

DOI: 10.30906/1026-2296-2025-32-2-96-107

ORIGIN OF POPULATIONS OF THE CANE TOAD *Rhinella marina* AND ITS PARASITIC TICK *Amblyomma dissimile* ON MARGARITA ISLAND (VENEZUELA)

Spartak N. Litvinchuk,^{1*} Polina K. Ibrogimova,²
Nikolay V. Anisimov,³ and Anton O. Svinin¹

Submitted February 16, 2025.

Invasive species pose a serious threat to global biodiversity. One of the most successful of these species is the cane toad *Rhinella marina*. Since 1842, it has become a popular biological pest control agent and has been deliberately introduced to many islands and Australia. However, due to its toxicity and potential impact on wildlife and domestic animals, cane toads are now considered one of the most dangerous invasive species. They were first recorded on Margarita Island in the Caribbean in 1993 and have become very numerous there. The toads are heavily infested with the *Amblyomma dissimile* tick, which can also be transmitted by reptiles and occasionally birds and mammals. The aim of our paper was to analyze possible routes of introduction of cane toads and their parasitic ticks to Margarita Island using a mitochondrial DNA molecular marker (barcoding). We have genetically confirmed for the first time that the local population of cane toads is indeed *R. marina*. We did not find any genetic variation in the population, which may indicate that it is new and experiencing a bottleneck. The ticks have probably been present on this island longer than the cane toads. This may be supported by the fact that they were known here before the arrival of *R. marina*. Our genetic analysis supports this hypothesis, as a unique haplotype was found in the local tick population.

Keywords: barcoding; Bufonidae; Caribbean; ectoparasitism; invasive species; Ixodidae.

INTRODUCTION

Invasive species pose a serious threat to global biodiversity (Reaser et al., 2007). One of the most successful invasive terrestrial vertebrates is the cane toad *Rhinella marina* (Linnaeus, 1758). The species was described as “*Rana marina*” by Carl Linnaeus, who listed its type locality as “America,” although the type specimen was probably collected in Suriname (Müller and Hellmich, 1936). It was long considered a widespread Neotropical species of the cosmopolitan genus *Bufo* Garsault, 1764, but is now regarded as one of about a dozen species in the *R. marina* complex that occasionally hybridize with each other (Maciel et al., 2010; Vallinoto

et al., 2010, 2017; Sequeira et al., 2011; Acevedo et al., 2016; Bessa-Silva et al., 2020; Pereyra et al., 2021; Riveira et al., 2022; Menéndez-Guerrero et al., 2024).

Since 1842, cane toads have become a popular biological pest control agent and have been deliberately introduced from their native range to most of the Antilles, Australia, Papua New Guinea, and numerous Pacific islands (Lever, 2001; Acevedo et al., 2016). However, due to their toxicity to predators and potential impact on wild and domestic animals, cane toads are now recognized as one of the World’s 100 most dangerous invasive species (Lowe et al., 2000).

The cane toad is fairly common in its native habitats in the Amazon Basin, the Guiana Region and on the neighboring islands of Trinidad and Tobago (Bodkin, 1918; Murphy, 1997), but has not previously been reported from Margarita Island located 19 km off the Caribbean Coast of mainland Venezuela (Roze, 1964). Margarita Lampo with colleagues (Lampo and Bayliss, 1996; Lampo and Medialdea, 1996; Lampo et al., 1998) were the first to record *R. marina* on the island in 1993 and

¹ Institute of Cytology of the Russian Academy of Sciences, St. Petersburg, Russia.

² Institute of Environmental and Agricultural Biology (X-BIO), University of Tyumen, Tyumen, Russia.

³ Institute of Pharmacy, Tyumen State Medical University, Tyumen, Russia.

* Corresponding author: litvinchukspartak@yandex.ru

provided data on its occurrences in coastal xerophytic shrublands near Macanao and Porlamar. Additionally, Rivas et al. (2005) observed a young individual on shores of La Pared Lagoon in 2004. Currently, the local population the cane toad has become quite numerous (Ugueto and Rivas, 2010; our data).

The invasion process can often be accompanied by an introduction of previously unknown into these regions parasites and infections. Cane toads can harbor several pathogenic viruses, bacteria, protozoa, and helminths (Delvignier and Freeland, 1988; Russo et al., 2021; Selechnik et al., 2017). They are also host to larvae, nymphs, and adults of two species of ectoparasitic ixodid ticks (*Amblyomma dissimile* Koch, 1844 and *A. rotundatum* Koch, 1844), which reduce the survival and fertility of these toads, significantly affecting their numbers and causing the death of young individuals (Bodkin, 1918; Schumaker and Barros, 1994; Guglielmone and Nava, 2010; Benavides-Montaño et al., 2018; Polo et al., 2020).

Amblyomma dissimile is distributed from the United States in the north to Argentina in the south and is commonly found, in addition to cane toads [*R. horribilis* (Wiegmann, 1833) and *R. marina*], on snakes, crocodiles, lizards, and turtles, of which most records of parasitism are associated with primary hosts such as the common green iguana *Iguana iguana* (Linnaeus, 1758), and the common boa *Boa constrictor* Linnaeus, 1758 (Guglielmone and Nava, 2010; Krebber Mogollón et al., 2017; Rodríguez-Vivas et al., 2023). This species of tick also occasionally infests various species of birds and mammals, including humans (Nava et al., 2007; Guglielmone and Nava, 2010; Pedrosa-Santos et al., 2020; Bermúdez et al., 2022). It can be dangerous for people and pets because it carries some pathogenic bacteria (e.g., *Rickettsia* da Rocha-Lima, 1916, *Anaplasma* Theiler, 1910, and *Ehrlichia* Moshkovski, 1945) and protozoa of the genus *Hepatozoon* Miller, 1908 (Jongejan, 1992; Paredes-León et al., 2008; Miranda et al., 2012, 2020; Luz et al., 2018; Ogrzewalska et al., 2018; Polo et al., 2020). On Margarita Island, the *A. dissimile* tick was first detected in 1967 in snakes found in vicinities of La Asunción Town (Jones et al., 1972). Lampo and Bayliss (1996) were the first to found the *A. dissimile* parasitism on cane toads here.

The aim of our paper was to analyze, using molecular markers, possible routes of invasion of cane toads and the *A. dissimile* tick to Margarita Island, Venezuela.

MATERIAL AND METHODS

Field observations were carried out by S. N. Litvinchuk and L. F. Litvinchuk from 12 to 22 February 2023 in Costa Caribe Beach Hotel & Resort (11.109 °N 63.961 °W, 6 m a.s.l.), Altigracia, Margarita Island, Venezuela. Herpetological surveys were conducted during walks around the hotel grounds (the route length is about 700 m). Several cane toads were crushed on a road by vehicles serving the hotel. Toes from six of these toads were used as samples for our molecular analysis. In addition, we collected five individuals of ticks attached to these toads for morphological and molecular analyses. The species identification of ticks was carried out on all five specimens based on their external morphology using taxonomic keys for adults and nymphs of species of the genus *Amblyomma* Koch, 1844 (Martins et al., 2010; Guzman-Cornejo et al., 2011). One of the main distinguishing characters of *A. dissimile* is the absence of an internal spur on coxa II and coxa III. After fixation in 70% ethanol, the ticks were examined and measured using a Zeiss Discovery V.8 stereodissecting microscope (Carl Zeiss AG, Oberkochen, Germany).

DNA was extracted from samples of toads and ticks using Magen HiPure Universal DNA Kit (Magen Biotech Co., China). For molecular study, we used sequences of the mitochondrial (mtDNA) cytochrome c oxidase subunit 1 gene (COI) which is commonly used for barcoding. The list of primers (Evrogen, Moscow, Russia) is given in Table 1. The PCR reaction mixture (25 µl) contained 2 – 4 µl of DNA isolated from samples, 0.5 µl of each forward and reverse primer, 12.5 µl of BioMaster HS-Taq PCR-color master mix (Biolabmix, Novosibirsk, Russia), and 9.5 – 7.5 µl of nuclease-free water.

To amplify the COI gene in toads, we used primers VUTF and VUTR (Table 1). The following protocol was applied: 95°C for 3 min, 35 cycles of amplification (95°C for 30 sec, 56°C for 30 sec, 72°C for 1 min), and 72°C for 5 min. To amplify the COI gene in ticks we used universal primers LCO1490 and HCO2198 (Table 1) with

TABLE 1. List of Primers

Primer name	Primer sequence (5' – 3')	Fragment size, bp	Source
LCO1490	GGT CAA CAA ATC ATA AAG ATA TTG G	658	Folmer et al. (1994)
HCO2198	TAA ACT TCA GGG TGA CCA AAA AAT CA		
VUTF	TGT AAA ACG ACG GCC AGT TCT CAA CCA AYC AYA ARG AYA TYG G	600	Lissovsky et al. (2010)
VUTR	CAG GAA ACA GCT ATG ACT ARA CTT CTG GRT GKC CRA ARA AYC A		

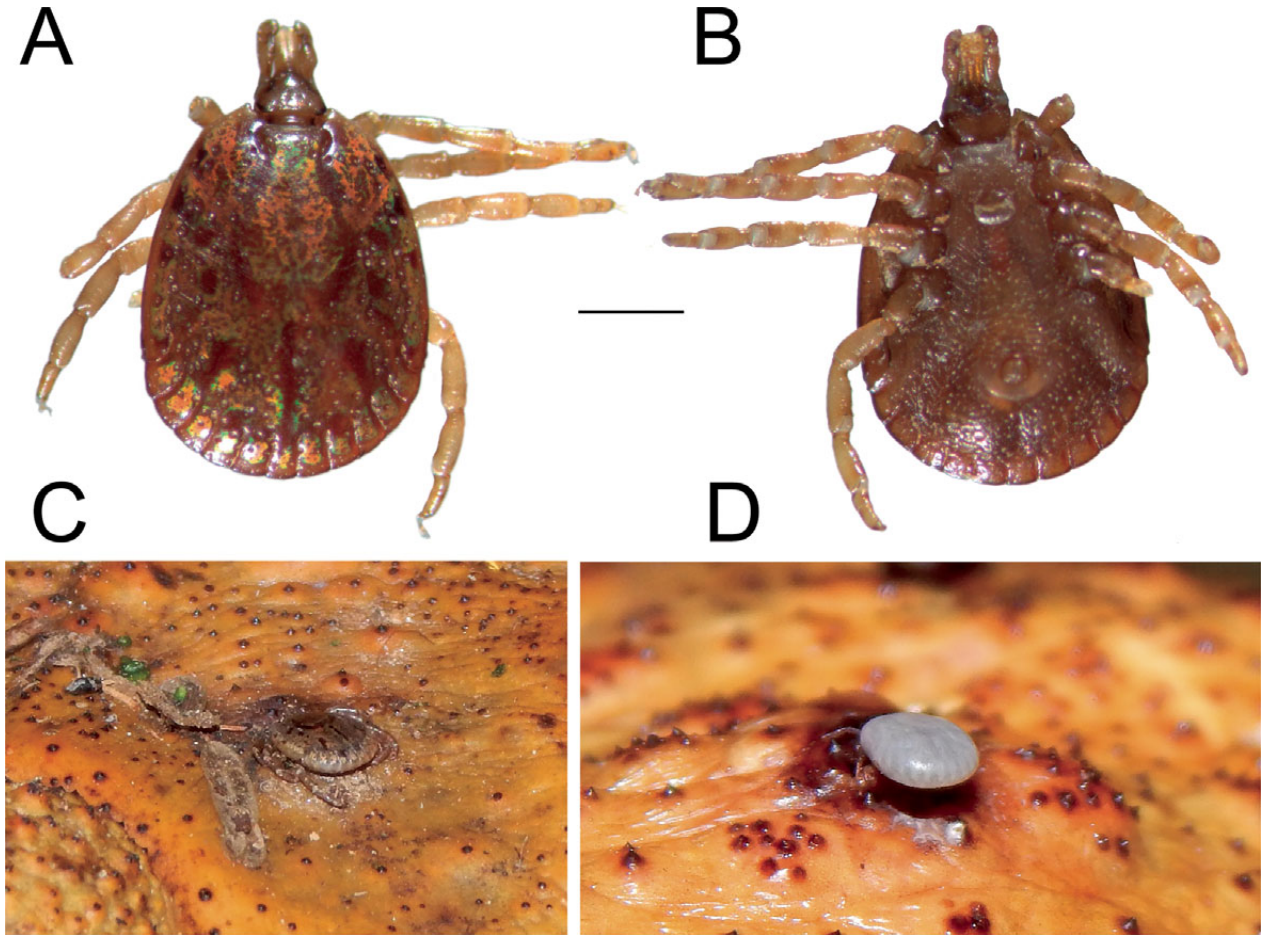


Fig. 1. Adult male of the *Amblyomma dissimile* tick from Margarita Island from dorsal (A) and ventral (B) views. Ticks attached to cane toads (C and D). Scale bar is 1000 μm .

following protocol: 95°C for 5 min, 40 cycles of amplification (94°C for 40 sec, 51°C for 1 min, 72°C for 1 min), and 72°C for 10 min. Nuclease-free water was used as a negative control. Resulting products were analyzed using 1.5% agarose gel with an addition of ethidium bromide and prepared for sequencing by purification using the Magen HiPure Gel DNA Mini Kit (China) and Sanger sequenced bidirectionally at Evrogene (Moscow, Russia) using Applied Biosystems 3500 Genetic Analyzer.

The screening of obtained sequences was performed using the BLAST algorithm available through the National Center for Biotechnology Information (Altschul et al., 1990). Chromatograms were analyzed using Chromas v. 2.6.6. The forward and reverse sequences were assembled in one sequence and aligned to relevant sequences obtained from the GenBank using MUSCLE in MEGA11. Maximum likelihood (ML) was used to estimate the phylogenetic tree. The most suitable evolutionary models were determined in IQ-TREE (Nguyen et al.,

2015) using the Bayesian Information Criterion (BIC) implemented in ModelFinder (Kalyaanamoorthy et al., 2017). The HKY + Γ + I model was identified as the most appropriate evolutionary model for the COI gene fragment in toads and the HKY + Γ model in ticks. ML analyses were performed using the IQ-TREE web server (Trifinopoulos et al., 2016) with 1000 UFBoot iterations (Minh et al., 2013; Hoang et al., 2018). Data were visualized and edited using FigTree v. 1.4. 3 and Inkscape 1.3.2. Haplotype network was constructed using PopART 1.7 (Leigh and Bryant, 2015). All sequences were deposited to the GenBank NCBI under the numbers PV037436-41 in toads and PV037434-5 in ticks (Tables 2 and 3). In our analysis of ticks, we did not use the sequences OR100687 and OR100611 (listed in GenBank as *A. dissimile*) because they actually belong to *A. marmoratum* Koch, 1844, nor the sequences OM912709 and ON138631 because they represent very short fragments.

TABLE 2. GenBank Numbers, Vouchers, and Localities of Toads of the *Rhinella marina* Complex Used in Molecular Analysis

GenBank	Voucher	Locality	Species	Source
PV037438	INCRAS.2023-78	Altagracia, Margarita Island, Venezuela	<i>marina</i>	Present paper
PV037439	INCRAS.2023-79	—»—	—»—	—»—
PV037440	INCRAS.2023-80	—»—	—»—	—»—
PV037441	INCRAS.2023-99	—»—	—»—	—»—
PV037436	INCRAS.2023-100	—»—	—»—	—»—
PV037437	INCRAS.2023-102	—»—	—»—	—»—
MZ675575*	ERS2169570	Oombulgurri, Australia	—»—	Edwards et al. (2018)
MZ895076*	ANWC A02522	Australia	—»—	Hahn et al. (2022)
MT583183	MAP T817	Brazil	<i>diptycha</i>	Koroiva et al. (2020)
MT583184	MAP T208	—»—	—»—	—»—
MT583185	MAP T3166	—»—	—»—	—»—
MW039424	CFBH14171	Pocone, Mato Grosso, Brazil	—»—	Benício et al. (2021)
KU494717	CFBHT08189	Cruz Machado, Parana, Brazil	<i>icterica</i>	Lyra et al. (2017)
KU494718	CFBHT08203	Ortigueira, Parana, Brazil	—»—	—»—
KU494719	CFBHT08162	Blumenau, Santa Catarina, Brazil	—»—	—»—
KU494720	CFBHT01218	Camanducaia, Minas Gerais, Brazil	—»—	—»—
KU494721	CFBHT08141	Quatro Barras, Parana, Brazil	—»—	—»—
KR012546	QCAZ: 50702	San Andres de Rocafuerte, Manabi, Ecuador	<i>bella</i>	Dos Santos et al. (2015)
KR012545	QCAZ: 50698	Puerto Cayo, Manabi, Ecuador	—»—	—»—
KU494706	TG094	Salvador, Bahia, Brazil	<i>crucifer</i>	Lyra et al. (2017)
KU494707	CFBHT09324	Serra dos Órgãos National Park, Rio de Janeiro, Brazil	—»—	—»—
KU494708	CFBHT00868	Santo Antonio do Pinhal, São Paulo, Brazil	—»—	—»—
KU494709	CFBHT06526	Santa Virginia, Sao Luis do Paraitinga, São Paulo, Brasil	—»—	—»—
KU494729	CFBHT14347	Serra da Bocaina, Sao Jose do Barreiro, São Paulo, Brazil	<i>ornata</i>	—»—
KU494730	CFBHT10355	Seropedica, Rio de Janeiro, Brazil	—»—	—»—
KU494731	CFBHT04380	Petropolis, Rio de Janeiro, Brazil	—»—	—»—
KU494732	CFBHT16750	Santa Virginia, Sao Luis do Paraitinga, São Paulo, Brasil	—»—	—»—
KU494733	CFBHT06170	Ilha Bela, São Paulo, Brazil	—»—	—»—
KU494734	CFBHT13389	Juiz de Fora, Minas Gerais, Brazil	—»—	—»—
KU494735	CFBHT11857	Tibagi, Parana, Brazil	—»—	—»—
KU494736	CFBHT09331	Serra dos Órgãos National Park, Rio de Janeiro, Brazil	—»—	—»—
KU494737	CFBHT13067	Trilha do Jequitiba e Arredores, Parque Estadual dos Três Picos, Rio de Janeiro, Brazil	—»—	—»—
KU494738	CFBHT14589	Paranapiacaba, SantoAndre, São Paulo, Brazil	—»—	—»—
MT990584	CFBH38700	Parque Estadual Turistico do Alto Ribeira, Apiai, São Paulo, Brazil	—»—	Benício et al. (2021)
MT888470	CH690-A04	Cenote Azul, Bacalar, Quintana Roo, Mexico	<i>horribilis</i>	Montes-Ortiz and Elias-Gutierrez (2018)
MT888465	CH690-A02	—»—	—»—	—»—
MT888262	CH690-A01	—»—	—»—	—»—
KP149217	AJC 3852	Finca El mirador, Puente Nacional, Santander, Colombia	—»—	Guarnizo et al. (2015)
KP149154	AJC 3850	—»—	—»—	—»—
KP149151	AJC 3851	—»—	—»—	—»—
KP149158	AJC 3877	Reserva Cabildo Verde, Sabana de Torres, Santander, Colombia	—»—	—»—
KP149135	AJC 3895	—»—	—»—	—»—
FJ766819	MVUP: 1802	Cocle, Panama	—»—	Crawford et al. (2010)
KT075329	USNM: Herp: 319280	Colon Island, Panama	—»—	Trizna M. G., Jr. (personal communication)

* Mitochondrion.

TABLE 3. GenBank Numbers, Vouchers, Localities, Hosts, and Developmental Stages Revealed in the Locality of the *Amblyomma dissimile* Tick Used in Molecular Analysis

GenBank	Voucher	Locality	Stage	Host	Source
PV037434	INCRAS.2023-#1	Altagracia, Margarita Island, Venezuela	A, N	<i>Rhinella marina</i> (Anura, Amphibia)	Present paper
PV037435	INCRAS.2023-#3	—»—	—»—	—»—	—»—
MH105049	—	Amapá, Brazil	A	—»—	Luz et al. (2018)
KF200114	CAS-T040	Colon, Barro Colorado Island, Panama	—	<i>Rhinella horribilis</i> (Anura, Amphibia)	Miller et al. (2016)
KF200116	CAS-T037	—»—	—»—	—»—	—»—
KF200117	CAS-T038	—»—	—»—	—»—	—»—
KF200168	CAS-T039	—»—	—»—	—»—	—»—
ON138623	D9	Santander, Colombia	A, N, L	<i>Iguana iguana</i> (Squamata, Reptilia)	Cotes-Perdomo et al. (2022)
ON138625	E9	—»—	—»—	—»—	—»—
ON138626	E10	—»—	—»—	—»—	—»—
ON138627	E11	—»—	—»—	—»—	—»—
ON138629	F4	—»—	—»—	—»—	—»—
ON138631	F9	—»—	—»—	—»—	—»—
ON138632	F10	—»—	—»—	—»—	—»—
ON138633	F11	—»—	—»—	—»—	—»—
ON138635	G4	—»—	—»—	—»—	—»—
ON138624	E3	—»—	—»—	<i>Leptodeira septentrionalis</i> (Squamata, Reptilia)	—»—
ON138628	F3	—»—	—»—	—»—	—»—
ON138634	F13	—»—	A	<i>Pseudoboa newwiedii</i> (Squamata, Reptilia)	—»—
ON138630	F7	—»—	—»—	<i>Boa constrictor</i> (Squamata, Reptilia)	—»—
MF095079	ADN A15	Bonda, Magdalena, Colombia	A, N	—»—	Santodomingo et al. (2018)
MF095087	ADN A62	Santa Marta, Magdalena, Colombia	N, L	<i>Cnemidophorus gagei</i> (Squamata, Reptilia)	—»—
MF095080	ADN A16	—»—	A, N	<i>Epicrates maurus</i> (Squamata, Reptilia)	—»—
MF095084	ADN A26	—»—	—»—	—»—	—»—
MF095083	ADN A24	Tayronaca, Magdalena, Colombia	N, L	<i>Basiliscus basiliscus</i> (Squamata, Reptilia)	—»—
MF095086	ADN A29	Rio Ancho, La Guajira, Colombia	A, N	<i>Trachemys callirostris</i> (Testudines, Reptilia)	—»—
MF095081	ADN A17	—»—	—»—	—»—	—»—
MF095085	ADN A27	—»—	A, N, L	<i>Chelonoidis carbonaria</i> (Testudines, Reptilia)	—»—
MF095082	ADN A18	—»—	—»—	<i>Rhinoclemmys melanosterna</i> (Testudines, Reptilia)	—»—
MF095078	ADN A6	—»—	—»—	—»—	—»—
KF200170	STRI-UP2	Amador, Panama	—	<i>Coccyzus minor</i> (Cuculiformes, Aves)	Miller et al. (2016)
OM912709	DL54	University of Magdalena, Colombia	A, N	Birds	Rodriguez et al. (2023)
OM912710	DL19	—»—	—	—»—	—»—
OM912711	DL89	—»—	—	—»—	—»—
MT000157	Tick 16	La Felisa, Antioquia, Colombia	A	<i>Homo sapiens</i> (Primates, Mammalia)	Ortiz-Giraldo et al. (2021)
MT000158	Tick17	—»—	—»—	—»—	—»—
OK086766	TM1	Magdalena, Colombia	—	<i>Tamandua mexicana</i> (Pilosa, Mammalia)	Rodriguez et al. (2022)
MH513219	dissimile0819	French Guiana	—	From vegetation	Binetruy et al. (2019)
MH513220	dissimile0836	—»—	—»—	—»—	—»—
MF363072	29-36Y1	Casanare, Yopal, Colombia	N, L	—»—	Rivera-Paez et al. (2018)
OR899804* (NC 085535)	T04	University of Magdalena, Santa Marta, Magdalena, Colombia	—	—	Uribe et al. (2024)

* Mitochondrion; A, adults; N, nymphs; L, larvae.

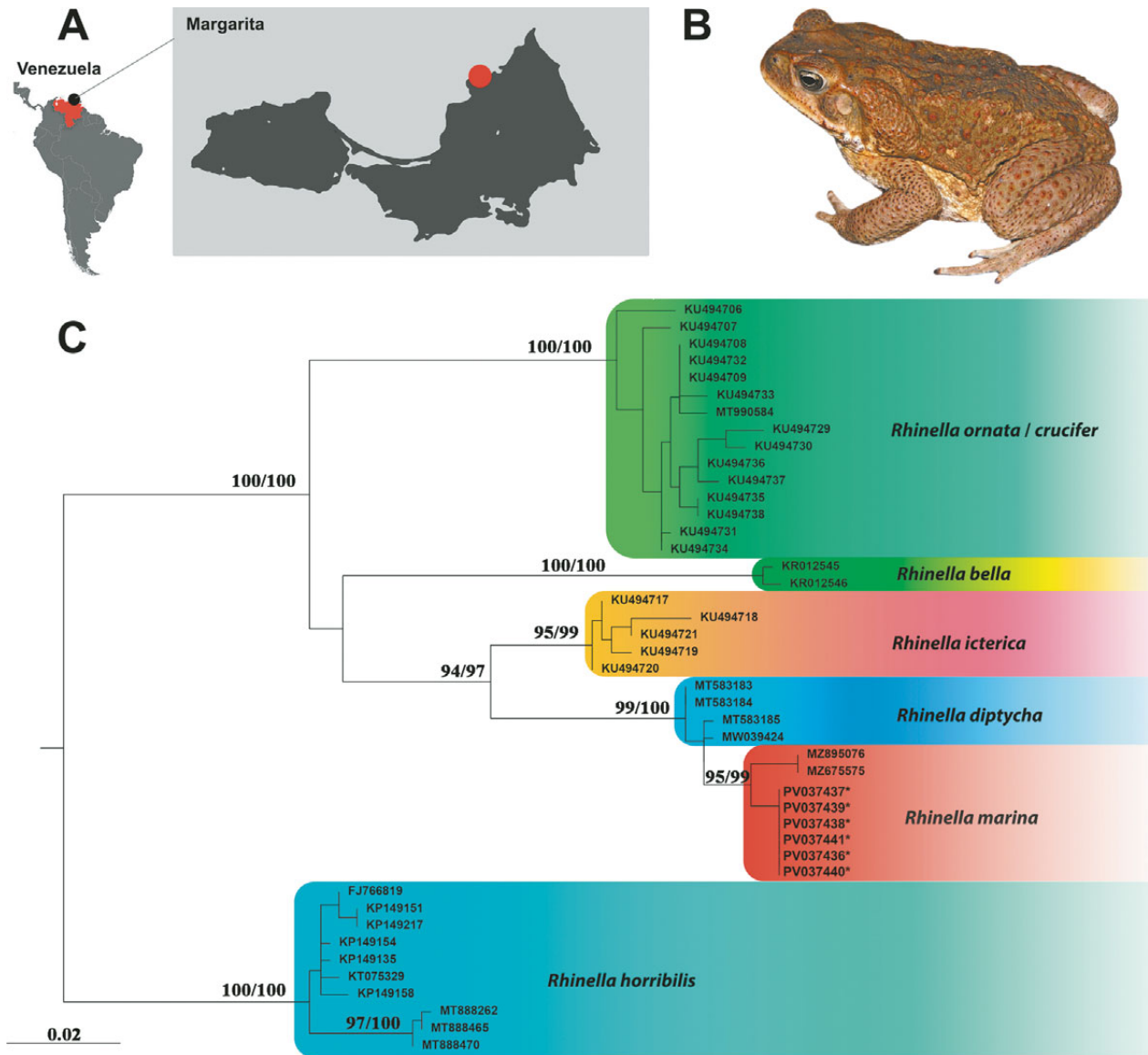


Fig. 2. Phylogenetic tree of species of the *Rhinella marina* complex based on fragments of the COI gene sequences, constructed using the Maximum Likelihood method. Numbers at nodes indicate SH-aLRT ($\geq 80\%$)/UFboot ($\geq 95\%$) supports. Sequences of *R. horribilis* were used as an outgroup. Our sequences are marked with an asterisk (*).

RESULTS

During a 10-day survey (five days and ten nights), we observed a total of 22 adult (including roadkill) and 52 yearling *R. marina* (maximum 7 adults and 10 juveniles per day; average 7.4 and range 1–12 adults and juveniles per day). A very cursory examination of adult cane toads revealed that about half of them had attached ticks, while only two juveniles were infested. Some adult toads had up to eight ticks at a time. Juveniles always had only one tick, which was attached to the head between eyes.

Beside cane toads, two additional species of amphibians were observed: 13 *Pleurodema brachyops* (Cope, 1869) and four *Leptodactylus fuscus* (Schneider, 1799). Among reptiles, lizards *Hemidactylus garnotii* Duméril et Bibron, 1836 and *Tropidurus hispidus* Spix, 1825 were numerous at night and during the day (respectively). Additionally, we observed at night eight *Gonatodes vittatus* Lichtenstein et Martens, 1856, and during the day two *Iguana iguana* Linnaeus, 1758, one *Ameiva bifrontata* Cope, 1862, four *Leptodeira annulata* Linnaeus, 1758, and one *Chelonoidis carbonarius* Spix, 1824. No ticks

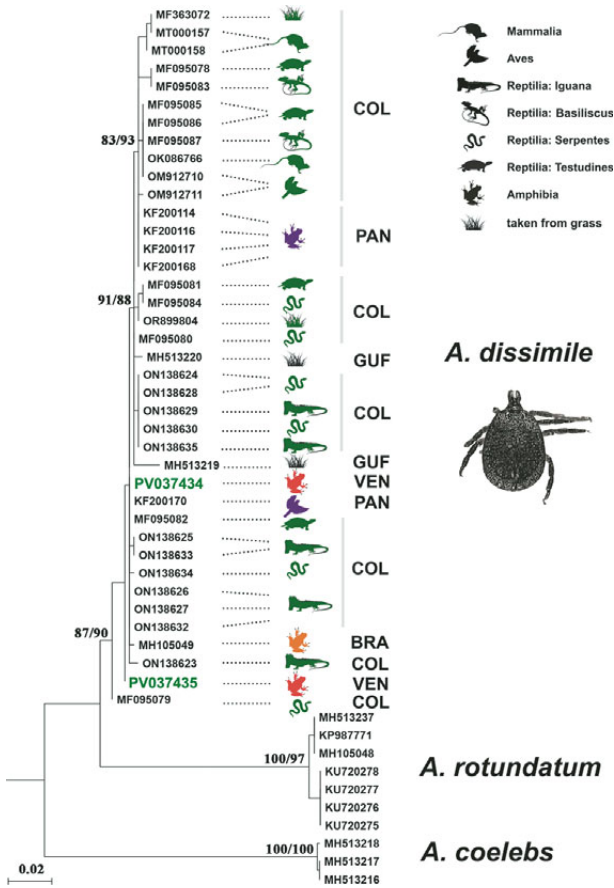


Fig. 3. Phylogenetic tree of *Amblyomma* ticks based on fragments of the COI gene sequences and constructed using the Maximum Likelihood method. Sequences of *Haemaphysalis kitaokai* (OP107280, OP935901, OP935902) were used as an outgroup. Numbers at nodes indicate SH-aLRT ($\geq 80\%$)/UFboot ($\geq 95\%$) supports. Our sequences are in green. COL is Colombia, BRA is Brazil, GUF is French Guiana, PAN is Panama, and VEN is Venezuela.

(except for chiggers on *Hemidactylus*) were found on these species of amphibians and reptiles.

Of *A. dissimile* ticks we studied, one was an adult male (Fig. 1) and the other four were nymphs. Morphological characteristics of nymphs were as follows: a body length ($n = 4$) ranged from 2271 to 5070 μm (mean \pm SE: 3415.8 ± 620.5); a body width was 1583 – 3709 μm (2373.5 ± 474.6); a gnathosoma length was 386 – 505 μm (437.8 ± 25.5); a gnathosoma width was 332 – 370 μm (350.3 ± 8.7); a scutum length was 101 – 553 μm (436.5 ± 111.8); and a scutum width was 844 – 1003 μm (911.5 ± 36.5). The male has the body length of 3540 μm , the body width of 2764 μm , the gnathosoma length of 848 μm , the gnathosoma width of 698 μm , a scutum length of 2930 μm , and the scutum width of 2348 μm .

Comparison of our COI sequences of cane toads with data from the NCBI database showed that only two Australian samples (MZ675575 and MZ895076) of *R. marina* were compatible. Two other COI sequences of the species from Brazil and Peru (KM024312 and EF532267, respectively) belong to a different part of the gene and do not completely overlap with our sequences. Comparison of our samples with *R. marina* from Australia (the species was introduced there in 1935 from French Guiana, successively via Martinique, Barbados, Jamaica, Puerto Rico and Hawaii; Selechnik et al., 2017) and other cane toad species showed that our samples are most closely related to *R. marina* (Fig. 2). All six specimens had identical sequences and showed seven nucleotide substitutions compared to Australian ones, corresponding to 98.9% similarity (634 out of 641 bp). The uncorrected genetic distance between cane toads from Margarita Island and those from Australia was 0.0125. Nucleotide diversity of the species was low ($P_i = 0.005$), as well as haplotype diversity ($H_d = 0.43 \pm 0.029$). The average number of pairwise nucleotide differences (k) was 3.429, and a total of eight polymorphic sites were identified in the species.

Molecular analysis of tick samples from Margarita Island revealed that one specimen belongs to the *A. dissimile* clade, while the second some differs from previously studied specimens of this species (Fig. 3). Both our samples were closely related to *A. dissimile* ticks collected from turtles and iguanas in Colombia (MF095082, ON138626–7, and ON138632) and from a bird in Panama (KF200170). One of our samples (PV037434) had sequences identical to these, but another sample (PV037434) differed from them by only one substitution (Fig. 4; 532 out of 533 bp, i.e., 99.8%).

A total of 19 different *A. dissimile* haplotypes were identified in samples of the species collected in Colombia, Panama, Venezuela, Brazil, and French Guiana. The most frequently encountered haplotypes were 1 (Panama), 2 (Colombia, Panama and Venezuela), 7 and 15 (Colombia) (Fig. 4). Our samples belong to the widespread haplotype 2 and the unique haplotype 18, revealed only on Margarita Island. We found no relationship between haplotypes and their distribution, as well as their preference for various hosts. Analysis of genetic variation among populations revealed relatively low levels of genetic diversity ($P_i = 0.0062$), but the haplotype diversity (H_d) was assessed at 0.939 ± 0.0004 , suggesting a high degree of genetic diversity within the species. The mean number of pairwise nucleotide differences (k) was found to be 3.277, and a total of 25 polymorphic sites were identified.

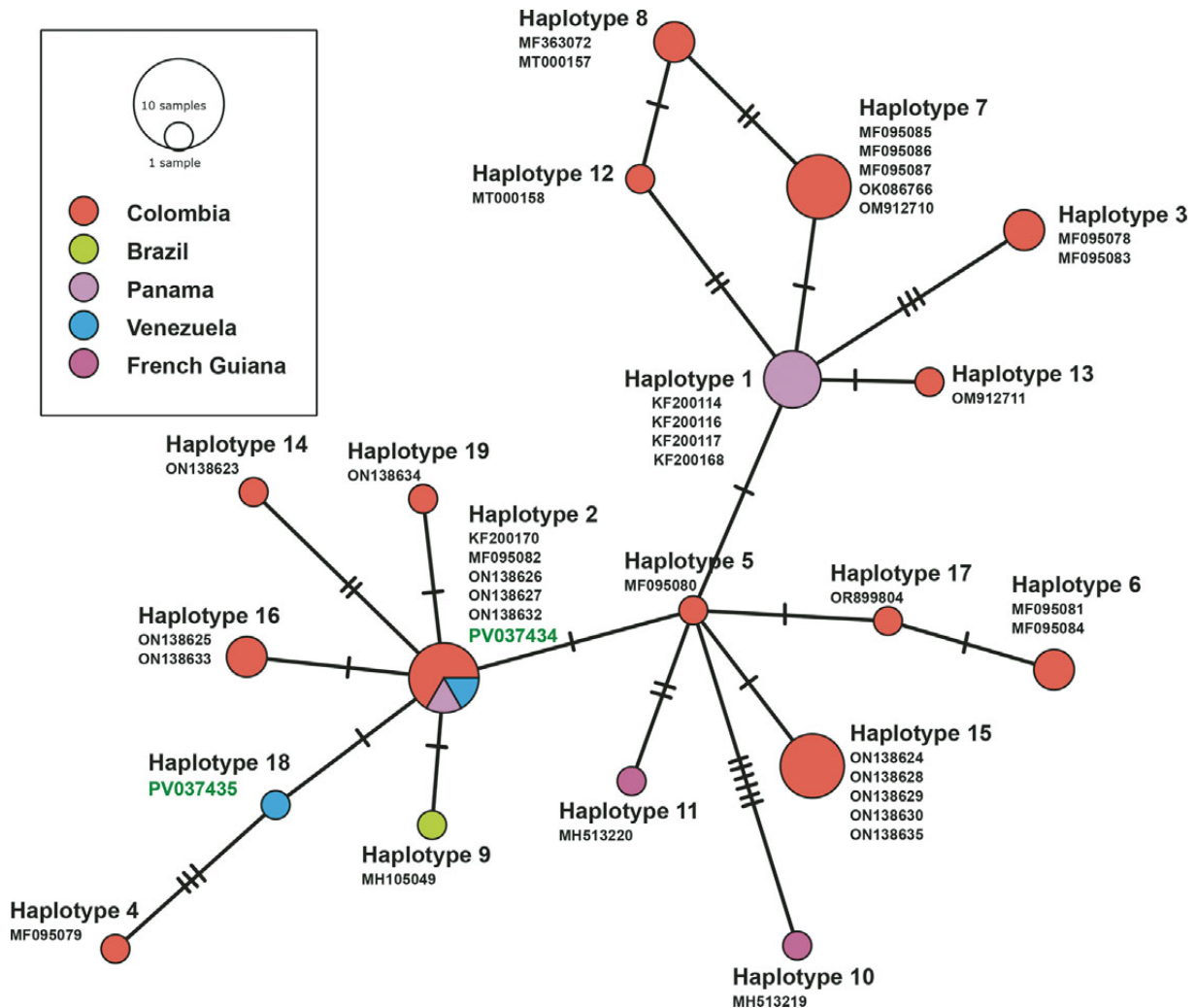


Fig. 4. Haplotype network of the *Amblyomma dissimile* tick based on fragments of the COI gene sequences. Our samples are in green.

DISCUSSION

The origin of the cane toad population on Margarita Island is still unknown. Lampo and Bayliss (1996) considered this population to be indigenous, but Jugueto and Rivas (2010) suggest that it is a recent introduction from mainland Venezuela. Results of our study support the latter authors, as we did not find any genetic variability here, which may indicate that this population arose relatively recently and is going through a “bottleneck” period. Furthermore, it should be noted that we have genetically confirmed for the first time that the local population is indeed *R. marina*, a species found throughout most of mainland Venezuela (Acevedo et al., 2016). Although populations from this country have not yet been studied using COI gene sequencing, our data clearly indicate that

the population of *R. marina* from Margarita Island is closely related the cane toad from Australia (originally from French Guiana).

It is also unclear how the cane toad population arose on Margarita Island. They may have arrived by swimming or floating down the sea channel between the island and the mainland, or they may have been introduced by humans. Indeed, overseas dispersal has been documented in several amphibian groups (Vences et al., 2003). In addition, it is known that cane toads living on sea coasts (the average salinity of the Caribbean Sea at Margarita Island is 38.3‰; Pereira-Ibarra and López-Monroy, 2021) have increased tolerance to salty seawater (for example, larvae of the closely related species *R. horribilis* can tolerate salinity up to 27.5‰; De León and Castillo, 2015) and can therefore stay in seawater for a long time. However, the sea strait between Margarita Island and

mainland is quite wide, which makes it more likely that these toads were introduced to the island by humans, either intentionally or accidentally.

Usually, parasites can also provide some information about migrations of their hosts (Barton, 1997; Dubey and Shine, 2008; Hartigan et al., 2010). However, *A. dissimile* ticks are usually absent from invasive populations of *R. marina* (perhaps, except on Española Island; Jackowska, 1972). This is probably due to conditions and duration of transportation of these toads, since after a few days ticks leave their hosts. In this sense, the population of invasive cane toads on Margarita Island, infested with these ticks, is an exception. This may be due to the fact that *A. dissimile* can travel long distances, for example, on birds (Martínez-Sánchez et al., 2020). In addition, ticks of the genus *Amblyomma* can be carried, for example, on reptiles imported to zoos or private collections (Lampo et al., 1998; González-Acuña et al., 2005; De Jesus et al., 2024). Invasion of ticks due to transportation of reptiles was observed, for example, in Hawaii (Kelehear et al., 2017), where *A. rotundatum* were accidentally introduced to a local zoo and appeared in the wild long after *R. marina* had been introduced there.

Amblyomma dissimile ticks have probably been present on Margarita Island longer than cane toads. This may be supported by the fact that these ticks were known here before the arrival of *R. marina* (first records in 1967 and 1993, respectively). Our genetic analysis supports this hypothesis, since a unique haplotype was detected in the local tick population. This assumption is also supported by differences in allozyme allele frequencies that have previously been found between populations from this island and mainland Venezuela (Lampo et al., 1998). Therefore, we speculate that the origin of the local tick population may be related to migrations of hosts other than toads, such as reptiles, birds or domestic mammals.

Acknowledgments. This research was funded in part by the Budgetary Program # FMFU-2024–0012 at the Institute of Cytology RAS. We are very grateful to L. F. Litvinchuk and D. V. Skorinov for their assistance in conducting research.

REFERENCES

- Acevedo A. A., Lampo M., and Cipriani R. (2016), “The cane or marine toad, *Rhinella marina* (Anura, Bufonidae): two genetically and morphologically distinct species,” *Zootaxa*, **4103**(6), 574 – 586.
- Altschul S. F., Gish W., Miller W., Myers E. W., and Lipman D. J. (1990), “Basic local alignment search tool,” *J. Mol. Biol.*, **215**, 403 – 410.
- Barton D. P. (1997), “Introduced animals and their parasites: the cane toad, *Bufo marinus*, in Australia,” *Austral. J. Ecol.*, **22**, 316 – 324.
- Benavides-Montaño J. A., Jaramillo-Cruz C. A., and Mesa-Cobo N. C. (2018), “Garrapatas Ixodidae (Acari) en el Valle del Cauca, Colombia,” *Bol. Cient. Centro Mus., Mus. Hist. Nat.*, **22**(1), 131 – 150.
- Benicio R. A., Provete D. B., Lyra M. L., Heino J., Haddad C. F. B., de Rossa-Feres C. D., and da Silva F. R. (2021), “Differential speciation rates, colonization time and niche conservatism affect community assembly across adjacent biogeographical regions,” *J. Biogeogr.*, **48**, 2211 – 2225.
- Bermúdez S. E., Domínguez L., Troyo A., and Venzal J. M. (2022), “Ticks infesting humans in Central America: a review of their relevance in public health,” *Curr. Res. Parasit. Vector-Borne Dis.*, **2**, 100065.
- Bessa-Silva A., Vallinoto M., Sampaio I., Flores-Villela O. A., Smith E. N., and Sequeira F. (2020), “The roles of vicariance and dispersal in the differentiation of two species of the *Rhinella marina* species complex,” *Mol. Phylog. Evol.*, **145**, 106723.
- Binetruy F., Chevillon C., de Thoisy B., Garnier S., and Duron O. (2019), “Survey of ticks in French Guiana,” *Ticks Tick-borne Dis.*, **10**(1), 77 – 85.
- Bodkin G. E. (1918), “The biology of *Amblyomma dissimile* Koch,” *Parasitology*, **11**, 10 – 17.
- Cotes-Perdomo A., Cárdenas-Carreño J., Hoyos J., González C., and Castro L. R. (2022), “Molecular detection of *Candidatus Rickettsia colombianensi* in ticks (Acari, Ixodidae) collected from herpetofauna in San Juan de Carare, Colombia,” *Int. J. Parasitol. Parasites Wildl.*, **19**, 110 – 114.
- Crawford A. J., Lips K. R., and Bermingham E. (2010), “Epidemic disease decimates amphibian abundance, species diversity, and evolutionary history in the highlands of central Panama,” *Proc. Natl. Acad. Sci. USA*, **107**(31), 13777 – 13782.
- De Jesus C. E., Harman M. E. A., Sutton A., Bredin S., Romagosa C. M., and Wisely S. M. (2024), “Spatially limited pathogen pollution in an invasive tick and host system,” *Biol. Invasions*, **26**, 2037 – 2047.
- De León L. F. and Castillo A. M. (2015), “*Rhinella marina* (Cane Toad). Salinity tolerance,” *Herpetol. Rev.*, **46**(2), 237 – 238.
- Delvignier B. L. J. and Freeland W. J. (1988), “Protozoan parasites of the cane toad, *Bufo marinus*, in Australia,” *Austral. J. Zool.*, **36**(3), 301 – 316.
- Dos Santos S. P., Ibáñez R., and Ron S. R. (2015), “Systematics of the *Rhinella margaritifera* complex (Anura, Bufonidae) from western Ecuador and Panama with insights in the biogeography of *Rhinella alata*,” *Zookeys*, **501**, 109 – 145.
- Dubey S. and Shine R. (2008), “Origin of the parasites of an invading species, the Australian cane toad (*Bufo marinus*): are the lungworms Australian or American?” *Mol. Ecol.*, **17**, 4418 – 4424.
- Edwards R. J., Tuipulotu D. E., Amos T. G., O’Meally D., Richardson M. F., Russell T. L., Vallinoto M., Carneiro M., Ferrand N., Wilkins M. R., Sequeira F., Rollins L. A., Holmes E. C., Shine R., and White P. A. (2018), “Draft genome assembly of the invasive cane toad, *Rhinella marina*,” *Gigascience*, **7**(9), giy095.

- Folmer O., Black M., Hoeh W., Lutz R., and Vrijenhoek R. (1994), "DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates," *Mol. Marine Biol. Biotech.*, **3**, 294 – 299.
- González-Acuña D., Beldoménico P. M., Venzal J. M., Fabry M., Keirans J. E., and Guglielmona A. A. (2005), "Reptile trade and the risk of exotic tick introductions into southern South American countries," *Exp. Appl. Acarol.*, **35**, 335 – 339.
- Guarnizo C. E., Paz A., Muñoz-Ortiz A., Flechas S. V., Méndez-Narváez J., and Crawford A. J. (2015), "DNA barcoding survey of anurans across the Eastern Cordillera of Colombia and the impact of the Andes on cryptic diversity," *PLoS ONE*, **10**(5), e0127312.
- Guglielmona A. A. and Nava S. (2010), "Hosts of *Amblyomma dissimile* Koch, 1844 and *Amblyomma rotundatum* Koch, 1844 (Acari: Ixodidae)," *Zootaxa*, **2541**, 27 – 49.
- Guzman-Cornejo C., Robbins R. G., Guglielmona A. A., Montiel-Parra G., and Pérez T. M. (2011), "The *Amblyomma* (Acari: Ixodida: Ixodidae) of Mexico: identification keys, distribution and hosts," *Zootaxa*, **2998**, 16 – 38.
- Hahn E. E., Alexander M. R., Grealy A., Stiller J., Gardner D. M., and Holleley C. E. (2022), "Unlocking inaccessible historical genomes preserved in formalin," *Mol. Ecol. Resour.*, **22**(6), 2130 – 2147.
- Hartigan A., Phalen D. N., and Šlapeta J. (2010), "Museum material reveals a frog parasite emergence after the invasion of the cane toad in Australia," *Paras. Vectors*, **3**, 50.
- Hoang D. T., Chernomor O., von Haeseler A., Minh B. Q., and Vinh L. S. (2018), "UFBoot2: improving the ultrafast bootstrap approximation," *Mol. Biol. Evol.*, **35**, 518 – 522.
- Jackowska S. (1972), "Lesions produced by ticks *Amblyomma dissimile* in *Bufo marinus* toads from the Dominican Republic," *Am. Zool.*, **12**, 731.
- Jones E. K., Clifford C. M., Keirans J. E., and Kohls G. M. (1972), "The ticks of Venezuela (Acarina: Ixodoidea) with a key to the species of *Amblyomma* in the Western Hemisphere," *Brigham Young Univ. Sci. Bull. Biol. Ser.*, **27**(4), 1 – 40.
- Jongejan F. (1992), "Experimental transmission of *Cowdria ruminatum* (Rickettsiales) by the American reptile tick *Amblyomma dissimile* Koch, 1844," *Exp. Appl. Acar.*, **15**, 117 – 121.
- Kalyaanamoorthy S., Minh B. Q., Wong T. K. F., von Haeseler A., and Jermini L. S. (2017), "ModelFinder: fast model selection for accurate phylogenetic estimates," *Nat. Methods*, **14**, 587 – 589.
- Kelehear C., Hudson C. M., Mertins J. W., and Shine R. (2017), "First report of exotic ticks (*Amblyomma rotundatum*) parasitizing invasive cane toads (*Rhinella marina*) on the Island of Hawai'i," *Ticks Tick-borne Dis.*, **8**, 330 – 333.
- Koroiva R., Rodrigues L. R. R., and Santana D. J. (2020), "DNA barcoding for identification of anuran species in the central region of South America," *PeerJ*, **8**, e10189.
- Krebber Mogollón H. R., Mestra Pineda A., Herrera Benavides Y. M., Causil Vargas L. (2017), "*Amblyomma dissimile* en *Boa constrictor* en cautiverio del Centro de Atención y Valoración de Fauna Silvestre de Montería (Córdoba, Colombia)," *Rev. Med. Vet.*, **2017**(35), 29 – 34.
- Lampo M. and Bayliss P. (1996), "Density estimates of cane toads from native populations based on mark-recapture data," *Wildlife Res.*, **23**, 305 – 315.
- Lampo M. and Medialdea V. (1996), "Allocation patterns in *Bufo marinus* from two habitats in Venezuela," *J. Trop. Ecol.*, **12**, 321 – 331.
- Lampo M., Rangel Y., and Mata A. (1998), "Population genetic structure of a three-host tick, *Amblyomma dissimile*, in eastern Venezuela," *J. Parasitol.*, **84**(6), 1137 – 1142.
- Leigh J. W. and Bryant D. (2015), "POPART: full-feature software for haplotype network construction," *Methods Ecol. Evol.*, **6**, 1110 – 1116.
- Lever C. (2001), *The Cane Toad: The History and Ecology of a Successful Colonist*, Westbury Academic and Scientific Publishing, Otley, West Yorkshire.
- Lissovsky A. A., Obolenskaya E. V., Abramson N. I., Dokuchaev N. E., Yakimenko V. V., Mal'kova M. G., Bogdanov A. S., and Ivanova N. V. (2010), "Geographic variation of *Microtus middendorffii* (Cricetidae, Arvicolinae, Rodentia) sensu lato studied by craniometrical and mitochondrial features," *Russ. J. Theriol.*, **9**(2), 71 – 81.
- Lowe S., Browne M., Boudjelas S., and De Poorter M. (2000), *100 of the World's Worst Invasive Alien Species A selection from the Global Invasive Species Database*, The Invasive Species Specialist Group (ISSG) a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN).
- Luz H. R., Silva-Santos E., Costa-Campos C. E., Acosta I., Martins T. F., Muñoz-Leal S., McIntosh D., Faccini J. L. H., and Labruna M. B. (2018), "Detection of *Rickettsia* spp. in ticks parasitizing toads (*Rhinella marina*) in the northern Brazilian Amazon," *Exp. Appl. Acarol.*, **75**(3), 309 – 318.
- Lyra M. L., Haddad C. F. B., and de Azeredo-Espin A. M. L. (2017), "Meeting the challenge of DNA barcoding Neotropical amphibians: polymerase chain reaction optimization and new COI primers," *Mol. Ecol. Resour.*, **17**(5), 966 – 980.
- Maciel N. M., Collevatti R. G., Colli G. R., and Schwartz E. F. (2010), "Late Miocene diversification and phylogenetic relationships of the huge toads in the *Rhinella marina* (Linnaeus, 1758) species group (Anura: Bufonidae)," *Mol. Phylog. Evol.*, **57**, 787 – 797.
- Martins T. F., Onofrio V. C., Barros-Battesti D. M., and Labruna M. B. (2010), "Nymphs of the genus *Amblyomma* (Acari: Ixodidae) of Brazil: descriptions, redescription, and identification key," *Ticks Tick-borne Dis.*, **1**, 75 – 99.
- Martínez-Sánchez E. T., Cardona-Romero M., Ortiz-Giraldo M., Tobón-Escobar W. D., López D. M., Ossa-López P. A., Pérez-Cárdenas J. E., Labruna M. B., Martins T. F., Rivera-Páez F. A., and Castaño-Villa G. J. (2020), "Associations between wild birds and hard ticks (Acari: Ixodidae) in Colombia," *Ticks Tick-borne Dis.*, **11**(6), 101534.
- Menéndez-Guerrero P. A., dos Santos S. P. L., Salazar-Nicholls M.-J., Green D. M., and Ron S. R. (2024), "Cryptic diversity in toads of the *Rhinella marina* species group (Anura, Bufonidae) with a subjectively beautiful new spe-

- cies from Western Ecuador,” *Zool. J. Linn. Soc.*, **202**, zlad197.
- Miller M. J., Esser H. J., Loaiza J. R., Herre E. A., Aguilar C., Quintero D., Alvarez E., and Bermingham E. (2016), “Molecular ecological insights into Neotropical bird-tick interactions,” *PLoS ONE*, **11**(5), e0155989.
- Minh B. Q., Nguyen M. A. T., and von Haeseler A. (2013), “Ultrafast approximation for phylogenetic bootstrap,” *Mol. Biol. Evol.*, **30**, 1188 – 1195.
- Miranda J., Portillo A., Oteo J. A., and Mattar S. (2012), “*Rickettsia* sp. strain *colombianensi* (Rickettsiales: Rickettsiaceae): a new proposed *Rickettsia* detected in *Amblyomma dissimile* (Acari: Ixodidae) from iguanas and free-living larvae ticks from vegetation,” *J. Med. Entomol.*, **49**, 960 – 965.
- Miranda J., Violet-Lozano L., Barrera S., Mattar S., Monsalve-Buriticá S., Rodas J., and Contreras V. (2020), “Candidatus *Rickettsia colombianensi* in ticks from reptiles in Córdoba, Colombia,” *Vet. World*, **13**(9), 1764 – 1770.
- Montes-Ortiz L. and Elías-Gutiérrez M. (2018), “Faunistic survey of the zooplankton community in an oligotrophic Sinkhole, Cenote Azul (Quintana Roo, Mexico), using different sampling methods, and documented with DNA barcodes,” *J. Limnol.*, **77**, 428 – 440.
- Murphy J. C. (1997), *Amphibians and Reptiles of Trinidad and Tobago*, Krieger Publishing, Malabar.
- Müller L. and Hellmich W. (1936), “Amphibien und Reptilien. I. Teil: Amphibia, Chelonia, Loricata,” in: *Wissenschaftliche Ergebnisse der Deutschen Gran Chaco-Expedition*, Strecker and Schröder, Stuttgart, pp. 1 – 120.
- Nava S., Lareschi M., Rebollo C., Usher C. B., Beati L., Robbins R. G., Durden L. A., Mangold A. J., and Guglielmo A. A. (2007), “The ticks (Acari: Ixodida: Argasidae, Ixodidae) of Paraguay,” *Ann. Trop. Med. Parasit.*, **101**, 255 – 270.
- Nguyen L.-T., Schmidt H. A., von Haeseler A., and Minh B. Q. (2015), “IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies,” *Mol. Biol. Evol.*, **32**, 268 – 274.
- Ogrzewalska M., Machado C., Rozental T., Forneas D., Cunha L. E., and De Lemos E. R. S. (2019), “Microorganisms in the ticks *Amblyomma dissimile* Koch 1844 and *Amblyomma rotundatum* Koch 1844 collected from snakes in Brazil,” *Med. Vet. Entomol.*, **33**, 154 – 161.
- Ortiz-Giraldo M., Tobón-Escobar W. D., Velásquez-Guarín D., Usma-Marín M. F., Ossa-López P. A., Ramírez-Chaves H. E., Carvajal-Agudelo J. D., and Rivera-Páez F. A. (2021), “Ticks (Acari: Ixodoidea) associated with mammals in Colombia: a historical review, molecular species confirmation, and establishment of new relationships,” *Parasitol. Res.*, **120**(2), 383 – 394.
- Paredes-León R., García-Prieto L., Guzmán-Cornejo C., León Règnon V., and Pérez T. M. (2008), “Metazoan parasites of Mexican amphibians and reptiles,” *Zootaxa*, **1904**, 1 – 166.
- Pedroso-Santos F., da Silva Santos E., Sanches P. R., Costa-Campos C. E., Luz H. R., and Faccini J. L. H. (2020), “First record of *Amblyomma dissimile* (Acari: Ixodidae) infesting the granular toad *Rhinella* major (Anura: Bufonidae) in the Eastern Amazon region,” *Herpetol. Notes*, **13**, 385 – 387.
- Pereira-Ibarra C. and López-Monroy F. (2021), “Trophic status of a tropical coastal lagoon in Margarita Island, Venezuela,” *Rev. Int. Cont. Ambiental*, **37**, 611 – 622.
- Pereyra M. O., Blotto B. L., Baldo D., Chaparro J. C., Ron S. R., Elias-Costa A. J., Iglesias P. P., Venegas P. J., Thomé M. T. C., Ospina-Sarria J. J., Maciel N. M., Rada M., Kolenc F., Borteiro C., Rivera-Correa M., Rojas-Runjaic F. J. M., Moravec J., De La Riva I., Wheeler W. C., Castroviejo-Fisher S., Grant T., Haddad C. F. B., and Faivovich J. (2021), “Evolution in the genus *Rhinella*: a total evidence phylogenetic analysis of Neotropical true toads (Anura: Bufonidae),” *Bull. Am. Mus. Nat. Hist.*, **447**, 1 – 155.
- Polo G., Luz H. R., Regolin A. L., Martins T. F., Winck G. R., Da Silva H. R., Onofrio V. C., Labruna M. B., and Faccini J. L. H. (2021), “Distribution modeling of *Amblyomma rotundatum* and *Amblyomma dissimile* in Brazil: estimates of environmental suitability,” *Parasitol. Res.*, **120**, 797 – 806.
- Reaser J. K., Meyerson L. A., Cronk Q., De Poorter M., Eldrege L. G., Green E., Kairo M., Latasi P., Mack R. N., Mauremootoo J., O’Dowd D., Orapa W., Sastroutomo S., Saunders A., Shine C., Thrainsson S., and Vaiutu L. (2007), “Ecological and socioeconomic impacts of invasive alien species in island ecosystems,” *Environ. Conserv.*, **34**(2), 98 – 111.
- Rivas G. F., Ugueto G., Rivero R., and Miralles A. (2005), “The herpetofauna of Isla de Margarita, Venezuela: new records and comments,” *Caribbean J. Sci.*, **41**, 346 – 351.
- Rivera D., Prates I., Firreno T. J., Jr., Rodrigues M. T., Caldwell J. P., and Fujita M. K. (2022), “Phylogenomics, introgression, and demographic history of South American true toads (*Rhinella*),” *Mol. Ecol.*, **31**, 978 – 992.
- Rivera-Páez F. A., Labruna M. B., Martins T. F., Perez J. E., Castaño-Villa G. J., Ossa-López P. A., Gil C. A., Sampieri B. R., Aricapa-Giraldo H. J., and Camargo-Mathias M. I. (2018), “Contributions to the knowledge of hard ticks (Acari: Ixodidae) in Colombia,” *Ticks Tick-borne Dis.*, **9**(1), 57 – 66.
- Rodriguez M. M., Oviedo A., Flores F. S., and Castro L. R. (2022), “First report of *Ornithodoros puertoricensis* (Argasidae) and *Amblyomma dissimile* (Ixodidae) on *Tamandua mexicana* (Myrmecophagidae),” *Syst. Appl. Acarol.*, **27**(8), 1495 – 1499.
- Rodriguez M. M., Oviedo A., Flores S. F., Tamaris-Turizo D. P., and Castro L. R. (2023), “Molecular detection of *Rickettsia* and other bacteria in ticks and birds in an urban fragment of tropical dry forest in Magdalena, Colombia,” *Life, Basel*, **13**(1), 145.
- Rodriguez-Vivas R. I., Ojeda-Chi M. M., Torres-Castro M. A., Sánchez-Montes S., Panti-May A., and Reyes-Novelo E. (2022), “*Amblyomma dissimile* (Acari: Ixodidae): Garrapata de anfibios y reptiles,” *Bioagrobiencias*, **15**, 55 – 64.
- Roze J. A. (1964), “La herpetología de la Isla de Margarita,” *Mem. Soc. Cienc. Nat. Salle*, **69**, 209 – 241.
- Russo A. G., Harding E. F., Yan G. J. H., Selechnik D., Ducatez S., DeVore J. L., Zhou J., Sarma R. R.,

- Lee Y. P., Richardson M. F., Shine R., Rollins L. A., and White P. A. (2021), "Discovery of novel viruses associated with the invasive cane toad (*Rhinella marina*) in its native and introduced ranges," *Front. Microbiol.*, **12**, 733631.
- Santodomingo A., Cotes-Perdomo A., Foley J., and Castro L. R. (2018), "Rickettsial infection in ticks (Acari: Ixodidae) from reptiles in the Colombian Caribbean," *Ticks Tick-borne Dis.*, **9**, 623 – 628.
- Schumaker T. T. S. and Barros D. M. (1994), "Notes on the biology of *Amblyoma dissimile* Koch, 1844 (Acaria: Ixodidae) on *Bufo marinus* (Linnaeus, 1758) from Brasil," *Mem. Inst. Oswaldo Cruz*, **89**(10), 29 – 31.
- Selechnik D., Rollins L. A., Brown G. P., Kelehear C., and Shine R. (2017), "The things they carried: the pathogenic effects of old and new parasites following the intercontinental invasion of the Australian cane toad (*Rhinella marina*)," *Int. J. Parasitol. Parasites Wildlife*, **6**, 375 – 385.
- Sequeira F., Sodr  D., Ferrand N., Bernardi J. A. R., Sampaio I., Schneider H., and Vallinoto M. (2011), "Hybridization and massive mtDNA unidirectional introgression between the closely related Neotropical toads *Rhinella marina* and *R. schneideri* inferred from mtDNA and nuclear markers," *BMC Evol. Biol.*, **11**, 264.
- Trifinopoulos J., Nguyen L.-T., von Haeseler A., and Minh B. Q. (2016), "W-IQ-TREE: a fast online phylogenetic tool for maximum likelihood analysis," *Nucl. Acids Res.*, **44**, W232 – W235.
- Ugueto G. N. and Rivas G. A. (2010), *Amphibians and Reptiles of Margarita, Coche and Cubagua*, Chimaira, Frankfurt am Main.
- Uribe J. E., Kelava S., Nava S. Cotes-Perdomo A. P., Castro L. R., Rivera-Pa z F. A., Perea S., Mans B. J., Gofton A., Teo E. J. M., Zardoya R., and Barker S. C. (2024), "New insights into the molecular phylogeny, biogeographical history, and diversification of *Amblyomma* ticks (Acari: Ixodidae) based on mitogenomes and nuclear sequences," *Parasit. Vectors*, **17**, 139.
- Vallinoto M., Sequeira F., Sodr  D., Bernardi J. A. R., Sampaio I., and Schneider H. (2010), "Phylogeny and biogeography of the *Rhinella marina* species complex (Amphibia, Bufonidae) revisited: implications for Neotropical diversification hypotheses," *Zool. Scripta*, **39**, 128 – 140.
- Vallinoto M., Cunha D. B., Bessa-Silva A., Sodr  D., and Sequeira F. (2017), "Deep divergence and hybridization among sympatric Neotropical toads," *Zool. J. Linn. Soc.*, **180**, 647 – 660.
- Vences M., Vieites D. R., Glaw F., Brinkmann H., Kosuch J., Veith M., and Meyer A. (2003), "Multiple overseas dispersal in amphibians," *Proc. Roy. Soc. B*, **270**, 2435 – 2442.