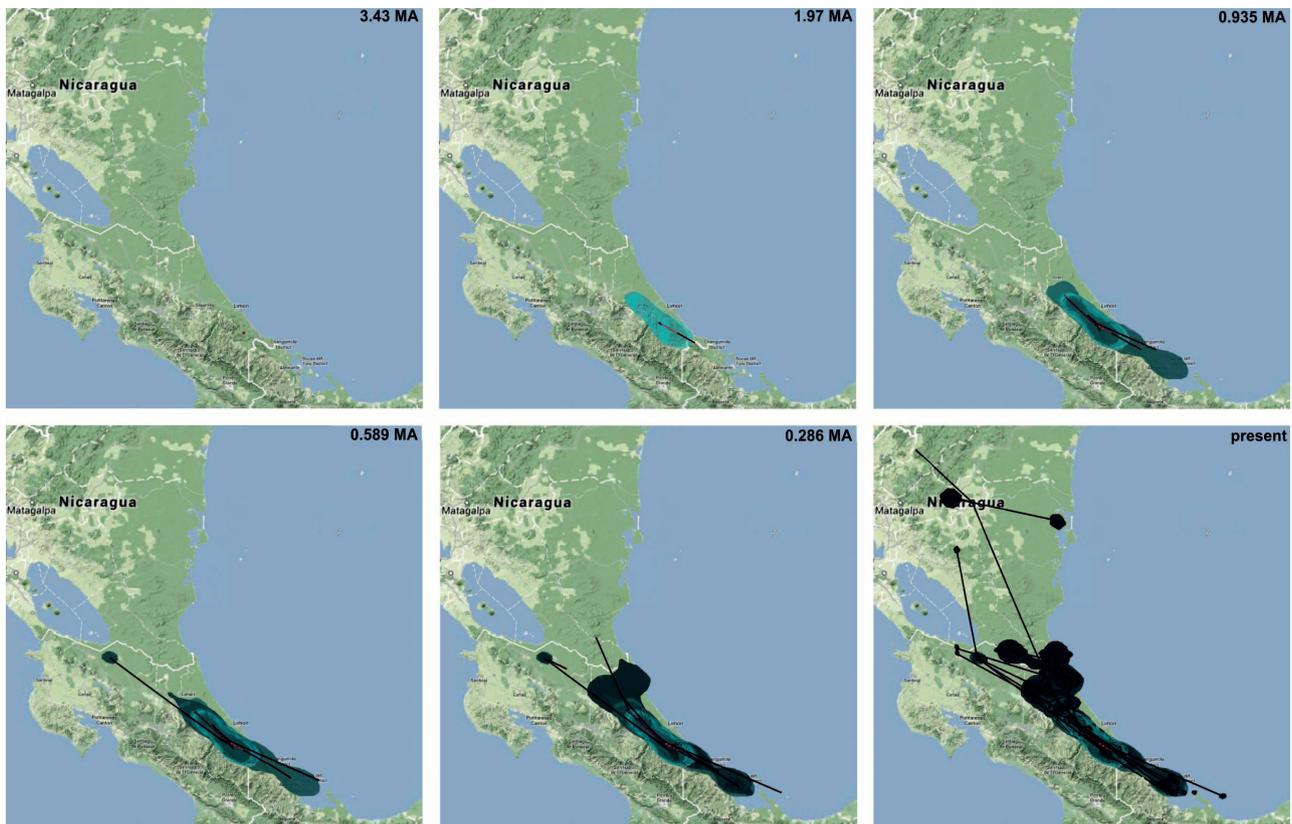


Figure 5. Phylogeographic reconstruction for Central American *Oophaga pumilio* populations using the Relaxed Random Walk Model. Green polygons and red lines indicate early events, whereas black lines and polygons more recent events. The red marking points out the Isla Bastimentos in Panama. MA – million years ago.

ern Nicaraguan populations of *O. pumilio* share haplotypes with Costa Rican populations, and that Nicaraguan populations overall do not differ strongly in colouration from the ones in Costa Rica, we find it very plausible that Nicaraguan populations indeed originate from a recent range expansion northwards.

The relationships among mitochondrial lineages and species of *Oophaga* also reveal the need for taxonomic revision. DALY & MYERS (1967) and MYERS & DALY (1976) suggested recognizing *O. pumilio* as a single polymorphic species, and this view has either been accepted or controversially discussed (SUMMERS et al. 1997, HAGEMANN & PRÖHL 2007, BATISTA & KÖHLER 2008, HAUSWALDT et al. 2011). The taxonomic integrity of *O. pumilio* as a single entity remains uncertain, especially relative to several other species in the genus distributed in Panama (*O. arborea*, *O. speciosa*, and *O. vicentei*). Alternatively, some (or all) of these other taxa could in fact represent morphs of *O. pumilio* and thus junior synonyms, or some populations considered as *O. pumilio* could in fact rather belong to one of those closely related species. The strawberry poison frog was originally described as *Dendrobates pumilio* SCHMIDT, 1857 from the Bocas del Toro province in Panama. DALY & MYERS (1967) applied this name to the small-sized poison frogs occurring from Nicaragua to northwestern Panama, and GRANT et al. (2006) suggested classifying this and related species, separate from *Dendrobates*, in the genus *Oophaga*. Three other names are currently considered to be junior synonyms of *O. pumilio*: *Dendrobates galindoi* TRAPIDO, 1953, described likewise from Bocas del Toro, *Dendrobates typographus* KEFERSTEIN, 1867, and *Dendrobates ignitus* COPE, 1874, with their type localities in Costa Rica and Nicaragua, respectively (see SAVAGE 1968, BATISTA & KÖHLER 2008). However, given the wide co-occurrence of the two haplotype lineages in *O. pumilio* (HAUSWALDT et al. 2011), we consider it to be rather unlikely that they represent distinct and independent evolutionary lineages, and that any of these junior synonyms would require elevation to species level. Inversely, as mentioned above, the identities, status and distribution ranges, especially of *O. arborea* and *O. speciosa*, but also of *O. vicentei*, require detailed revision. A first step that has to be taken towards such a revision is a comprehensive mitochondrial phylogeny of all *Oophaga* species, using longer DNA sequences, in order to obtain a strongly supported phylogenetic tree depicting the mitochondrial relationships among these frogs. Using phylogenomic methods such as RAD sequencing (e.g., RUBIN et al. 2012) would then allow to reconstruct the true evolutionary relationships among species and populations, and to understand if, when, and where phenomena of mitochondrial introgression might have taken place in this genus.

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References

- ABRAMOFF, M. D., P. J. MAGALHAES & S. J. RAM (2004): Image processing with ImageJ. – *Biophotonics International*, **11**: 36–43.
- BATISTA, A. & G. KÖHLER (2008). Variation in *Oophaga pumilio* (Amphibia: Anura: Dendrobatidae) in western Panama. – *Salamandra*, **44**: 225–234.
- BELT, T. (1874): *The Naturalist in Nicaragua*. – London (John Murray): 403 pp.
- BIELEJEC, F., A. RAMBAUT, M. A. SUCHARD & P. LEMEY (2011): SPREAD: spatial phylogenetic reconstruction of evolutionary dynamics. – *Bioinformatics*, **27**: 2910–2912.
- CITES (2013): CITES Species database. – <http://www.cites.org/eng/resources/species.html>. Accessed 8 September 2013.
- COPE, E. D. (1874): Description of some species of reptiles obtained by Dr. John F. Bransford, assistant surgeon United States Navy, while attached to the Nicaragua surveying expedition in 1873. – *Proceedings of the Academy of Natural Sciences of Philadelphia*, **26**: 64–72.
- DALY, J. W. & C. W. MYERS (1967): Toxicity of Panamanian poison frogs (*Dendrobates*): Some biological and chemical aspects. – *Science*, **156**: 970–973.
- DARRIBA, D., G. L. TABOADA, R. DOALLO & D. POSADA (2012): jModelTest 2: more models, new heuristics and parallel computing. – *Nature Methods*, **9**: 772.
- DONNELLY, M. A. (1989): Effects of reproductive resource supplementation on space-use patterns in *Dendrobates pumilio*. – *Oecologia*, **81**: 212–218.
- DRUMMOND, A. J., S. Y. W. HO, M. J. PHILLIPS & A. RAMBAUT (2006): Relaxed phylogenetics and dating with confidence. *PLoS Biology*, **4**: e88.
- DRUMMOND, A. J., M. A. SUCHARD, D. XIE & A. RAMBAUT (2012): Bayesian phylogenetics with BEAUti and the BEAST 1.7. – *Molecular Biology and Evolution*, **29**: 1969–1973.
- GAIGE, H. T., N. HARTWEG & L. C. STUART (1937): Notes on a collection of amphibians and reptiles from eastern Nicaragua. – *Occasional Papers Museum of Zoology University of Michigan*, **357**: 1–18.
- GALINDO URIBE, D., J. SUNYER, A. AMÉZQUITA, G. KÖHLER, H. PRÖHL & M. VENCES (2013): Auf der Suche nach Erdbeerfröschen in Nicaragua. – *Terraria/Elaphe*, **42**: 48–53.

- GRANT, T., D. R. FROST, J. P. CALDWELL, R. GAGLIARDO, C. F. B. HADDAD, P. J. R. KOK, D. B. MEANS, B. P. NOONAN, W. E. SCHARGEL & W. C. WHEELER (2006): Phylogenetic systematics of dart-poison frogs and their relatives (Amphibia: Athesphatana: Dendrobatidae). – *Bulletin of the American Museum of Natural History*, **299**: 1–262.
- HAGEMANN, S. & H. PRÖHL (2007): Mitochondrial paraphyly in a polymorphic poison frog species (Dendrobatidae; *D. pumilio*). – *Molecular Phylogenetics and Evolution*, **45**: 740–747.
- HAUSWALDT, J. S., A.-K. LUDEWIG, M. VENCES & H. PRÖHL (2011): Widespread co-occurrence of divergent mitochondrial haplotype lineages in a Central American species of poison frog (*Oophaga pumilio*). – *Journal of Biogeography*, **38**: 711–726.
- KEFERSTEIN, W. (1867): Ueber einige neue oder seltene Batrachier aus Australien und dem tropischen Amerika. – *Nachrichten von der Georg-Augusts Universität und der Königlichen Gesellschaft der Wissenschaften zu Göttingen*, **18**: 341–361.
- KÖHLER, G. (2001): Anfíbios y Reptiles de Nicaragua. – *Offenbach (Herpeton)*: 208 pp.
- LEMEY, P., A. RAMBAUT, J. J. WELCH & M. A. SUCHARD (2010): Phylogeography takes a relaxed random walk in continuous space and time. – *Molecular Biology and Evolution*, **27**: 1877–1885.
- MAAN, M. E. & M. E. CUMMINGS (2009): Sexual dimorphism and directional sexual selection on aposematic signals in a poison frog. – *Proceedings of the National Academy of Sciences of the U.S.A.*, **105**: 19072–19077.
- MEBS D., W. POGODA, A. BATISTA, M. PONCE, G. KÖHLER & G. KAUERT (2008): Variability of alkaloid profiles in *Oophaga pumilio* (Amphibia: Anura: Dendrobatidae) from western Panama and southern Nicaragua. – *Salamandra*, **44**(4): 241–247.
- MORITZ, C., C. J. SCHNEIDER & D. B. WAKE (1992): Evolutionary relationships within the *Ensatina eschscholtzii* complex confirm the ring species interpretation. – *Systematic Biology*, **41**(3): 273–291.
- MYERS, C. W. & J. DALY (1976): Preliminary evaluation of skin toxins and vocalizations in taxonomic and evolutionary studies of poison dart frogs (Dendrobatidae). – *Bulletin of the American Museum of Natural History*, **157**: 173–262.
- MYERS, C. W., J. W. DALY & V. MARTÍNEZ (1984): An arboreal poison frog (*Dendrobates*) from Western Panama. *American Museum Novitates*, **2783**: 1–20.
- NOBLE, G. K. (1918): The amphibians collected by the American Museum expeditions to Nicaragua in 1916. – *Bulletin of the American Museum of Natural History*, **38**(10): 311–347.
- PRÖHL, H. & W. HÖDL (1999): Parental investment, potential reproductive rates and mating system in the strawberry dart-poison frog *Dendrobates pumilio*. – *Behavioural Ecology and Sociobiology*, **46**: 215–220.
- PRÖHL, H. & T. OSTROWSKI (2011): Behavioural elements reflect phenotypic colour divergence in a poison frog. – *Evolutionary Ecology*, **25**: 993–1015.
- RONQUIST, F. & J. P. HUELSENBECK (2003): MrBayes 3: Bayesian phylogenetic inference under mixed models. – *Bioinformatics*, **19**: 1572–1574.
- RUBIN, B. E. R., R. H. REE & C. S. MOREAU (2012): Inferring phylogenies from RAD sequence data. – *PLoS One*, **7**: e33394.
- SANTOS J. C. & D. C. CANNATELLA (2011): Phenotypic integration emerges from aposematism and scale in poison frogs. – *Proceedings of the National Academy of Sciences of the U.S.A.*, **108**: 6175–6180.
- SAPORITO, R. A., M. A. DONNELLY, P. JAIN, H. M. GARRAFFO, T. F. SPANDE & J. W. DALY (2007): Spatial and temporal patterns of alkaloid variation in the poison frog *Oophaga pumilio* in Costa Rica and Panama over 30 years. – *Toxicon*, **50**: 757–778.
- SAVAGE, J. M. (1968): The dendrobatid frogs of Central America. – *Copeia*, **1968**: 745–776.
- SAVAGE, J. M. (1973): Herpetological collections made by Dr. John F. Bransford, Assistant Surgeon, U.S.N. during the Nicaragua and Panama Canal Surveys (1872–1885). – *Journal of Herpetology*, **7**: 35–38.
- SAVAGE, J. M. (2002): The amphibians and reptiles of Costa Rica: a herpetofauna between two continents, between two seas. – Chicago and London (Univ. Chicago Press): 934 pp.
- SCHMIDT, O. (1857): Diagnosen neuer Frösche des zoologischen Cabinets zu Krakau. – *Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften in Wien*, **24**: 10–15.
- SOLÍS, F., R. IBÁÑEZ, C. JARAMILLO, G. CHAVES, J. SAVAGE, G. KÖHLER & N. COX (2013): *Oophaga pumilio*. – in: IUCN 2013. IUCN Red List of Threatened Species. Version 2013.1. – www.iucnredlist.org, downloaded on 01 September 2013.
- STYNOSKI, J. L. (2009): Discrimination of offspring by indirect recognition in an egg-feeding dendrobatid frog, *Oophaga pumilio*. – *Animal Behaviour*, **78**: 1351–1356.
- SUMMERS, K., E. BERMINGHAM, L. WEIGT, S. MCCAFFERTY & L. DAHLSTROM (1997): Phenotypic and genetic divergence in three species of dart-poison frogs with contrasting parental behavior. – *Journal of Heredity*, **88**: 8–13.
- SUNYER, J. & G. KÖHLER (2010): Conservation status of the herpetofauna of Nicaragua. – pp. 488–509 in: WILSON, L. D., J. H. TOWNSEND & J. D. JOHNSON (eds): Conservation of Mesoamerican amphibians and reptiles. – Eagle Mountain Publishing, Utah, USA.
- SUNYER, J., G. PÁIZ, M. DEHLING & G. KÖHLER (2009): A collection of amphibians from Río San Juan, southeastern Nicaragua. – *Herpetology Notes*, **2**: 189–202.
- TRAPIDO, H. (1953): A new frog from Panama, *Dendrobates galindoi*. – *Fieldiana Zoology*, **34**: 181–187.
- VILLA, J. D. (1981): Three albino snakes. – *Herpetological Review*, **12**: 81.
- VILLA, J. D. (1972): Anfíbios de Nicaragua. – Managua (Instituto Geográfico Nacional & Banco Central de Nicaragua), 216 pp.
- WANG, I. J. & H. B. SHAFFER (2008): Rapid color evolution in an aposematic species: a phylogenetic analysis of color variation in the strikingly polymorphic strawberry poison-dart frog. – *Evolution*, **62**: 2742–2759.
- WONG, S., H. PARADA & P. M. NARINS (2009): Heterospecific acoustic interference: effects on calling in the frog *Oophaga pumilio* in Nicaragua. – *Biotropica*, **41**: 74–80.