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Research

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World Travelers: Parthenogenesis and Ecological Tolerance Enable Multiple Colonization Events by the Widespread Short-Tailed Whipscorpion, *Stenochrus portoricensis* (Schizomida: Hubbardiidae)

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Abstract

Whereas morphology remains a powerful tool for the diagnosis and description of short-tailed whip scorpions, or schizomids (Order Schizomida Petrunkevitch, 1945), especially when adults of both sexes are available, the systematics of some schizomid taxa is difficult to resolve due to a lack of characters in these morphologically conserved arachnids. *Stenochrus portoricensis* Chamberlin, 1922, defined on a single character of the female spermathecae, is the most widespread schizomid in the New World. Numerous records in the Neotropics, from the southern United States to Brazil, throughout the Caribbean, and further afield, including the Galapagos Islands and Europe, raise the question as to whether *S. portoricensis* is indeed a single widespread species or a complex of multiple species with conserved morphology? The present study uses a multilocus dataset and the broadest geographical sample currently available to address the phylogeography of *S. portoricensis* with molecular divergence dating and ancestral area reconstruction of all currently known species of *Stenochrus* Chamberlin, 1922. Analyses recovered *S. portoricensis* as paraphyletic. Two species previously synonymized are revalidated and transferred to *Stenochrus*. Population structure analyses recovered the remaining samples of *S. portoricensis* as a single monophyletic species with low genetic divergence and comprising two subclades. Ancestral area reconstruction suggests a Mesoamerican origin for *Stenochrus*, which contains a widespread species, recently introduced to multiple localities. Introductions to Europe and the Caribbean occurred from a single clade in the Yucatán Peninsula, Mexico, within which genetic divergence is minimal, confirming the hypothesis of multiple independent introductions with successful colonization facilitated by parthenogenetic reproduction.

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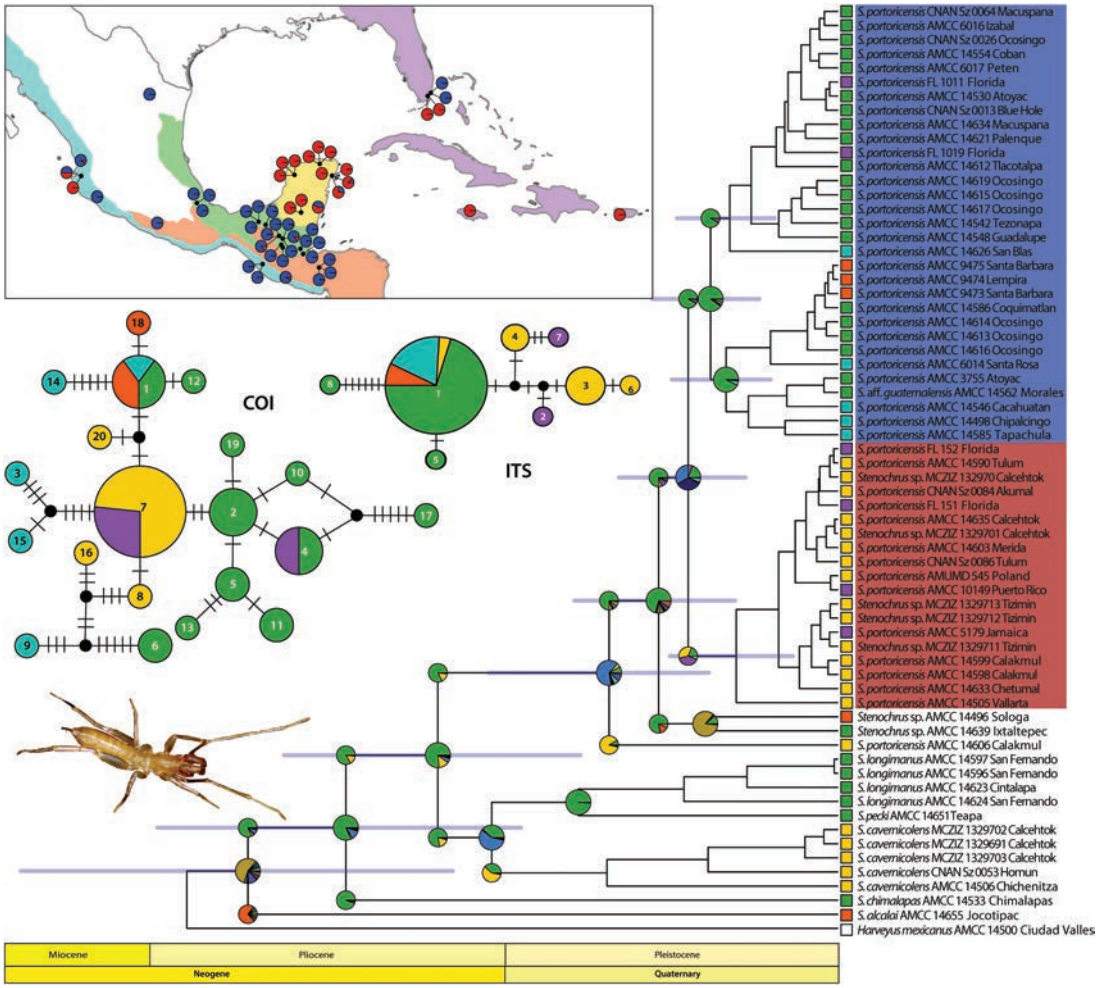
Graphical Abstract



Key words: phylogeography, biogeography, Pleistocene, Uropygi, invasive species

Several recent studies focused on phylogeography and species delimitation in arachnids, especially spiders and harvestmen (Order Opiliones Sundevall, 1833), with an ability to colonize new environments through long-distance dispersal (Hamilton et al. 2011, Satler et al. 2013, Didomenico and Hedin 2016, Ortiz and Francke 2016, Harrison et al. 2017, Burns et al. 2018, Chamberland et al. 2020). Fewer studies investigated the phylogeography of less diverse, or minor arachnid orders (Harvey 2002, Clouse et al. 2017, Schramm et al. 2021), which are characterized by limited vagility and often restricted distributions, and include many short-range endemics (Edward and Harvey 2008, Didomenico and Hedin 2016, Abrams et al. 2019). Short-tailed whip scorpions, or schizomids (Order Schizomida Petrunkevitch, 1945), are one example of the latter. Recent studies revealed more diversity than previously suspected, with the distributions of most species narrowly restricted, often to single localities (Harms et al. 2018, Abrams et al. 2019, Monjaraz-Ruedas et al. 2020).

Few schizomid species are widely distributed or reported to have been introduced to areas beyond their known distributions. One example from Southeast Asia concerns an undescribed species of *Orientzomus* Cokendolpher and Tsurusaki, 1994 distributed across the islands of Micronesia, suggesting they may have colonized the archipelago by long-distance dispersal, leading to marked genetic differentiation (Clouse et al. 2017). Other examples include *Bucinozomus hortuspalmarum* Armas and Rehfeldt, 2015, *Ovozomus lunatus* (Gravely, 1911), *Schizomus crassicaudatus* (Pickard-Cambridge, 1872), and *Zomus bagnallii* (Jackson, 1908), reported from greenhouses and botanical gardens in France, Germany, and India, and apparently introduced from Southeast Asia (Reddell and Cokendolpher 1995; Harvey 2003, 2011; Armas and Rehfeldt 2015). Although originally described from a greenhouse in Germany, a specimen of *B. hortuspalmarum* was collected in Singapore by the last author (R. Monjaraz-Ruedas and L. Prendini, personal observation).

The most widespread Neotropical schizomid, *Stenochrus portoricensis* Chamberlin, 1922, is distributed from the southern United States to South America and throughout the Caribbean, but also recorded from Europe (often in greenhouses), again suggesting anthropogenic introductions (Korenko et al. 2009, Christophoryová et al. 2013, Clouse et al. 2017).

Stenochrus portoricensis was originally described from Coamo Springs, Puerto Rico, based on a single female, diagnosed by reduction of the lateral lobes in comparison with the median lobes, of the female spermathecae. A revised diagnosis was provided by Rowland and Reddell (1977), who subsequently proposed several synonyms

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(Rowland and Reddell 1977, 1980; Reddell and Cokendolpher 1995), due primarily to the absence of adult males and to similarities in the only diagnostic character (spermathecae). Among the species synonymized with *S. portoricensis* were *Schizomus antilus* Hilton, 1933 from near Havana, Cuba; *Schizomus cavernicolens* Chamberlin and Ivie, 1938 from caves in Calcehtok, Yucatán, Mexico; *Schizomus floridanus* Muma, 1967 from Hammock, Dade County, FL; and *Schizomus longimanus* Rowland, 1971 from caves in the vicinity of Tuxtla Gutierrez, Chiapas, Mexico. Rowland and Reddell (1980) again revised the diagnosis based on new material from Muna, Yucatán, Mexico, in which adults of both sexes were present, describing the male of *S. portoricensis* for the first time.

To date, *S. portoricensis* has been reported from many Neotropical localities, mostly in Mexico, the United States, the Caribbean, Central and South America, including the Galapagos Islands, and in Europe, e.g., in the Czech Republic, Germany, Poland, Slovakia, and the U.K. (Reddell and Cokendolpher 1995; Tourinho and Kury 1999; Armas 2004, 2010; Blick et al. 2006; Korenko et al. 2009; Armas and Víquez 2010; Zawierucha et al. 2013; Christophoryová et al. 2013; Barranco et al. 2014; Teruel and Questel 2019). Most of these records were based on the morphology of the female spermathecae, and usually in the absence of males, excepting some from Mexico, in which males were reported, specifically, in *Schizomus longimanus*, and in other populations from Chiapas and Muna, Yucatán.

The absence of males from most localities and the conserved morphology of the female spermathecae, taken together with observations that *S. portoricensis* populations often inhabit disturbed habitats, have led to hypotheses that the species was introduced into many localities, and that thelytokous parthenogenetic reproduction enabled single adult females to colonize and reproduce (Korenko et al. 2009, Clouse et al. 2017).

Molecular phylogenetics and phylogeography of schizomids have recently begun to be explored. Most studies revealed more genetic divergence within schizomid populations than expected based on morphology, i.e., cryptic diversity (Clouse et al. 2017, Harms et al. 2018, Abrams et al. 2019, Monjaraz-Ruedas et al. 2020) raising questions as to whether populations of *S. portoricensis* around the world belong to distinct lineages, possibly species, or a single facultatively parthenogenetic, introduced species. Furthermore, if *S. portoricensis* is indeed an introduced species, where is the original source population? Also, as *S. portoricensis* is the type species of *Stenochrus* Chamberlin, 1922, are all species included within *Stenochrus* congeneric or is *S. portoricensis* a distinct Caribbean lineage with no continental affinities, suggesting that other, currently recognized species belong to a different genus or genera?

The present study evaluates the phylogeography of *S. portoricensis* and its close relatives (Monjaraz-Ruedas et al. 2019, 2020) using multilocus data from two nuclear and two mitochondrial markers for the broadest geographical sample currently available. Genetic clustering and tree-based approaches are used to evaluate genetic structure and haplotype diversity among populations and to investigate species limits. Based on other studies of short-range endemics, in which Extensive population structure is often observed, it is hypothesized that *S. portoricensis* would present high genetic diversity and extensive population structure if it is a species complex, but low genetic diversity and limited population structure if it is a single widespread species. The biogeography of *Stenochrus* and the widespread *S. portoricensis* are also discussed based on molecular divergence dating and ancestral range reconstruction. Finally, the life history and habitat preferences for some populations of *S. portoricensis* are discussed.

Materials and Methods

Taxon Sampling and Material

An attempt was made to broadly cover the known distribution of *S. portoricensis*, a challenge given the large number of records across the New World and Europe. Most ingroup taxon samples included in the study originated from the Caribbean and Mexico, the areas with the greatest concentration of records for this species, but samples were unavailable from Cuba and South America (e.g., Brazil, Colombia, Ecuador, and the Galapagos Islands). Exemplars of all described species of *Stenochrus* were included, along with undescribed species from which tissue samples were available, e.g., *Stenochrus* sp. AMCC 14496 and AMCC 14639. Six outgroup taxa were included, based on previous work on *Stenochrus* by Monjaraz-Ruedas et al. (2020), and the tree rooted on *Harveyus mexicanus* (Rowland, 1971).

Two datasets were created for the study. The first dataset, used to test the monophyly and phylogenetic placement of *S. portoricensis*, included sequences from 66 samples, twelve of which were included in Clouse et al. (2017), one in Zawierucha et al. (2013), and 22 in Monjaraz-Ruedas et al. (2020), in addition to sequences newly generated from 31 samples (Table 1). The second dataset, used for phylogeographic analyses of *S. portoricensis*, comprised sequences from 50 samples (Table 1).

Specimens newly sequenced in this study were collected by hand or using an aspirator and preserved in 96% ethanol for DNA isolation. Tissue samples and material examined are deposited in the Ambrose Monell Cryocollection (AMCC) at the American Museum of Natural History (AMNH), New York, United States, and the Colección Nacional de Arácnidos (CNAN) at the Instituto de Biología, Universidad Nacional Autónoma de México (IBUNAM), Mexico City, Mexico.

DNA Extraction, Amplification, Sequencing, and Alignment

DNA was insolated using the DNeasy Tissue kit (Qiagen, Valencia, CA). Extractions were prepared from the entire specimen when several individuals were available whereas leg pairs II–IV were used when only a singleton was available.

DNA sequences were generated from two nuclear gene loci, the Internal Transcribed Spacer (hereafter, ITS) and 28S rDNA (hereafter, 28S), and two mitochondrial gene loci, 12S rDNA (hereafter, 12S) and Cytochrome *c* Oxidase Subunit I (hereafter, COI). Polymerase chain reaction (PCR) amplification was performed with primers and protocols described by Monjaraz-Ruedas et al. (2020), using Illustra Hot Start Mix RTG beads (GE Healthcare, Little Chalfont, Buckinghamshire, U.K.) in a 25 µl reaction comprising 21 µl de-ionized water, 1 µl forward and reverse primers, and 2 µl DNA. PCR products were Sanger-dideoxy sequenced using an ABI Prism 3730 XL DNA Sequencer (Perkin-Elmer, Melville, NY) at the AMNH Sackler Institute of Comparative Genomics, and a 3500 XL Genetic Analyzer (Life Technologies, Foster City, CA) at the Laboratorio Nacional de Biodiversidad (LANABIO), IBUNAM. A total of 108 sequences were newly generated from 31 samples for this study; 16 sequences could not be obtained from 10 samples and were treated as missing data (Table 1).

Double-stranded sequences were edited and assembled into consensus sequences using Sequencher ver. 5.4.6 (Gene Codes Corporation, Ann Arbor, MI). Edited consensus sequences of the four gene loci were aligned using MAFFT ver. 6 (Katoh et al. 2002, 2009; Katoh and Standley 2013). The 28S and COI loci were aligned using the L-INS-i

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Table 1. Tissue samples, provenance data and GenBank accession codes for DNA sequences from the Internal Transcribed Spacer (ITS), 28S rDNA (28S), 12S rDNA (12S) and Cytochrome *c* Oxidase Subunit I (COI) used for phylogenetic analysis of the short-tailed whipscorpion, *Stenochrus portoricensis* Chamberlin, 1922 (Schizomida: Hubbardiidae Cook, 1899). Samples deposited in the following collections: the Ambrose Monell Cryocollection (AMCC) at the American Museum of Natural History (AMNH), New York; the Colección Nacional de Arácnidos (CNAN) at the Instituto de Biología, Universidad Nacional Autónoma de México (IBUNAM), Mexico City; the Museum of Comparative Zoology (MCZ), Harvard University, Cambridge, MA; the Department of Animal Taxonomy and Ecology, Adam Mickiewicz University, Poznań, Poland (AMUMD). Samples highlighted in boldface indicate newly generated sequences for this study.

Species	Tissue Sample	ITS	28S	12S	COI	Country	Administrative Division	Locality	Latitude	Longitude
Harveyus mexicanus	AMCC [LP 14500]	MN087610	MN087535	MN087574	MN097730	Mexico	San Luis Potosí	Ciudad Valles, Sotano de la Tinaja	22.076	-98.9778
S. aff. *guatemalensis*	AMCC [LP 14562]	MK849651	MK849721	MK849686	MK801863	Guatemala	Izabal	Morales, Finca Firmeza, Sendero Anfibios	15.4067	-88.6963
S. alcalai	AMCC [LP 14655]	MK849650	MK849720	MK849685	MK801862	Mexico	Oaxaca	San Pedro Jocotipac, El Tanque	17.7929	-97.1056
S. cavernicolens	AMCC [LP 14506]	MN087628	MN087553	MN087589	MN097748	Mexico	Yucatan	Chichenitza, Ruinas de Chichenitza	20.6804	-88.5694
S. cavernicolens	**CNAN DNA Sz53**	**OL336274**	**–**	**–**	**OK641877**	**Mexico**	**Yucatan**	**Homun, Cueva Santa Maria**	**20.7472**	**-89.2966**
S. cavernicolens	MCZ IZ-132969	–	KY573826	KY573443	KY573308	Mexico	Yucatán	Grutas de Calcehtok (X'pukil), Calcehtok	20.5507	-89.9124
S. cavernicolens	MCZ IZ-132970 106724.2	–	KY573829	KY573446	KY573310	Mexico	Yucatán	Grutas de Calcehtok (X'pukil), Calcehtok	20.5507	-89.9124
S. cavernicolens	MCZ IZ-132970 106724.3	–	KY573830	KY573447	KY573311	Mexico	Yucatán	Grutas de Calcehtok (X'pukil), Calcehtok	20.5507	-89.9124
S. chimalapas	AMCC [LP 14533]	MK849652	MK849722	MK849687	MK801864	Mexico	Oaxaca	Santa Maria Chimalapas, Cueva de Escolapa	16.8506	-94.7481
S. longimanus	**AMCC [LP 14596]**	**OL336255**	**OL336308**	**OL336284**	**OK641857**	**Mexico**	**Chiapas**	**San Fernando, Cueva de la Mano, Ejido Miguel Hidalgo**	**16.8644**	**-93.2477**
S. longimanus	**AMCC [LP 14597]**	**OL336256**	**OL336309**	**–**	**OK641858**	**Mexico**	**Chiapas**	**San Fernando, Cueva de la Mano, Ejido Miguel Hidalgo**	**16.8644**	**-93.2477**
S. longimanus	**AMCC [LP 14623]**	**OL336265**	**OL336318**	**OL336293**	**OK641867**	**Mexico**	**Chiapas**	**Cintalapa, Fuera de la Cueva Ejidal**	**16.8717**	**-93.7113**
S. longimanus	AMCC [LP 14624]	MN087638	MN087563	MN087599	MN097758	Mexico	Chiapas	San Fernando, Cueva de la Cañada, Ejido Benito Juarez	16.8973	-93.1331
S. pecki	AMCC [LP 14651]	MN087626	MN087551	MN087587	MN097746	Mexico	Tabasco	Teapa, Grutas del Cocona	17.5636	-92.9289
S. portoricensis	AMCC [LP 10149]	MK849655	MK849725	MK849689	MK801867	United States	Puerto Rico	Bosque Estatal Susua, old campsite	18.0674	-66.8973
S. portoricensis	**AMCC [LP 14498]**	**OL336248**	**OL336301**	**–**	**OK641851**	**Mexico**	**Guerrero**	**Chilpancingo, Cueva del Borrego, Omiltemi**	**17.5552**	**-99.6547**
S. portoricensis	AMCC [LP 14505]	MN087627	MN087552	MN087588	MN097747	Mexico	Jalisco	Puerto Vallarta, Calle Olas Altas (Final)	20.5989	-105.237
S. portoricensis	**AMCC [LP 14530]**	**OL336249**	**OL336302**	**OL336278**	**OK641852**	**Mexico**	**Veracruz**	**Atoyac, Rancho San Fermín**	**18.9083**	**-96.8195**
S. portoricensis	**AMCC [LP 14542]**	**OL336250**	**OL336303**	**OL336279**	**OK641853**	**Mexico**	**Veracruz**	**Tezonapa, Presidio**	**18.675**	**-96.7834**
S. portoricensis	AMCC [LP 14546]	MN087629	MN087554	MN087590	MN097749	Mexico	Chiapas	Cacahuatan, Puente Faja de Oro	15.0389	-92.1715
S. portoricensis	**AMCC [LP 14548]**	**OL336251**	**OL336304**	**OL336280**	**OK641854**	**Mexico**	**Nuevo León**	**Guadalupe, Fraccionamiento Bosques del Rey**	**25.6311**	**-100.202**
S. portoricensis	**AMCC [LP 14554]**	**OL336252**	**OL336305**	**OL336281**	**OK641855**	**Guatemala**	**Alta Verapaz**	**Coban, Reserva Natural Chahunpek**	**16.0098**	**-90.649**
S. portoricensis	**AMCC [LP 14585]**	**OL336253**	**OL336306**	**OL336282**	**OK641856**	**Mexico**	**Chiapas**	**Tapachula, Restaurante Campestre 'La Changa'**	**14.9997**	**-92.2347**
S. portoricensis	**AMCC [LP 14586]**	**OL336254**	**OL336307**	**OL336283**	**–**	**Mexico**	**Colima**	**Coquimatlán, Cueva Parque Ecológico 'El Palapo'**	**19.1991**	**-103.914**
S. portoricensis	AMCC [LP 14590]	MN087630	MN087555	MN087591	MN097750	Mexico	Quintana Roo	Xpu-Ha, Sistema Dos Amores	20.5116	-87.2843
S. portoricensis	AMCC [LP 14598]	MN087631	MN087556	MN087592	MN097751	Mexico	Campeche	Calakmul, Grutas Actum Kin, Cristobal Colon	18.216	-89.4818
S. portoricensis	AMCC [LP 14599]	MN087632	MN087557	MN087593	MN097752	Mexico	Campeche	Calakmul, Grutas Actum Kin, Cristobal Colon	18.216	-89.4818

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Table 1. Continued

Species	Tissue Sample	ITS	28S	12S	COI	Country	Administrative Division	Locality	Latitude	Longitude
S. portoricensis	AMCC [LP 14603]	MN087635	MN087560	MN087596	MN097755	Mexico	Yucatan	Merida, Km 172 Autopista Merida–Cancun	20.7637	-88.0569
S. portoricensis	AMCC [LP 14606]	MN087636	MN087561	MN087597	MN097756	Mexico	Campeche	Calakmul, Grutas Xibalba, Ejido Cristobal Colon	18.2024	-89.4758
S. portoricensis	**AMCC [LP 14612]**	**OL336257**	**OL336310**	**OL336285**	**OK641859**	**Mexico**	**Tabasco**	**Tlacotalpa, Sendero a la Gruta de Villa Luz**	**17.4454**	**-92.7615**
S. portoricensis	**AMCC [LP 14613]**	**OL336258**	**OL336311**	**OL336286**	**OK641860**	**Mexico**	**Chiapas**	**Ocosingo, Arroyo Nayte, Sierra la Cojolita**	**16.7941**	**-91.0423**
S. portoricensis	**AMCC [LP 14614]**	**OL336259**	**OL336312**	**OL336287**	**OK641861**	**Mexico**	**Chiapas**	**Ocosingo, Arroyo Nayte, Sierra la Cojolita**	**16.7941**	**-91.0423**
S. portoricensis	**AMCC [LP 14615]**	**OL336260**	**OL336313**	**OL336288**	**OK641862**	**Mexico**	**Chiapas**	**Ocosingo, El Aserradero**	**16.7853**	**-91.0382**
S. portoricensis	**AMCC [LP 14616]**	**OL336261**	**OL336314**	**OL336289**	**OK641863**	**Mexico**	**Chiapas**	**Ocosingo, El Aserradero**	**16.7853**	**-91.0382**
S. portoricensis	**AMCC [LP 14617]**	**OL336262**	**OL336315**	**OL336290**	**OK641864**	**Mexico**	**Chiapas**	**Ocosingo, El Aserradero**	**16.7853**	**-91.0382**
S. portoricensis	**AMCC [LP 14619]**	**OL336263**	**OL336316**	**OL336291**	**OK641865**	**Mexico**	**Chiapas**	**Ocosingo, Cueva Grande, Reserva Chan-Kin**	**16.6914**	**-90.824**
S. portoricensis	**AMCC [LP 14621]**	**OL336264**	**OL336317**	**OL336292**	**OK641866**	**Mexico**	**Chiapas**	**Palenque, Centro de Convenciones de Ruinas de Palenque**	**17.32**	**-92.0215**
S. portoricensis	**AMCC [LP 14626]**	**OL336266**	**OL336319**	**OL336294**	**OK641868**	**Mexico**	**Nayarit**	**San Blas, Cueva del Naranjo**	**21.4794**	**-105.078**
S. portoricensis	AMCC [LP 14633]	MN087640	MN087565	MN087600	MN097760	Mexico	Quintana Roo	Chetumal, Campus de ECOSUR	18.5443	-88.2641
S. portoricensis	**AMCC [LP 14634]**	**OL336267**	**OL336320**	**OL336295**	**OK641869**	**Mexico**	**Tabasco**	**Macuspana, Grutas Ixtaha, Ejido Paloma**	**17.6213**	**-92.4793**
S. portoricensis	AMCC [LP 14635]	MN087641	MN087566	MN087601	MN097761	Mexico	Yucatan	Opichen, Grutas de Actun Spukil, Clacehtok	20.5512	-89.9121
S. portoricensis	AMCC [LP 3755]	MN087642	MN087567	MN087602	MN097762	México	Veracruz	Paso del Macho, Cueva del Cabrito	19.0394	-96.8311
S. portoricensis	AMCC [LP 5179]	MN087643	MN087568	MN087603	MN097763	Jamaica	St. Ann District	Ulster Spring, ca. 1 km N	18.3242	-77.5132
S. portoricensis	AMCC [LP 6014]	MN999744	MN999736	MN999751	MN996832	Guatemala	Santa Rosa	El Papayo	14.1381	-90.5644
S. portoricensis	**AMCC [LP 6016]**	**OL336269**	**OL336322**	**OL336297**	**OK641871**	**Guatemala**	**Izabal**	**Rio Sauce, 15 km E El Estor**	**15.5602**	**-89.2849**
S. portoricensis	**AMCC [LP 6017]**	**OL336270**	**OL336323**	**OL336298**	**OK641872**	**Guatemala**	**Petén**	**Rio Secoyo, 15 km S St. Luis**	**16.0271**	**-89.3233**
S. portoricensis	**AMCC [LP 9473]**	**–**	**OL336324**	**OL336299**	**OK641873**	**Honduras**	**Santa Barbara Department**	**Santa Rita, 3.4 km SW San Francisco de Ojuera**	**14.7355**	**-88.2212**
S. portoricensis	**AMCC [LP 9474]**	**OL336271**	**OL336325**	**OL336300**	**OK641874**	**Honduras**	**Lempira Department**	**La Iguala?, La Telegrafía**	**14.766**	**-88.2419**
S. portoricensis	AMCC [LP 9475]	MN999743	MN999735	MN999750	MN996831	Honduras	Santa Barbara Department	Santa Barbara, San Antonio, 1 km NE Río Grande de Otoro	14.7744	-88.1708
S. portoricensis	AMUMD 545	–	–	–	JX280414	Poland	Poznan	Poznan Palm House	–	–
S. portoricensis	**CNAN DNA Sz13**	**OL336272**	**–**	**–**	**OK641875**	**Belize**	**Cayo District**	**Blue Hole National Park**	**17.1471**	**-88.6748**
S. portoricensis	**CNAN DNA Sz26**	**OL336273**	**–**	**–**	**OK641876**	**Mexico**	**Chiapas**	**Marquez de Comillas, Playón de la Gloria, Sendero Rockera**	**16.1502**	**-90.8465**
S. portoricensis	**CNAN DNA Sz64**	**OL336275**	**–**	**–**	**OK641878**	**Mexico**	**Tabasco**	**Macuspana, Fuera de la Cueva de Agua Blanca**	**17.62**	**-92.47**
S. portoricensis	**CNAN DNA Sz84**	**OL336276**	**–**	**–**	**OK641879**	**Mexico**	**Quintana Roo**	**Akumal, Cueva Imix**	**20.5135**	**-87.2854**
S. portoricensis	**CNAN DNA Sz86**	**OL336277**	**–**	**–**	**OK641880**	**Mexico**	**Quintana Roo**	**Xpu-Ha, Cueva Xibalba**	**20.5115**	**-83.2843**
S. portoricensis	FL10.11	–	KY573906	–	KY573387	United States	Florida	Fairchild Tropical Garden, Miami	25.6753	-80.2711
S. portoricensis	FL10.19	–	KY573395	–	KY573460	United States	Florida	Fairchild Tropical Garden, Miami	25.6753	-80.2711
S. portoricensis	FL15.1	–	KY573909	KY573461	KY573402	United States	Florida	Fruit and Spice Park, Homestead	25.5348	-80.4936
S. portoricensis	FL15.2	–	KY573910	KY573462	KY573403	United States	Florida	Fruit and Spice Park, Homestead	25.5348	-80.4936

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Table 1. Continued

Species	Tissue Sample	ITS	28S	12S	COI	Country	Administrative Division	Locality	Latitude	Longitude
S. portoricensis	MCZ IZ-132970 106724.4	–	KY573831	KY573448	KY573312	Mexico	Yucatán	Grutas de Calcehtok (X'pukil), Calcehtok	20.5507	-89.9124
S. portoricensis	MCZ IZ-132970 106724.5	–	KY573832	KY573449	KY573313	Mexico	Yucatán	Grutas de Calcehtok (X'pukil), Calcehtok	20.5507	-89.9124
Stenochrus sp.	AMCC [LP 14496]	MN087644	MN087569	MN087604	MN097764	Mexico	Oaxaca	San Andres Sologa, 6 km S San Andres Sologa	17.2579	-96.2399
***Stenochrus* sp.**	**AMCC [LP 14639]**	**OL336268**	**OL336321**	**OL336296**	**OK641870**	**Mexico**	**Oaxaca**	**Asunción Ixtaltepec, Arroyo Taté, Lázaro Cárdenas**	**16.7716**	**-94.8418**
Stenochrus sp.	MCZ IZ-132971 106725.1	–	KY573834	–	KY573315	Mexico	Yucatán	Kikil, Municipio Tizimini	21.1923	-88.1685
Stenochrus sp.	MCZ IZ-132971 106725.2	–	KY573835	–	KY573316	Mexico	Yucatán	Kikil, Municipio Tizimini	21.1923	-88.1685
Stenochrus sp.	MCZ IZ-132971 106725.3	–	KY573836	–	KY573317	Mexico	Yucatán	Kikil, Municipio Tizimini	21.1923	-88.1685

strategy, whereas the ITS and 12S markers were aligned using the G-INS-i strategy (Katoh et al. 2005, Swain 2018). Ambiguous regions within the ITS and 12S alignments were trimmed using GBlocks ver. 0.91b (Castresana 2000). The protein-coding locus, COI, was translated into amino acids to assess its quality by identifying stop codons in Mesquite ver. 3.0.4 (Maddison and Maddison 2019).

Phylogenetic Analysis

The COI dataset was the most complete of the four loci, with only one sequence missing, followed by the 28S, with eight sequences missing. Most of the missing data was concentrated in the 12S and ITS loci, with 13 and 14 sequences missing, respectively, due to the inclusion of sequences from previous studies which omitted some of the loci used in the present study, but added otherwise unavailable localities (Zawierucha et al. 2013, Clouse et al. 2017).

The aligned ITS, 28S, 12S, and COI sequences were concatenated using Mesquite ver. 3.0.4 (Maddison and Maddison 2019) and analyzed simultaneously. PartitionFinder ver. 2 (Lanfear et al. 2012) suggested a single data partition with the TRN+I+G model as the most suitable for phylogenetic analyses with Maximum Likelihood (hereafter, ML) and Bayesian inference (hereafter, BI). However, problems encountered in chain mixing when using this model with BI, were resolved by implementing a more complex partitioning scheme, comprising six data partitions (ITS, 28S, 12S, and COI first, second, and third codon positions), with the GTR+I+G model for each partition, in the analyses with ML and BI.

Analyses with ML were conducted using RAxML-HPC2 ver. 8.2.10 with XSEDE (Stamatakis 2014) on the CIPRES Science Gateway ver. 3.3 online portal (Miller et al. 2010). Optimal trees were computed with the **-f a** command for rapid bootstrap analysis and search for the best-scoring tree in one run, computing 1,000 bootstrap replicates, using the GTRGAMMAI model.

Analyses with BI were conducted using MrBayes ver. 3.2.6 with XSEDE (Huelsenbeck and Ronquist 2001) on the CIPRES portal. Each analysis comprised four simultaneous runs, with four chains default for 5,000,000 generations, sampling every 1,000 trees. The initial 25% of sampled trees were discarded as burn-in. Effective sample size (EES > 200) for each parameter was checked in Tracer ver. 1.7 (Rambaut et al. 2018). In addition to the multilocus analysis, the COI dataset, the most complete of the four loci, was analyzed separately with ML and BI.

Population Structure and Genetic Diversity

Analyses of population structure and genetic diversity were performed on the second dataset, comprising 50 samples of *S. portoricensis* s. str. (Fig. 1), and excluding outgroup taxa, including other species of *Stenochrus* and three clades of *S. portoricensis* considered to be heterospecific (see below).

Two genetic clustering programs, STRUCTURE ver. 2.3.2 (Pritchard et al. 2000) and Bayesian Analysis of Population Structure (BAPS) ver. 6 (Corander et al. 2008), were used to analyze and explore the genetic population structure within *S. portoricensis*. As STRUCTURE requires a specific, formatted matrix, the concatenated data matrix was transformed into a *genind* object, using the R package *adegenet* (Jombart 2008) to retain polymorphic sites only. STRUCTURE analyses were run using an admixture model, considering between 1 and 10 genetic clusters (K = 1–10), with 10 independent runs per K value. A Markov Chain Monte Carlo (MCMC) algorithm was run for 100,000 steps, with the first 10,000 discarded as burn-in. The optimum K value was assessed with the ΔK method of Evanno

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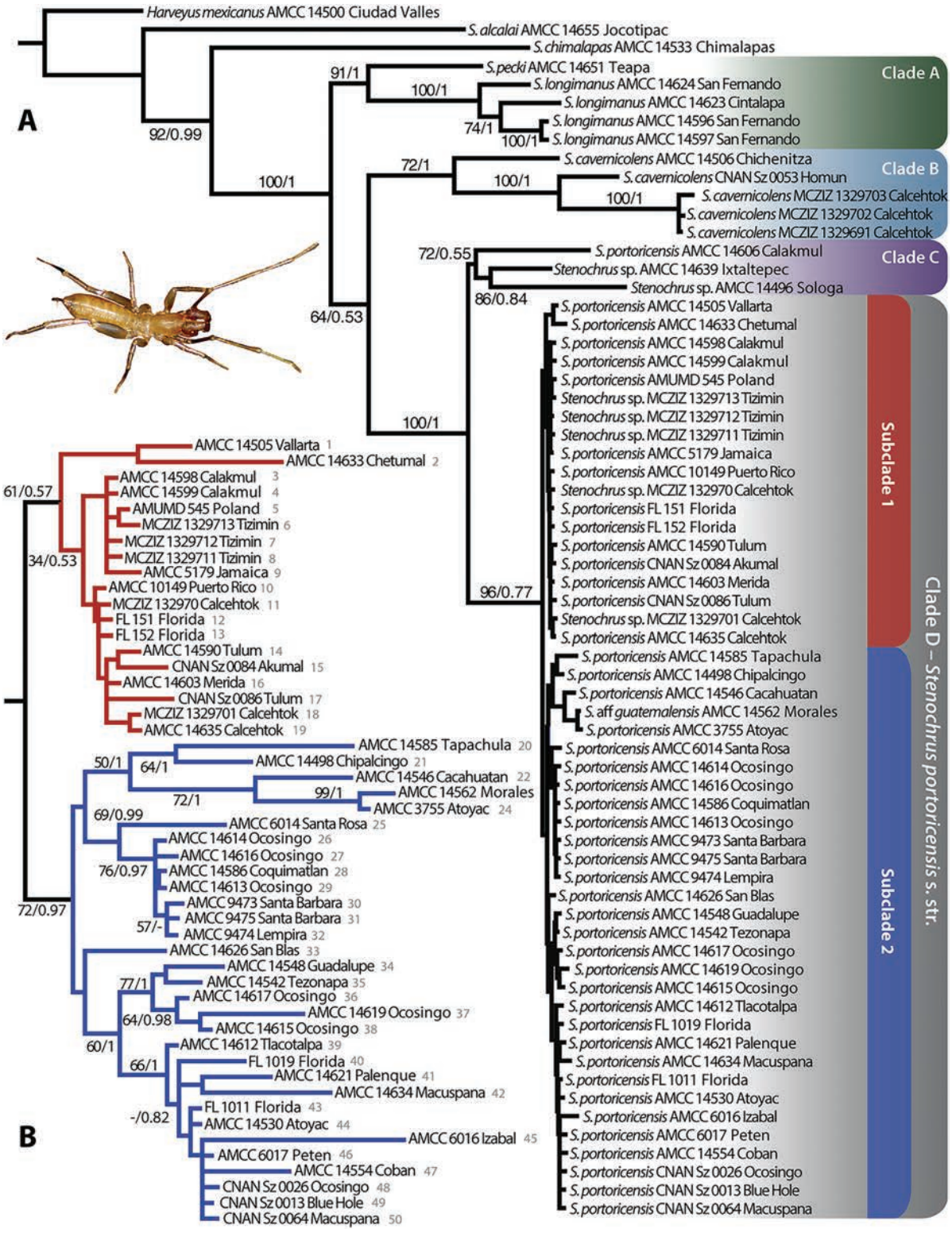

Fig. 1. (A) Phylogenetic relationships of the short-tailed whipscorpion, *Stenochrus portoricensis* Chamberlin, 1922 (Schizomida: Hubbardiidae Cook, 1899), obtained by analysis of the concatenated data matrix with Maximum Likelihood. Colored areas represent clades recovered in phylogenetic and phylogeographic analyses: Clade A (dark green), *Stenochrus longimanus* (Rowland, 1971), comb. nov.; Clade B (blue), *Stenochrus cavernicolens* (Chamberlin and Ivie, 1938), comb. nov.; Clade C (purple), *Stenochrus* sp.; Clade D (grey), *S. portoricensis* s. str. Subclades of *S. portoricensis* recovered with structure analyses: Subclade 1 (red), Subclade 2 (dark blue). (B) Close-up of Clade D. Numbers on branches represent bootstraps and posterior probabilities above 50%; numbers in grey to right of species indicate position matching STRUCTURE analysis order. Inset schizomid: *S. portoricensis* female from Chichenitza, Yucatán, Mexico.

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et al. (2005), using Structure Harvester (Earl and vonHoldt 2012). Data were summarized using the *FullSearch* algorithm of CLUMPP (Jakobsson and Rosenberg 2007) and visualized with DISTRUCT (Rosenberg 2004).

The BAPS algorithm allows for clustering of individuals with linked loci using a concatenated alignment by specifying a boundaries file for each locus. The concatenated dataset was converted to BAPS format using PGDSpider ver. 2.1.1.5 (Lischer and Excoffier 2012) and non-spatial clustering of individuals with linked loci performed in BAPS, assuming between 1 to 10 genetic clusters (K = 1–10), with 10 independent runs per K value.

The demographic history of *S. portoricensis* was addressed using the COI and ITS data sets only, from 50 samples comprising Clade D of *S. portoricensis* (see Fig. 1). Three samples, *S. portoricensis* AMCC 14586 Colima, *S. portoricensis* AMUMD545 Poland, and *S. portoricensis* CNAN Sz0013 Belize, were removed from the COI dataset due to missing data. In order to facilitate demographic analysis, interpretation of the results, and for capturing most of the genetic variation within *S. portoricensis*, samples were geographically arranged and assigned to 16 broad regions, i.e., the countries or territories of the Bahamas, Belize, Guatemala, Honduras, Jamaica, Puerto Rico, and the following states and areas of Mexico: Chiapas (Ocosingo, Pacific, and Palenque), Guerrero, Jalisco, Nuevo Leon, Quintana Roo, Tabasco, Veracruz, and Yucatán. Additionally, analyses were performed across the four major subclades of *S. portoricensis* which recovered the highest support values (Fig. 1).

Data were imported into DnaSP ver. 6 (Rozas et al. 2017) for haplotype estimation and exported as Nexus files for haplotype network reconstruction. The ITS dataset was phased using PHASE ver. 2.1 (Stephens et al. 2001) to infer haplotypes of diploid data. Phylogeographic relationships among the haplotypes were reconstructed using the median-joining algorithm (Bandelt et al. 1999) in PopArt ver. 1.7 (Leigh and Bryant 2015).

Haplotype diversity (Hd), nucleotide diversity (π), number of segregating sites (S), and sequence diversity (K) were calculated for both loci, and the two subclades of *S. portoricensis* using DnaSP (Fig. 1). In addition to the analyses with BAPS and STRUCTURE, an analysis of hierarchical molecular variance (AMOVA) was performed at three levels among subclades (Fig. 1B), among populations within subclades, and among samples; populations were represented by 14 broad regions, i.e., the Bahamas, Guatemala, Honduras, Jamaica, Puerto Rico, and the following states and areas of Mexico: Chiapas (Ocosingo, Pacific), Guerrero, Jalisco–Nayarit, Nuevo Leon, Quintana Roo, Tabasco, Veracruz, and Yucatán. The AMOVA was run with default settings using the R package *poppr* (Kamvar et al. 2014). Genetic distances were computed for the COI dataset using the R package *adegenet* (Jombart 2008).

Phylogenetic Dating and Ancestral Range Reconstruction

In order to test hypotheses for the current distribution of *S. portoricensis*, the ancestral range of *Stenochrus* was reconstructed using the entire dataset (66 samples). According to Clouse et al. (2017), the most recent common ancestor for *Stenochrus* diverged about 92 Ma. However, species delimitation analyses suggest these date estimates for the internal clades of Schizomida are too old for studies at the population/species interface and a gene-rate approach is preferred (Harms et al. 2018, Abrams et al. 2019). A conservative approach was therefore taken in the present study, applying the unpartitioned COI clock rate of 3.36%/Myr proposed for insects (Papadopoulou et al. 2010, Didomenico and Hedin 2016).

Divergence times were estimated using BEAST ver. 2.4.8 (Bouckaert et al. 2014) on two datasets, nuclear (ITS and 28S) and mitochondrial (COI and 12S) with model TN93 for each partition. Site and clock models were unlinked across partitions, a relaxed clock with a lognormal prior distribution was set with a Yule model process as tree prior. The *ucld.mean* of the clock model was set to a COI rate mutation of 0.0169 substitutions/site/myr. Two runs of 5×10^7 generations were specified and merged in LogCombiner ver. 2.4.8 (Bouckaert et al. 2014), with the initial 25% discarded as burn-in. Convergence of the chains and effective sample sizes were checked in Tracer ver. 1.7.1 (Rambaut et al. 2018). Trees were computed using TreeAnnotator ver. 2.4.8 (Bouckaert et al. 2014). Trees were edited using the R package *strap* (Bell and Lloyd 2015) and Adobe Illustrator 2021.

Ancestral range reconstruction was estimated using the R package BioGeoBEARS (Matzke 2014) using the BEAST maximum clade credibility tree as input and specifying the following models: DEC and DEC+J, DIVALIKE and DIVALIKE+J, and BAYAREALIKE and BAYAREALIKE+J, followed by statistical comparison for the best fitting model using the likelihood ratio-test (LRT).

Terminal taxa were scored for presence/absence in a matrix of five geographical regions corresponding to the Mexican biogeographical provinces proposed by Morrone (2014) and Morrone et al. (2017): Antillean, Pacific Lowlands, Veracruzan, Yucatan, and Transition Zone (Fig. 3). A maximum number of three areas were set for the analyses.

As BioGeoBEARS requires each tip on the tree to represent a species/population, and the dataset for *S. portoricensis* contains populations comprising one or more individuals, the BEAST tree was pruned to four subclades (Fig. 1) representing most of the genetic diversity and geographical range of the species, based on results of the analyses of population structure, including the haplotype networks.

Nomenclature

This paper has been registered in Zoobank (www.zoobank.org), the official register of the International Commission on Zoological Nomenclature. The LSID (Life Science Identifier) number of the publication is: urn:lsid:zoobank.org:pub:ADF09C2F-8537-42E6-AD72-B405432CE01E

Results

Phylogenetic Analyses

Analyses of the concatenated multilocus dataset with ML and BI obtained similar relationships to a previous study of *Stenochrus* phylogeny (Monjaraz-Ruedas et al. 2020). *Stenochrus portoricensis* was rendered paraphyletic by *Stenochrus pecki* Rowland, 1973 from Tabasco, Mexico, and two undescribed species from Oaxaca, *Stenochrus* sp. AMCC 14639 and AMCC 14496 (Fig. 1A). Both analyses recovered four main clades in addition to *Stenochrus alcalai* Monjaraz-Ruedas and Francke, 2018 and *Stenochrus chimalapas* Monjaraz-Ruedas and Francke, 2018. Clade A comprised *S. pecki* and samples of *S. portoricensis* from caves in Chiapas. Clade B comprised samples of *S. portoricensis* from caves in the Yucatán (Calcehtok and Chichenitza). Clade C comprised two undescribed species of *Stenochrus* and a sample of *S. portoricensis* from Campeche. Clade D comprised 50 samples of *S. portoricensis* from across Mexico and the Caribbean, grouped into two subclades (Fig. 1B). The extremely short branch lengths in Clade D, compared to the other three clades, are consistent with low genetic divergence among the samples.

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Separate analyses of the COI dataset were congruent with analyses of the concatenated dataset in recovering Clades A and B, but differed in obtaining a single clade, comprising members of Clades C and D, neither of which were monophyletic.

Samples initially identified as *S. portoricensis* in Clade A were collected exclusively from caves in the vicinity of Tuxtla Gutierrez, Chiapas, Mexico. Populations from these caves are represented by both sexes and characterized by marked sexual dimorphism in the pedipalps of the males, which are greatly elongated in comparison with females, as in other species of *Stenochrus* (Rowland and Reddell 1980, Armas 1989, Santos et al. 2013, Monjaraz-Ruedas and Francke 2015). Based on morphological examination (see 'Taxonomy'), these specimens are conspecific with the types of *Schizomus longimanus* Rowland, 1971, collected from Cueva de Cerro Hueco, 3 km SE of Tuxtla Gutierrez, and synonymized with *S. portoricensis* by Rowland and Reddell (1977), justifying its revalidation and transfer to *Stenochrus*, as *Schizomus longimanus* (Rowland, 1971), **comb. nov.**

Similarly, samples of Clade B are cavernicolous populations which are conspecific with the types of *Schizomus cavernicolens* Chamberlin and Ivie, 1938, from Xkyc Cave [= Actun Xkyc], Calcehtok, Yucatan, Mexico, also synonymized with *S. portoricensis* by Rowland and Reddell (1977), justifying its revalidation and transfer to *Stenochrus*, as *Stenochrus cavernicolens* (Chamberlin and Ivie, 1938), **comb. nov.** (see 'Taxonomy').

Clade C comprises two undescribed species of *Stenochrus*, each differing morphologically from *S. portoricensis* for which males are known. One sample in Clade C, *S. portoricensis* AMCC 14606, requires further examination to determine whether it constitutes a morphologically diagnosable new species.

Samples in Clade D, which includes a sample from Puerto Rico, the island on which the type locality of *S. portoricensis* is situated, are considered conspecific with one another due to the low genetic distances among them (Fig. 4). Consequently, Clade D is regarded as *S. portoricensis* s. str.

Stenochrus aff. *guatemalensis* AMCC 14562, previously included in the study of Monjaraz-Ruedas et al. (2020) was also placed within Clade D, suggesting it is conspecific with *S. portoricensis*. However, the question as to whether *S. guatemalensis* (Chamberlin, 1922) is a junior synonym of *S. portoricensis* awaits further investigation, due to the ambiguous type locality, which could refer to several different places in Guatemala.

Genetic Diversity and Population Structure

The COI alignment comprised 47 sequences and was 928 nucleotide base-pairs (bp) in length. The alignment was 444 bp in length after removal of missing data, with 33 variable sites, 20 parsimony informative sites, and 30 segregating sites, resulting in 20 haplotypes (Table 1; Fig. 2). Genetic diversity was high (Hd = 0.880 ± 0.038) ranging from 0.49 in Subclade 1 to 0.96 in Subclade 2, whereas nucleotide diversity was low (π = 0.01080 = 1%, K = 4.79) ranging from 0.014 in Subclade 2 to 0.0022 in Subclade 1 (Table 2). The number of haplotypes varied from four in Subclade 1 to 16 in Subclade 2. No shared or endemic haplotypes, unique to a single locality or region, were observed. The most common haplotype (H7), present in 32% of the samples, corresponds to Subclade 1 (= STRUCTURE cluster 1), and was restricted to the Caribbean archipelago and the Yucatán Peninsula (Fig. 2). Haplotypes in Subclade 2 (= STRUCTURE cluster 2) were distributed mainly along the Pacific coast and in the mountain ranges of Chiapas (Fig. 2).

The ITS alignment of 39 sequences was 795 bp in length. The alignment was 766 bp in length after removal of missing data, with 15 variable sites, four parsimony informative sites, and 15 segregating sites, resulting in eight haplotypes (Table 2; Fig. 2). Genetic diversity was considerably lower than in the COI dataset (Hd = 0.514 ± 0.094), ranging from 0.140 in Subclade 2 to 0.8 in Subclade 1. Nucleotide diversity was even lower than in the COI dataset (π = 0.00214 = 0.2%, K = 1.6), ranging from 0.0006 in Subclade 2 to 0.003 in Subclade 1 (Table 2). There was also considerably less diversity in the number of haplotypes. The most common haplotype (H1) occurred in 69% of the samples and was shared among the two subclades. The most abundant and common haplotype, H1, was widely distributed in Mexico, the Yucatán Peninsula, and Central America (Fig. 2). Subclade 1 included the greatest haplotype diversity (Hd = 0.836 ± 0.089) with five haplotypes, restricted to the Caribbean and the Yucatán Peninsula, except for a single sample, *S. portoricensis* AMCC 14505, from Jalisco (Fig. 2). Subclade 2 contained low haplotype diversity (Hd = 0.140 ± 0.087) and included only three haplotypes distributed in Guatemala and Mexico (Fig. 2C).

Analyses of population structure recovered low genetic differentiation (Fig. 2A and B), consistent with the short branch lengths in the phylogenetic analyses and the large number of shared haplotypes among clades and populations in the haplotype networks (Fig. 2C and D). Bayesian clustering analysis with STRUCTURE recovered two clusters (K = 2, ΔK = 523.8914, Fig. 2A). The first STRUCTURE cluster corresponds to Subclade 1 (red in Fig. 2) and the second to Subclade 2 (blue in Fig. 2) recovered by the phylogenetic analyses with ML and BI (Fig. 1B). The two clusters recovered by STRUCTURE were also congruent with distribution and haplotypes (Fig. 2B). Cluster 1 (Subclade 1; red in Fig. 2C) is mostly restricted to the Caribbean and the Yucatán Peninsula, with the exception of samples from Jalisco and Nayarit, whereas cluster 2 (Subclade 2; blue in Fig. 2C) is mostly restricted to the North American continent, except for two records in Florida. Population structure analyses with BAPS recovered three clusters (K = 3, *log*ML = –2094.5097, *P* = 0.99, Fig. 2B). The first cluster was congruent with the STRUCTURE analyses. The second and third clusters recovered by the BAPS analyses (blue in Fig. 2A) correspond to the second cluster in the STRUCTURE analyses. The third cluster in the BAPS analyses (green in Fig. 2A) is consistent with the genetic distances (Fig. 4) in Subclade 2, which contains the greatest genetic distances and is distributed mainly along the Pacific coast of Mexico.

AMOVA corroborated the hypothesis of low population structure when samples were compared among populations and subclades, with most of the genetic variation (64.3%) explained within samples, suggesting low genetic differentiation among populations and subclades (Table 3).

Divergence Time and Ancestral Range Estimation

BEAST analyses estimated the divergence time for the most recent common ancestor (MRCA) of *Stenochrus* to about 5 Ma (95% HPD: 3.3–7.2 Ma), whereas the MRCA for *S. portoricensis* was estimated to about 1.38 Ma (95% HPD: 0.83–2.07 Ma). Consistent with the low genetic divergence observed, these estimates are considerably younger than previous dates proposed by Clouse et al. (2017) of around 70 Ma for the MRCA of a clade comprising representatives of *Stenochrus* and about 45 Ma for *S. portoricensis*.

The BEAST analyses recovered a different topology from the ML and BI analyses of the concatenated matrix (Fig. 1), in which *S. longimanus* and the *S. pecki* clade (Clade A) were placed sister to *S. cavernicolens* (Clade B) (Fig. 3). Clade C was not monophyletic

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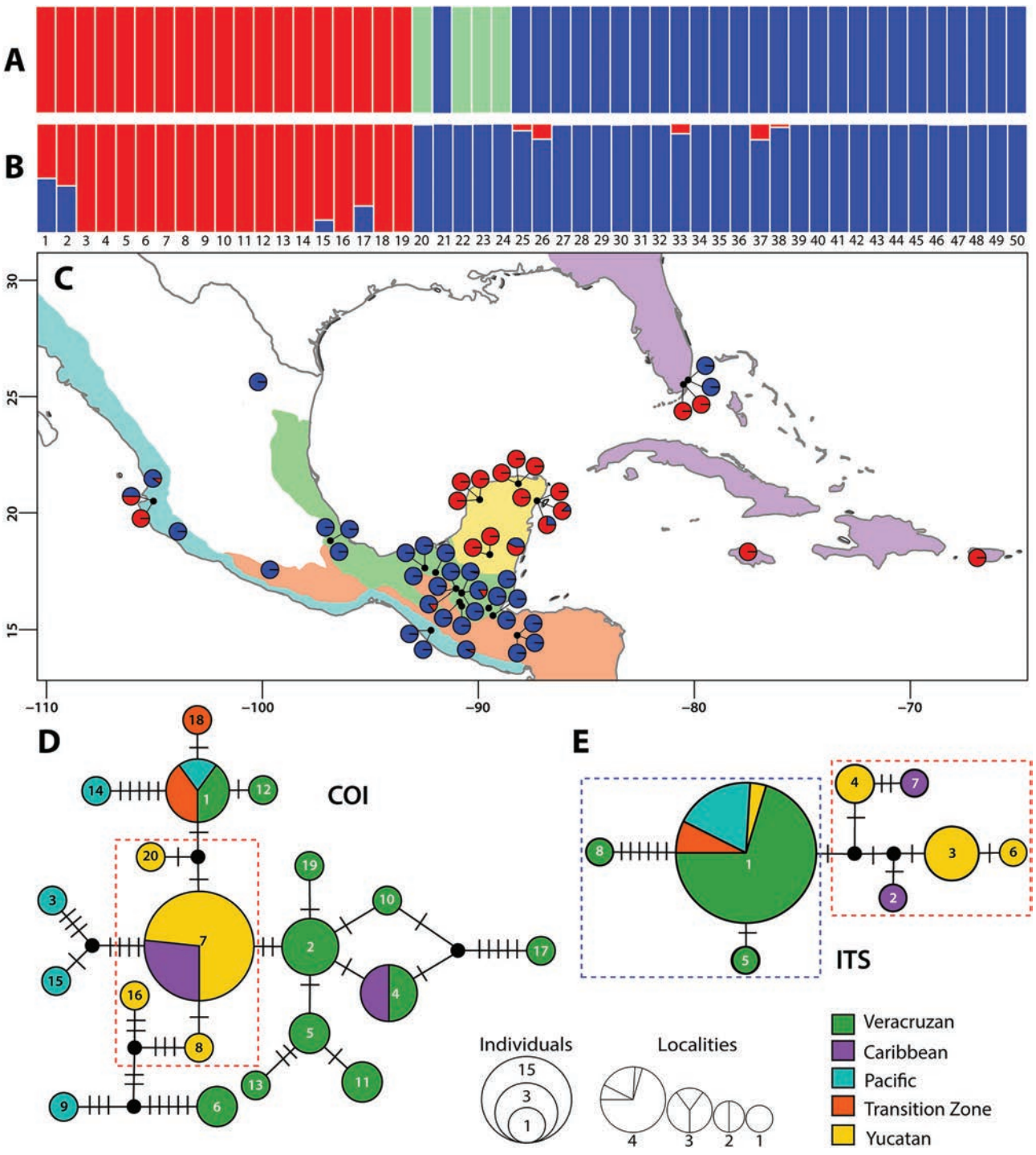

Fig. 2. Phylogeographical results for Clade D of the short-tailed whipscorpion, *Stenochrus portoricensis* Chamberlin, 1922 (Schizomida: Hubbardiidae Cook, 1899). (A, B) Bar plots of results recovered by analyses with BAPS (A) and STRUCTURE (B). (C) Map of North America showing pie charts of individuals with corresponding subclade assignations recovered by analyses with STRUCTURE. (D, E) Median-joining network obtained from Cytochrome *c* Oxidase Subunit I, COI (D) and Internal Transcribed Spacer, ITS (E): black circles represent median vectors presumed to be unsampled or missing intermediates; hashmarks represent number of mutations between haplotypes; numbers inside circles denote haplotypes; colors denote regions used for ancestral range estimation; circle size proportional to frequencies; dotted line enclosure haplotypes correspond to Subclades 1 and 2 in Fig. 1.

in the BEAST analyses (Fig. 3) and the relationships of Subclade 1 of Clade D, were also incongruent, with sample AMCC 14505 Vallarta placed sister to the entire Subclade 1 rather than sister to AMCC 14633 Chetumal. Most of the topological incongruence occurs in the area with the lowest support values (Fig. 1), suggesting more data from different loci, as well as more samples for these clades could improve of the support and stability of this part of the tree.

Model selection under LRT for ancestral range estimation using BioGeoBears suggested DIVALIKE as the best fit to explain the current distribution of *Stenochrus* (Table 4). Models applying the *j* dispersal parameter fit better than without it, suggesting the

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Table 2. Molecular diversity and population demographic statistics of two loci, Cytochrome *c* Oxidase Subunit I (COI) and Internal Transcribed Spacer (ITS), recovered by delimitation analysis of the short-tailed whipscorpion, *Stenochrus portoricensis* Chamberlin, 1922 (Schizomida: Hubbardiidae Cook, 1899)

	Subclade	*n*	*H*	Hd ± (SD)	π	*S*	*K*
COI	Subclade 1	18	4	0.49 ± 0.142	0.00222	8	0.98
	Subclade 2	29	16	0.963 ± 0.019	0.01408	31	6.25
	Total	47	20	0.880 ± 0.038	0.01080	33	4.79
ITS	Subclade 1	11	5	0.836 ± 0.089	0.00328	8	2.5
	Subclade 2	28	3	0.140 ± 0.087	0.00065	7	0.5
	Total	39	8	0.514 ± 0.094	0.00214	15	1.64

Abbreviations: *n*, number of samples; *H*, number of haplotypes; Hd, haplotype diversity (± standard deviation); π, nucleotide diversity; *S*, number of segregating sites; *K*, average nucleotide differences.

Table 3. Analysis of molecular variance (AMOVA) showing population genetic structure in the short-tailed whipscorpion, *Stenochrus portoricensis* Chamberlin, 1922 (Schizomida: Hubbardiidae Cook, 1899)

AMOVA	df	SSq	MSq	% Variation	*P*
Between subclades	1	130.26	130.26	9.61	0.005
Within clades	14	615.95	44.00	26.12	0.026
Among samples	31	649.14	20.94	64.27	0.111
Total	46	1395.35	30.33	100	

SSq, sum of squares; MSq, mean of squares.

possibility of jump- or long-distance dispersal. Due to statistical concerns with the *j* parameter (Ree and Sanmartín 2018), however, as well as similar results, only DIVALIKE is discussed hereafter (Fig. 3). The ancestral area for *Stenochrus* was estimated as a combination of the Veracruzan Province and the Transition Zone, i.e., the mountains of southern Mexico and Guatemala (Fig. 3), whereas the ancestral area for *S. portoricensis* was estimated as a combination of the Veracruzan and Yucatán provinces followed by the colonization (introductions, see below) of the Caribbean for Subclade 1 and dispersal to mountain regions (Transition Zone) and possible human introductions to the Pacific coast and the Caribbean for Subclade 2. These results must be treated with caution, however, due to the many anthropogenic introductions of *S. portoricensis* (see below).

Discussion

Phylogenetic Relationships

Phylogenetic analyses of the genus *Stenochrus*, including the largest sample of *S. portoricensis* presented to date, revealed the presence of more than one species-level clade in what used to be considered a single species with a conserved morphology. At least two distinct clades comprising samples initially identified as *S. portoricensis*, were recovered, corresponding to *S. cavernicolens* and *S. longimanus*, two species previously in synonymy, with sexual populations, and males that can be differentiated by morphological characters (see 'Taxonomy'). However, most samples of *S. portoricensis* belong to a single clade, represented by females that are presumably parthenogenetic.

Samples in Clade D, which contains a sample from Puerto Rico, AMCC 10149 (Table 1; Fig. 1), are considered conspecific with *S. portoricensis* s. str. based on similar genetic distances and morphology, which matches the holotype.

Additional synonyms of *S. portoricensis*, e.g., *S. antilus* or *Schizomus loreto* Armas, 1977, require molecular evaluation, whereas some clades that appear to be genetically distinct from typical *S. portoricensis* require morphological evaluation to determine whether males are present, and characters may permit their diagnosis.

Considering that the first male of *S. portoricensis* was described from Muna in the Yucatán Peninsula, near Calcehtok, the type locality of *S. cavernicolens*, it is possible that the male described and assigned to *S. portoricensis* by Rowland and Reddell (1977) is actually the male of *S. cavernicolens* and the male of *S. portoricensis*, if it exists, remains unknown.

Specimens from Florida, conspecific with the junior synonym, *Schizomus floridanus* Muma, 1967, described from Hammock Dade County, were recovered within the *S. portoricensis* clade, confirming the validity of this synonym. The same could apply to Cuban forms currently synonymized with *S. portoricensis*, and the many records from elsewhere in the world. For example, samples from Poland included in the present study, are almost identical to samples from Mexico (Fig. 4). However, other cavernicolous sexual populations from Mexican caves in Chiapas and Veracruz, for which males are available, but not molecular data, may prove to be valid species.

Biogeography of *Stenochrus*

Stenochrus was estimated to have diverged around 5 Ma (95% HPD: 7.2–3.3 Ma) in southern Mexico. Most diversity in the genus is currently distributed in the mountain ranges of Oaxaca and southern Chiapas, suggesting that the Yucatán Peninsula was subsequently colonized from the mountain ranges of southern Mexico. Southern Mexico was characterized by high volcanic activity during the Miocene and Pliocene (23–2.6 Ma) with the separation of the Isthmus of Tehuantepec serving as an important barrier for faunal dispersal during the Late Miocene (Mastretta-Yanes et al. 2015), which may have influenced the current distribution and diversity of *Stenochrus* in this area.

It has been suggested that Pleistocene glaciations played an important role in the current distribution of the Nearctic fauna and flora in Mexico (Moreno-Letelier and Piñero 2009, Hamilton et al. 2011, Rodríguez-Gómez et al. 2013, Graham et al. 2020, Schramm et al. 2021), a region also considered an important interchange between the Nearctic and Neotropical biotas (Sanmartín et al. 2001, Devitt 2006, Graham et al. 2020, Schramm et al. 2021). *Harveyus* Monjaraz-Ruedas et al. 2019, the putative sister group of *Stenochrus* is a Nearctic genus distributed in the northern Mexican state of San Luis Potosí, suggesting that *Stenochrus* could be a Nearctic lineage that dispersed south during the Pleistocene glaciations, as conditions became warmer and drier, and became isolated in refugia such as caves or mountains. Such a scenario might explain the current distribution of *Stenochrus* in caves along the coast of the Gulf of Mexico and mountain ranges in Oaxaca and Chiapas, as well as its association with the Nearctic fauna, rather than the Mesoamerican fauna as previously believed (Rowland 1975). As suggested by Monjaraz-Ruedas et al. (2020), some Caribbean schizomid taxa, e.g., *Antillostenochrus* Armas and Teruel, 2002, resemble *Stenochrus* morphologically, suggesting a close relationship. However, the Caribbean schizomid fauna appears to be more closely related to the fauna of South America based on morphology (Reddell and Cokendolpher 1995, Armas and Abud-Antun 2002, Teruel 2018), suggesting a different evolutionary history in which the Caribbean islands were colonized from South America as suggested for other arachnids (Esposito et al. 2015,

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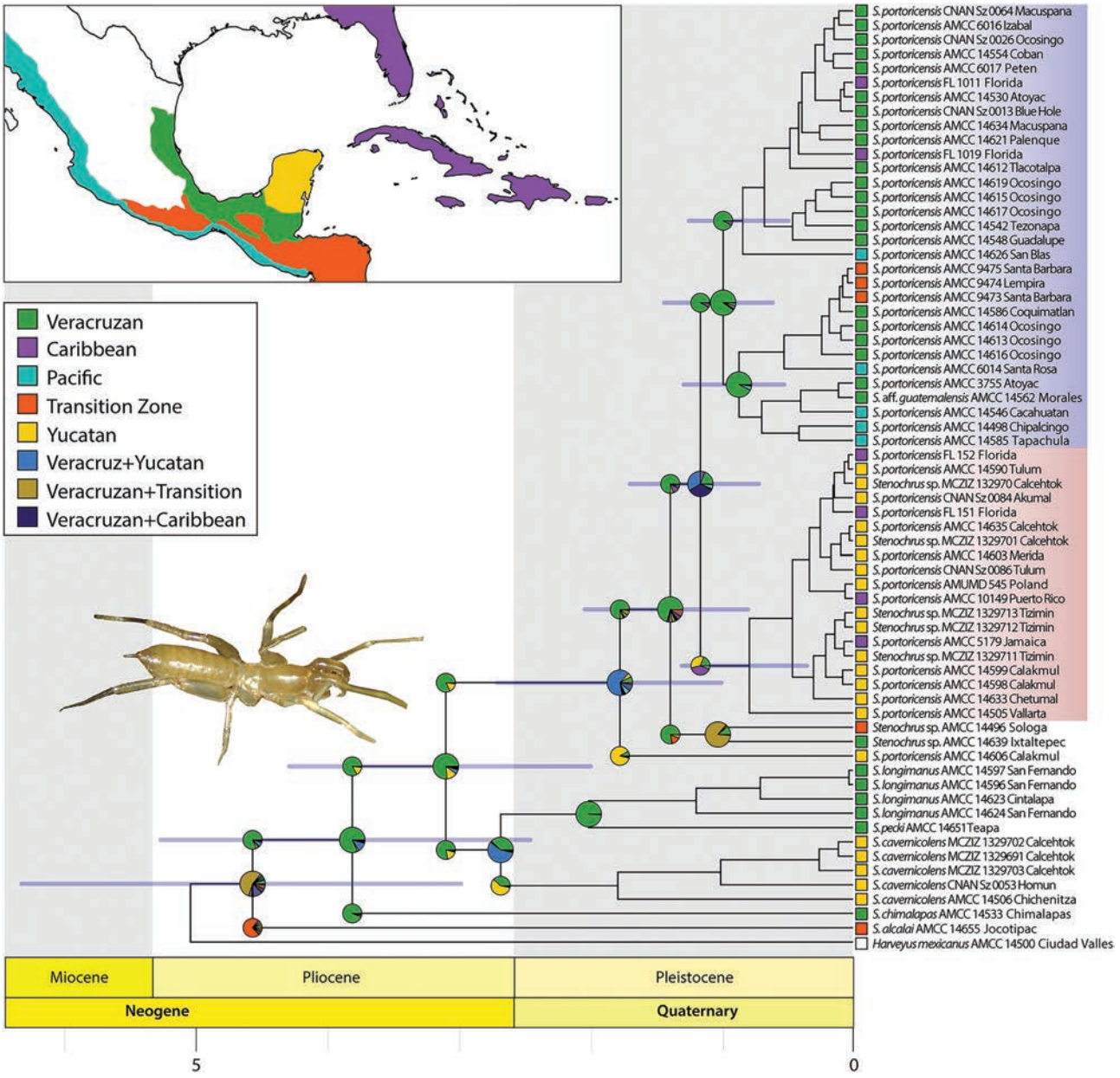

Fig. 3. Dated phylogeny and ancestral range estimation of the short-tailed whipscorpion, *Stenochrus portoricensis* Chamberlin, 1922 (Schizomida: Hubbardiidae Cook, 1899) under DIVALIKE model. Pie charts illustrate probabilities for ancestral range reconstruction at each node; area assignation represented by squares at tips; colors correspond to areas in map of Mexico and the Caribbean (upper left). Blue lines at nodes represent 95% credibility intervals for molecular dating. Inset schizomid: *S. portoricensis* female from Ocosingo, Chiapas, Mexico.

Esposito and Prendini 2019, Chamberland et al. 2020). A phylogenetic analysis including samples of other Caribbean schizomid genera is needed to test these hypotheses.

Stenochrus portoricensis is abundant in the lowlands of Chiapas, Tabasco, and the Yucatán Peninsula, extending southwards along the coast to the lowlands of Belize, Guatemala, and Honduras. Ancestral area reconstruction suggests it diverged from the MRCA around 1.7 Ma and colonized the lowlands of the Veracruzan Province, on one side, and the Yucatán Peninsula, on the other, explaining the current distributions of the two subclades recovered in the analyses (Fig. 2). The current distribution of the species can be explained by subsequent multiple introductions, from different places, into the Caribbean and other parts of Mexico and Central America.

Table 4. Statistics of ancestral range estimation model-testing for the short-tailed whipscorpion, *Stenochrus portoricensis* Chamberlin, 1922 (Schizomida: Hubbardiidae Cook, 1899)

Model	LnL	*n*	AIC
DEC	–37.13	2	78.26
DEC+J	–32.75	3	71.5
DIVALIKE	**–36.49**	**2**	**76.98**
DIVALIKE+J	**–32.41**	**3**	**70.83**
BAYAREALIKE	–37.3	2	78.59
BAYAREALIKE+J	–32.75	3	71.5

Text highlighted in boldface indicate best fitting model. LnL, log likelihood; *n*, number of free parameters; AIC, Akaike information criterion.

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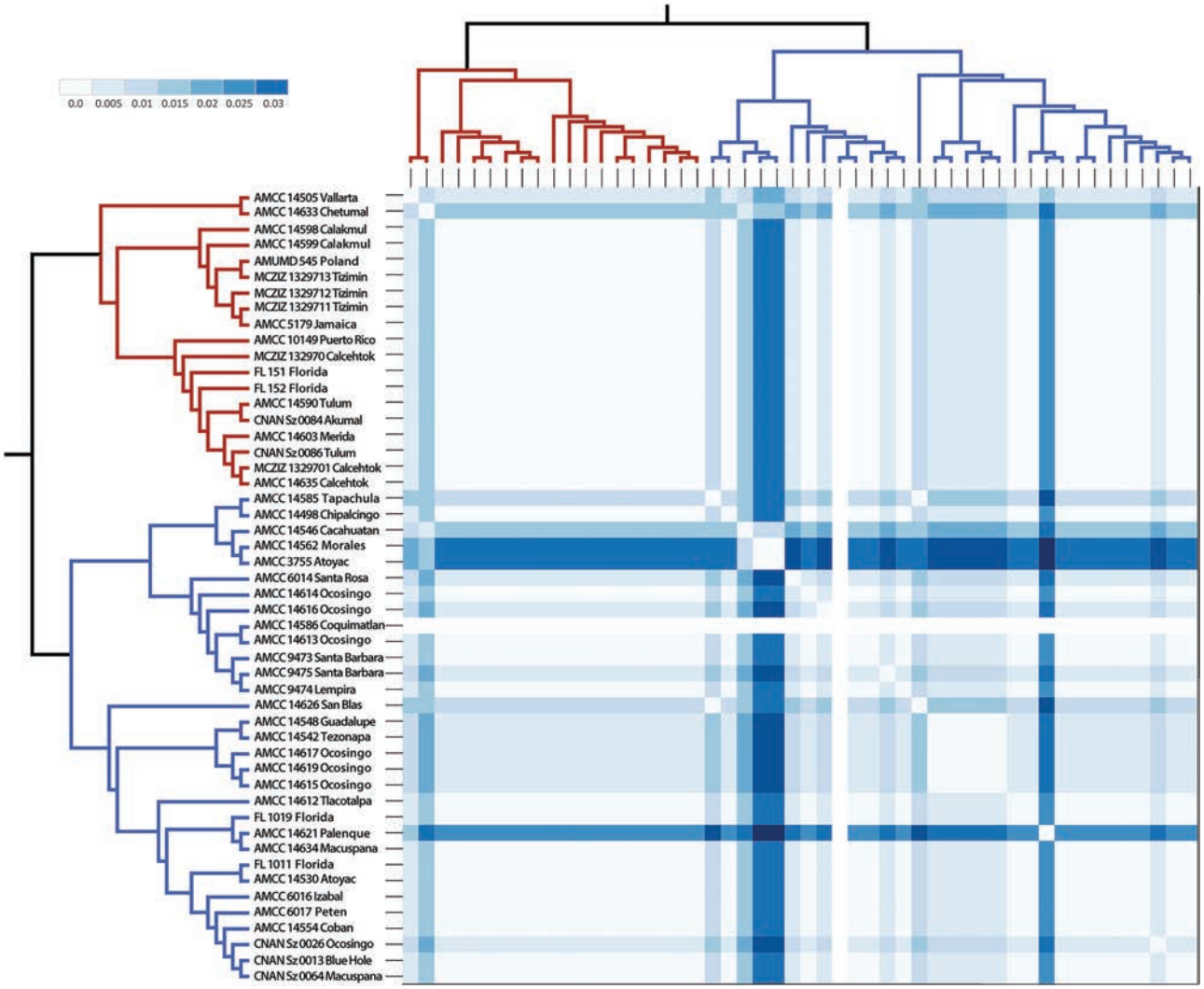

Fig. 4. Matrix of uncorrected genetic *p*-distances among samples of the short-tailed whipscorpion, *Stenochrus portoricensis* Chamberlin, 1922 (Schizomida: Hubbardiidae Cook, 1899), ordered by subclades of Clade D.

Given the higher genetic diversity within Subclade 2 (see below) and the results of the ancestral area reconstruction, members of Subclade 1 could have been introduced from the lowlands of Chiapas and Tabasco to the Yucatán Peninsula, creating a founder population that initially remained isolated from members of Subclade 2, followed by subsequent introductions from members of Subclade 1 in the Yucatán to the Caribbean, the Pacific coast of Jalisco and Nayarit, and Europe. Such a hypothesis could explain how the species achieved its widespread distribution in such a short time, as well as the low genetic divergence across its distribution.

Phylogeography of *S. portoricensis*

Phylogenetic studies confirm that many schizomids are short-range endemics (Edward and Harvey 2008), species restricted to single localities or habitats (e.g., a cave system or biome with no apparent barriers). As such, unique haplotypes per population are expected. However, ITS and COI did not recover unique haplotypes for a single locality in the present study. Although surprising for a group of organisms with low vagility, it is impossible to compare with other taxa endemic to specific localities, as this is the first study addressing haplotype diversity in schizomids.

The population structure analysis revealed low genetic structure in *S. portoricensis*, consistent with the haplotype networks (Fig. 2D and E) and phylogenetic analyses, as well as with results of the AMOVA, in which most of the genetic variation is within and not among populations (Table 3). Invasive or introduced species tend to possess less genetic diversity due to bottlenecks and/or founder events resulting from the colonization of new habitats by a few, or single individuals. However, cases of introduced species with high genetic diversity were the result of multiple re-introductions from the original source population (Morim et al. 2019, Urquía et al. 2019). When comparing the subclades of *S. portoricensis*, less genetic diversity is evident in Subclade 1 than Subclade 2 (Table 2). Additionally, Subclade 1 is mostly restricted to the Yucatán Peninsula and the Caribbean (Fig. 2C), suggesting several introductions may have occurred from the Caribbean to the Yucatán or vice versa, and from either source to other, more distant localities such as Jalisco and Europe. Additional evidence for multiple introductions of *S. portoricensis* is the percentage of admixture in both clusters (Subclades 1 and 2) suggested by the STRUCTURE analyses for samples 1, 2, 15, 17, 25, 26, 33, 37, and 38 (Fig. 2B) which implies hybridization between individuals of the two different clusters introduced from two different localities. The p-distances of the samples

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from Poland (Fig. 4), although not included in the haplotype network, are identical to those from the samples of Subclade 1 (Fig. 4). Several introductions of Subclade 1 to different localities are also supported by the ITS haplotype network, which recovered higher genetic diversity (Subclade 1, Hd = 0.83) than the COI (Subclade 1, Hd = 0.49), with five different haplotypes restricted to the Yucatán Peninsula and the Caribbean (Table 2; Fig. 2).

Subclade 2 has a different evolutionary history which is nevertheless concordant with that of Subclade 1. Subclade 2 contains more genetic diversity in the COI, but less in the ITS, suggesting it is independent from Subclade 1 and restricted to southeastern Mexico, Guatemala, and Honduras. Although the haplotype networks reveal introductions, there have been fewer in comparison with Subclade 1, and these introductions occurred in continental areas, as well as in Florida. A common pattern observed among samples from Florida and Jalisco is the presence of haplotypes from both subclades, suggesting two different introductions from at least two different subclades.

Genetic and nucleotide diversity are also a measure of divergence time, as ancient lineages have had more time to accumulate diversity than recent lineages which are still evolving and adapting to new habitats (Nei and Tajima 1981). Subclade 2 contains more genetic and nucleotide diversity in the COI dataset (Table 2), greater genetic distances (Fig. 4), and a more complex phylogenetic structure, as detected by the BAPS analyses and COI haplotype networks, than Subclade 1. This is consistent with the phylogenetic hypothesis of *Stenochrus* and the ancestral area reconstruction, which suggest that *S. portoricensis* originated in Mesoamerica and was subsequently introduced to the Caribbean and elsewhere in the New World and Europe. Therefore, *S. portoricensis* is probably not closely related to the schizomid fauna endemic to the Caribbean, although a comprehensive analysis including more Caribbean taxa is needed to test this hypothesis more rigorously.

Invasive Species

Stenochrus portoricensis has been repeatedly observed in disturbed areas, inhabiting areas with very dry conditions, even preferring disturbed areas over apparently more suitable habitat in close proximity (R. Monjaraz-Ruedas, personal observation). Additionally, females of *S. portoricensis* have shown tolerance to desiccation, evidenced first-hand when a female from the Yucatán Peninsula, maintained in captivity, was kept alive for nine months under suboptimal conditions (dry surface and low humidity), and successfully bore offspring (R. Monjaraz-Ruedas, personal observation). In comparison, other schizomid species are difficult to maintain in captivity for long, with many unable to survive a trip from the collection locality to the laboratory. This apparent tolerance of disturbance, together with the absence of males, led several authors to suggest that *S. portoricensis* is a facultative parthenogen (Korenko et al. 2009, Clouse et al. 2017, Teruel and Questel 2019). Reproduction by parthenogenesis would be advantageous for allowing this species to colonize new environments and establish itself in marginal habitats.

The combination of parthenogenesis and ecological tolerance to dry or disturbed habitats increases the possibility of transporting and introducing *S. portoricensis* into the Caribbean and Europe. *Stenochrus portoricensis* has been reported from Germany, the Czech Republic, Poland, and Slovakia, in all cases, from greenhouses (Korenko et al. 2009), suggesting specimens were transported in soil or cultivated pot plants. A record of *S. portoricensis* from an aqueduct in Spain, comprising 52 females and no males (Barranco et al. 2014), suggests the species was able not only to survive but to establish itself in that environment.

Taxonomy

Family Hubbardiidae Cook, 1899
Subfamily Hubbardiinae Cook, 1899
Genus *Stenochrus* Chamberlin, 1922

Type Species: Stenochrus portoricensis Chamberlin, 1922, by original designation.

Included Species: Stenochrus alcalai Monjaraz-Ruedas and Francke, 2018; *Stenochrus cavernicolens* (Chamberlin and Ivie, 1938), comb. nov.; *Stenochrus chimalapas* Monjaraz-Ruedas and Francke, 2018; *Stenochrus gruta* Monjaraz-Ruedas and Francke, 2018; *Stenochrus guatemalensis* (Chamberlin, 1922); *Stenochrus leon* Armas, 1995; *Stenochrus longimanus* (Rowland, 1971), comb. nov.; *Stenochrus pecki* (Rowland, 1973a); *Stenochrus portoricensis* Chamberlin, 1922.

Stenochrus cavernicolens (Chamberlin and Ivie, 1938), comb. nov.

Schizomus cavernicolens Chamberlin and Ivie, 1938: 102, 103, figs. 4–7; Gertsch, 1940: 4; Takashima, 1941: 94; Pearse, 1945: 153; Cárdenas-Figueroa, 1950: 154; Rémy, 1961: 406; Nicholas, 1962: 181; Vandel, 1964: 116; 1965: 93; Reddell, 1971: 28; Rowland, 1971a: 117; 1973c: 135; Brignoli, 1974: 149; Rowland, 1975b: 186; Reddell, 1977: 230; Rowland and Reddell, 1977: 87; 1980: 14; Camilo and Cokendolpher, 1988: 55; Reddell and Cokendolpher, 1995: 110.

Stenochrus portoricensis Chamberlin, 1922: Rowland and Reddell, 1977: 87.

Type Material: Schizomus cavernicolens: MEXICO: Yucatán: Municipio Opichén: Xkyc Cave [Actun Xkyc], 6.viii.1936, A.S. Pearse, holotype ♀ (AMNH) [examined].

Remarks: Chamberlin (1922) described *S. portoricensis* based on a single female from Puerto Rico. Chamberlin and Ivie (1938) described *Schizomus cavernicolens* again based from a single female. Rowland and Reddell (1977) described the female spermathecae and male of *Stenochrus portoricensis*, based on samples from caves in Muna, Yucatán, Mexico. The analyses presented herein suggest that populations from caves in the Yucatán are conspecific with *S. cavernicolens*, implying that the male described by Rowland and Reddell (1977) was in fact the male of *S. cavernicolens*, not *S. portoricensis*, the male of which is unknown. Although no morphological differences between the females of the two species are apparent, Rowland and Reddell (1977) mentioned statistical differences in the dimensions of the spermathecae, allowing them to differentiate populations from Chiapas and the Yucatán from populations in the Galapagos Islands and Florida, and suggesting that the females of the two species may be possible to differentiate with morphometrics. In light of the molecular evidence and male morphology, which suggests that members of clade B (Fig. 1) are conspecific with the type material, *Schizomus cavernicolens* is hereby revalidated and transferred to *Stenochrus*.

Distribution: Stenochrus cavernicolens is associated with caves in the vicinity of Opichén, southwestern Yucatán, Mexico, including Calcehtok cave, the locality at which material used in the present study, was collected (Table 1).

Stenochrus longimanus (Rowland, 1971), comb. nov.

Schizomus longimanus Rowland, 1971a: 119, 120, 124, 125, figs. 4–6, 17; Brignoli, 1973: 6–9, figs. 1, 2; Reddell, 1973: 38;

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Rowland, 1973a: 13, 16, 22, fig. 21; 1973c: 135, 137; Brignoli, 1974: 143, 144, 146, 147, 151, fig. 1b; Sbordoni et al., 1974: 19; Rowland, 1975b: 186; 1975b: 14, 15, 17, 19, 20; Dumitresco, 1977: 157; Rowland and Reddell, 1977: 87; 1980: 14; Camilo and Cokendolpher, 1988: 55; Armas, 1989a: 23; Reddell and Cokendolpher, 1995: 5, 99, 110, 111.

Stenochrus portoricensis Chamberlin, 1922: Rowland and Reddell, 1977:87.

Type Material: Schizomus longimanus: MEXICO: Chiapas: Municipio Tuxtla Gutierrez: Cueva Cerro Hueco, 3 km SE of Tuxtla Gutierrez, 18.viii.1967, J. Reddell, J. Fish, and M. Tandy, holotype ♂ and allotype ♀ (AMNH) [examined].

Remarks: Rowland (1971) described *Schizomus longimanus* based on male morphology, primarily the presence of an elongated propeltidium, elongated leg I, and elongation of the pedipalp femur and patella. However, Rowland and Reddell (1977) synonymized *S. longimanus* with *S. portoricensis* after examining populations of the latter from Campeche, which exhibit similar elongation. Males of *S. longimanus* resemble males of *S. cavernicolens* in flagellar shape but differ in the shape of the pedipalps, which are elongated in males of *S. longimanus*. However, dimorphism of the male pedipalps is variable in many schizomid species and should be used with caution. As with females of *S. cavernicolens*, Rowland and Reddell (1977) observed differences in the size of the female spermathecae of *S. longimanus*. In light of the molecular evidence and male morphology, which suggests that members of clade A (Fig. 1) are conspecific with the type material, *Schizomus longimanus* is hereby revalidated and transferred to *Stenochrus*.

Distribution: Stenochrus longimanus is associated with caves in the vicinity of Tuxtla Gutierrez, Mexico (Table 1).

Conclusion

The phylogeography of *S. portoricensis* merits further evaluation, including more extensive sampling of specific populations, especially those known to include multiple individuals in a specific habitat, as in Spain (52 females), and the Galapagos Islands (32 females). Some of these may deserve specific recognition, as demonstrated in the present study with *S. cavernicolens* and *S. longimanus*. However, other populations, e.g., Ocosingo in Chiapas (ca. 80 females), are now known to contain more than a single haplotype at the locality, revealing a more complex demographic history that should be assessed in future.

Future work which confirms thelytokous parthenogenetic reproduction in this species would corroborate and support the hypothesis presented here as to how *S. portoricensis* became widespread. Molecular analysis of samples from Europe and South America will help to clarify the sources of introduction for these populations.

An integrative approach to schizomid systematics and evolution has proven helpful for the recognition and delimitation of taxa, as well as for elucidating their complex evolutionary history. This approach will continue to be fruitful for answering evolutionary questions in many narrowly endemic organisms with high levels of cryptic diversity.

Supplementary Data

Supplementary data are available at *Insect Systematics and Diversity* online.

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Author Contributions

RMR: Conceptualization; Formal analysis; Writing—review and editing. OFF: Conceptualization; Funding acquisition; Resources; Supervision; Writing—review & editing. LP: Conceptualization; Funding acquisition; Resources; Writing—review and editing.

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