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Integrative systematics untangles the evolutionary history of *Stenochrus* (Schizomida: Hubbardiidae), a neglected junkyard genus of North American short-tailed whipscorpions

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Until recently, the Nearctic short-tailed whipscorpion genus, *Stenochrus* Chamberlin, 1922, included 27 species distributed primarily in Mexico, the USA and Central America. Morphological disparity among its species, associated with their adaptation to diverse habitats, raised the question as to whether *Stenochrus* was monophyletic. The phylogenetic relationships among short-tailed whipscorpions have only recently begun to be explored, and the monophyly of *Stenochrus* had never been tested. The present contribution provides the first phylogeny of *Stenochrus* and related genera, based on 61 morphological characters and 2991 aligned DNA nucleotides from two nuclear and two mitochondrial gene markers, for 73 terminal taxa. Separate and simultaneous analyses of the morphological and molecular data sets were conducted with Bayesian Inference, Maximum Likelihood, and parsimony with equal and implied weighting. Terminals represented only by morphological data ('orphans') were included in some analyses for evaluation of their phylogenetic positions. As previously defined, *Stenochrus sensu* Reddell & Cokendolpher (1991, 1995) was consistently polyphyletic and comprised eight monophyletic clades, justifying its reclassification into eight genera including *Heteroschizomus* Rowland, 1973, revalidated from synonymy with *Stenochrus* by Monjaraz-Ruedas *et al.* (2019). Rowland & Reddell's (1980) *mexicanus* and *pecki* species groups were consistently paraphyletic. Orphans grouped with the most morphologically similar taxa.

ADDITIONAL KEYWORDS: Arachnida – biodiversity – phylogenetics – Uropygi.

INTRODUCTION

The order Schizomida Petrunkevitch, 1945, commonly known as the short-tailed whipscorpions, schizomids or tartarids, is among the less diverse, or minor, arachnid orders (Harvey, 2003). It currently includes two families: the Protoschizomidae Rowland, 1975, endemic to North America, comprising two extant and one fossil genera with 16 species, and the Hubbardiidae Cook, 1899, with 65 extant genera and 345 species worldwide (Harvey, 2003, 2013; Monjaraz-Ruedas *et al.*, 2019). There are 33 genera of Hubbardiidae (Reddell & Cokendolpher, 1995; Harvey, 2013; Monjaraz-Ruedas *et al.*, 2019) in the New World.

Until quite recently, the systematics of short-tailed whipscorpions focused principally on the description of new genera and species, using a limited set of morphological character systems (Reddell & Cokendolpher, 1995; Harvey, 2003; Monjaraz-Ruedas & Francke, 2015; Monjaraz-Ruedas *et al.*, 2016, 2017). The morphological characters used in schizomid taxonomy have undergone many changes in the past decade, with the relevance of phylogenetically informative characters increasingly recognized (Monjaraz-Ruedas & Francke, 2016; Monjaraz-Ruedas *et al.*, 2016; Villarreal *et al.*, 2016). However, few phylogenetic

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analyses of the relationships among schizomids have been conducted to date, and few genera have been tested for monophyly. Five phylogenetic analyses were based solely on morphological characters (Rowland, 1975; Cokendolpher & Reddell, 1992; Monjaraz-Ruedas & Francke, 2016, 2017; Monjaraz-Ruedas *et al.*, 2017), whereas four were based mostly or entirely on DNA sequences (Harvey *et al.*, 2008; Clouse *et al.*, 2017; Harms *et al.* 2018; Abrams *et al.*, 2019).

Rowland (1975) presented the first phylogenetic analysis of schizomids, based on twelve morphological characters scored for all New World species, most of which were assigned to *Schizomus* Cook, 1899 at the time. Based on Rowland's (1975) unpublished analysis, Rowland & Reddell (1979a, b, 1980, 1981) proposed seven species groups of *Schizomus* in the New World, i.e., the *brasiliensis*, *briggsi*, *dumitrescoae*, *goodnightorum*, *mexicanus*, *pecki* and *simonis* groups. The *briggsi* group was placed sister to a monophyletic group comprising two reciprocally monophyletic subgroups, one comprising the *brasiliensis*, *dumitrescoae* and *simonis* groups, the other comprising the *goodnightorum*, *mexicanus* and *pecki* groups. Rowland & Reddell (1980, 1981) assigned sixteen species of *Schizomus* from the USA, Mexico and Guatemala to the *goodnightorum*, *mexicanus* and *pecki* groups. However, further studies by Reddell & Cokendolpher (1991, 1995) demonstrated that *Schizomus* and another schizomid genus, *Trithyreus* Kraepelin, 1899, are actually restricted to the Old World, leading Reddell & Cokendolpher (1991) to revalidate *Stenochrus* Chamberlin, 1922 and synonymize *Heteroschizomus* Rowland, 1973 with it.

After establishing that *Schizomus* does not occur in the New World, Reddell & Cokendolpher (1991, 1995) assigned Rowland's (1975) species groups to other existing genera and created new genera to accommodate the rest. Species of the *simonis* group were transferred to *Hansenochrus* Reddell & Cokendolpher, 1995; the *briggsi* group to *Hubbardia* Cook, 1899; and the *goodnightorum*, *mexicanus* and *pecki* groups to *Stenochrus*, with two species of the *mexicanus* group accommodated in *Sotanostenochrus* Reddell & Cokendolpher, 1991. *Pacal* Reddell & Cokendolpher, 1995 and *Surazomus* Reddell & Cokendolpher, 1995 were created to accommodate species of the *brasiliensis* group; and *Rowlandius* Reddell & Cokendolpher, 1995 to accommodate the *dumitrescoae* group.

When the present study began, *Stenochrus* was the most speciose schizomid genus in North America and the third most speciose in the New World (Reddell & Cokendolpher, 1995; Harvey, 2013), containing 27 species, distributed mostly in the Nearctic region, from the southern USA, through Mexico to Central America (Fig. 1). One cosmopolitan species, *Stenochrus portoricensis* Chamberlin, 1922, was reported from North, Central and South America and the Caribbean, as well as several countries in Europe (Korenko *et al.*, 2009; Christophoryová *et al.*, 2013; Harvey, 2013; Šestáková *et al.*, 2017) (Fig. 1A), where they were introduced. Ever since *Stenochrus* was redescribed by Reddell & Cokendolpher (1991), new species from Mexico and Central America have been placed within the genus, based on a single diagnostic character (in the female spermathecae, the lateral pair of lobes reduced compared to the median pair), while ignoring many other differences. Over time, *Stenochrus* became the 'junkyard' for North American schizomids, comprising a plethora of morphologically disparate species, differing in body size, male flagellar shape, setal patterns, and sexual dimorphism, including homeomorphic and heteromorphic pedipalps in males. This disparity among *Stenochrus* species, along with their diverse habitats, including caves, rainforest, tropical dry forest, and pine and oak forest above 2000 m, raised the question as to whether the genus was monophyletic.

Given the paucity of phylogenetic analyses on schizomids, it is unsurprising that the monophyly and phylogenetic relationships of *Stenochrus sensu* Reddell & Cokendolpher (1991, 1995) had never been tested, beyond the unpublished analysis of Rowland (1975), and the inclusion of exemplar species in recent analyses of schizomid phylogeny based on morphology (Monjaraz-Ruedas & Francke, 2016, 2017) or DNA sequences (Clouse *et al.*, 2017). *Stenochrus* was paraphyletic in a phylogenetic analysis of *Mayazomus* Reddell & Cokendolpher, 1995 based on 130 morphological characters (Monjaraz-Ruedas & Francke, 2016), which included exemplar species of *Stenochrus* as outgroups, along with outgroup exemplars of *Hansenochrus*, *Hubbardia* and *Rowlandius* from North, Central and South America, once included in the *Schizomus* species groups of Rowland & Reddell (1979a, b, 1980, 1981). *Stenochrus* was also paraphyletic in the molecular analysis of Clouse *et al.* (2017), based on two nuclear and two mitochondrial gene markers for 240 samples, which included several individuals of *S. portoricensis*, one *Stenochrus sbordonii* (Brignoli, 1973), and several unidentified schizomids from Mexico; *Hubbardia* grouped sister to all other hubbardiid taxa, and *S. sbordonii* sister to the remainder. In contrast, *Stenochrus* was monophyletic in a morphological phylogenetic analysis of *Olmecazomus* Monjaraz-Ruedas *et al.*, 2019, which included different exemplar species of *Stenochrus* from the *Mayazomus* analysis (Monjaraz-Ruedas & Francke, 2017), i.e. *Stenochrus pecki* Rowland, 1973 and *S. portoricensis*.

The present contribution provides the first phylogeny of *Stenochrus* and related genera, based on 61 morphological characters and 2991 aligned DNA nucleotides from two markers in the nuclear genome,

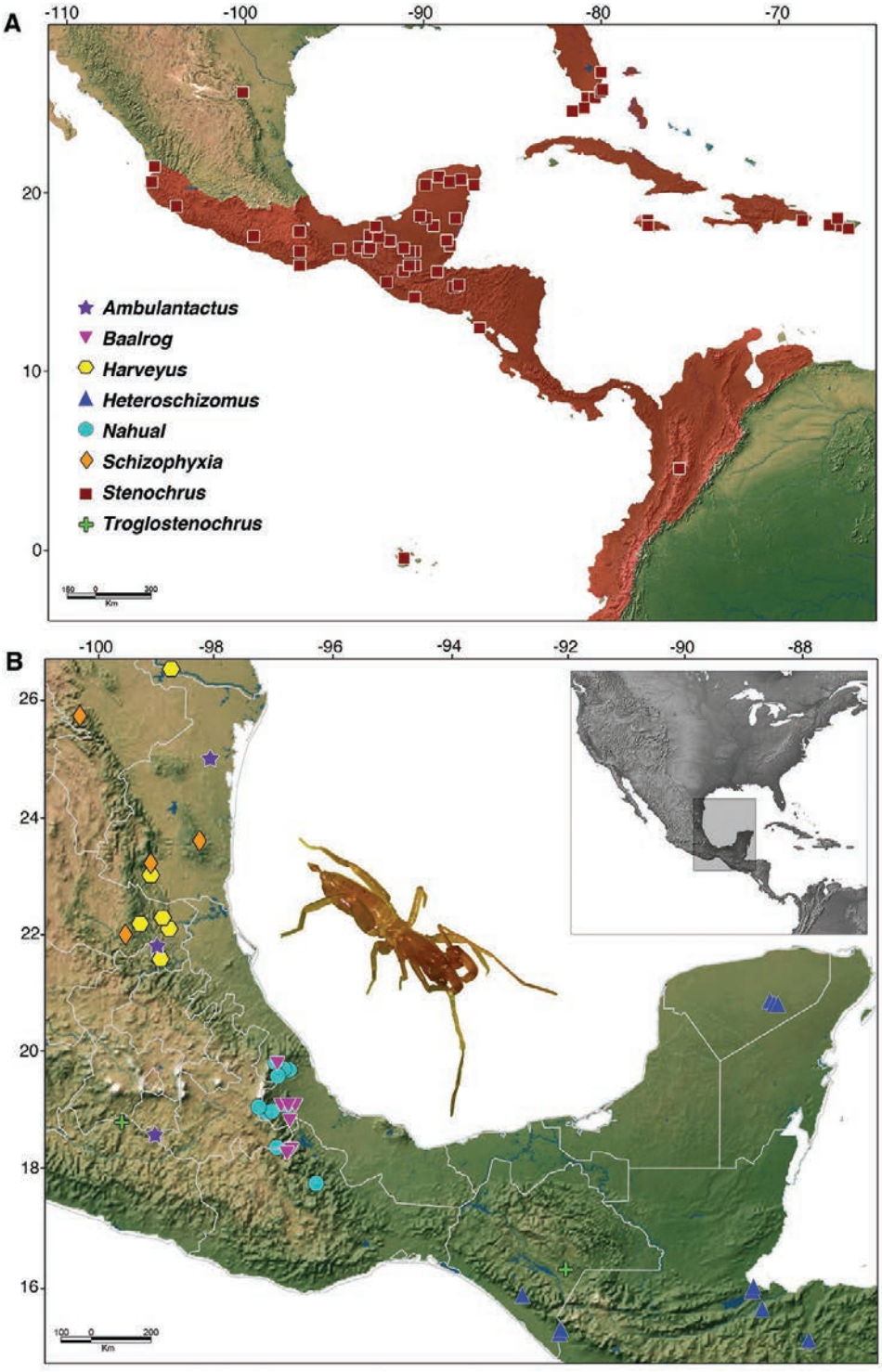

Figure 1. Map of the Caribbean, Central America and Mexico, plotting known locality records of the short-tailed whipscorpion genus *Stenochrus* Chamberlin, 1922 and related genera (Schizomida: Hubbardiidae Cook, 1899), based on data from museum collections and the literature. A, distribution of *Stenochrus* Chamberlin, 1922, as redefined by Monjaraz-Ruedas *et al.* (2019). B, distributions of *Ambulantactus* Monjaraz-Ruedas *et al.*, 2019, *Baalrog* Monjaraz-Ruedas *et al.*, 2019, *Harveyus* Monjaraz-Ruedas *et al.*, 2019, *Heteroschizomus* Rowland, 1973, *Nahual* Monjaraz-Ruedas *et al.*, 2019, *Schizophyxia* Monjaraz-Ruedas *et al.*, 2019, and *Troglostenochrus* Monjaraz-Ruedas *et al.*, 2019.

the internal transcribed spacer (ITS) and 28S rDNA, and two markers in the mitochondrial genome, 12S rDNA and cytochome *c* oxidase subunit I (*COI*), for a comprehensive sample of 73 taxa. Separate and simultaneous analyses of the morphological and molecular data sets were conducted with different optimality criteria and analytical parameters: Bayesian Inference, Maximum Likelihood and parsimony with equal and implied weighting. Terminals represented only by morphological data (termed 'orphans') were included in some analyses to test alternative phylogenetic positions. *Stenochrus sensu* Reddell & Cokendolpher (1991, 1995) was consistently polyphyletic, and comprised eight monophyletic groups, justifying its reclassification into eight genera (Fig. 1B), including *Heteroschizomus*, revalidated from synonymy under *Stenochrus*, by Monjaraz-Ruedas *et al.* (2019).

MATERIALS AND METHODS

Taxon sampling and material examined

The revised generic classification of North American schizomids proposed by Monjaraz-Ruedas *et al.* (2019), wherein *Stenochrus sensu* Reddell & Cokendolpher (1991, 1995) was redefined to include only seven species and its remaining species assigned to seven other genera, is followed in the present contribution. The ingroup consisted of 61 terminal taxa representing 26 (96%) of the 27 species formerly assigned to *Stenochrus* by Reddell & Cokendolpher (1991, 1995), Harvey (2003), Armas & Cruz-López (2009), Armas & Víquez (2010), Monjaraz-Ruedas (2012) and Monjaraz-Ruedas & Francke (2015, 2016, 2017, 2018) (Supporting Information, Appendices S1, S2). Tissue samples for DNA extraction could not be obtained for six species formerly assigned to *Stenochrus*: *Ambulantactus davisi* (Gertsch, 1940); *Heteroschizomus meambar* Armas & Víquez, 2010; *Schizophyxia bartolo* (Rowland, 1973); *Stenochrus guatemalensis* Chamberlin, 1922; *Stenochrus leon* Armas, 1995; and *Troglostenochrus palaciosi* (Reddell & Cokendolpher, 1986). *Ambulantactus davisi* is known only from the type specimens from a single locality in northern Tamaulipas, Mexico, to which access was problematic due to the prevailing lack of security. Attempts to collect fresh material of *S. bartolo*, *S. guatemalensis* and *T. palaciosi*, were unsuccessful. The type locality of *S. guatemalensis* could not be unambiguously identified (there are at least three places with the same name in that country); however, the sole female described by Chamberlin (1922) closely resembles *S. portoricensis*, suggesting it may be just another record of this species, which is already reported from Guatemala. Some specimens collected in Guatemala, with similar female spermathecae, were included in the analysis as *Stenochrus* cf. *guatemalensis*. The type locality of *T. palaciosi* was never located. The original collector could not locate the cave either during a subsequent attempt. Finally, it was impossible to collect *H. meambar* and *S. leon* from Central America. Despite the absence of molecular data, all except one of these taxa, hereafter referred to as 'orphans', were included in the simultaneous analyses of the morphological and molecular data, to test their phylogenetic positions based solely on morphology. Type specimens of all species were examined, except for *H. meambar* and *S. leon*. *Stenochrus leon* was omitted from the morphological matrix due to the absence of specimens for examination and the limited information available from the inadequate original description (Appendices 1, 2).

The outgroup comprised 18 terminal taxa representing eight of the most diverse New World genera of schizomids. Outgroup selection was intended to test the monophyly of *Stenochrus*, and guided by previous phylogenetic studies of schizomids by Rowland (1975), Monjaraz-Ruedas & Francke (2016) and Clouse *et al.* (2017) (Supporting Information, Appendices S1, S2). The analyses were rooted on *Hubbardia*, placed sister to all other New World schizomid exemplars in the phylogenies of Rowland (1975), Monjaraz-Ruedas & Francke (2016) and Clouse *et al.* (2017). The Central and South American genera *Hansenochrus* Reddell & Cokendolpher, 1995, *Rowlandius* Reddell & Cokendolpher, 1995 and *Surazomus* Reddell & Cokendolpher, 1995 were included based on the analyses of Rowland (1975) in which these genera were placed sister to a clade comprising the *goodnighthorum*, *mexicanus* and *pecki* groups of *Stenochrus sensu* Reddell & Cokendolpher (1991, 1995). The analysis of Clouse *et al.* (2017) also placed *Surazomus* sister to a clade of Central and North American schizomids. The inclusion of *Mayazomus* and *Olmecazomus* was guided by the analyses of Monjaraz-Ruedas & Francke (2016, 2017), in which *Stenochrus mexicanus* (Rowland, 1971) was placed sister to a clade comprising *Mayazomus* and *Rowlandius*. The inclusion of *Olmecazomus* and *Sotanostenochrus* was also based on their morphological similarity to *Stenochrus sensu* Reddell & Cokendolpher (1991, 1995). The shapes of the male pygidial flagella and female spermathecae of *Olmecazomus* resemble those of *Stenochrus* whereas the species of *Sotanostenochrus* resemble *Stenochrus mexicanus*, both obligate cavernicoles which are codistributed. *Pacal* was included based on morphological similarities with some species of *Rowlandius*, *Surazomus* and *Stenochrus sensu* Reddell & Cokendolpher (1991, 1995), its Neotropical distribution, and the presence of epigean and hypogean species.

Specimens were collected by hand or using an aspirator, and preserved in 80% ethanol for morphological study, with one or two preserved in 96% ethanol for DNA isolation. Material examined is deposited in the American Museum of Natural History (AMNH), New York, including the Ambrose Monell Cryocollection (AMCC); the Colección Nacional de Arácnidos (CNAN) at the Instituto de Biología, Universidad Nacional Autónoma de México (IBUNAM), Mexico City; and the Texas Memorial Museum (TMM), University of Texas, Austin.

Morphological data

A morphological data matrix, comprising 61 morphological characters scored for 79 terminal taxa (Appendices 1, 2), was prepared using Mesquite v.3.0.4 (Maddison & Maddison, 2018), and deposited in Morphobank (https://morphobank.org) with accession number P3464. Forty-three multistate and 18 binary characters were modified from Monjaraz-Ruedas & Francke (2016, 2017) and treated as non-additive (Fitch, 1971) to avoid *a priori* character state transformations. The matrix included character systems considered important for species recognition, e.g. chelicerae, propeltidium, pygidial flagellum shape, pedipalp setation, pedipalp dimorphism, enlargement of the opisthosoma, and female spermathecae. However, several characters from previous matrices (e.g. Monjaraz-Ruedas et al. 2016, 2017) were excluded, specifically those which were constant (e.g. setation of the chelicerae and opisthosoma), variable only at the family level, or highly variable at the species level (e.g. number of teeth on the cheliceral movable finger).

Morphological terminology for legs and pedipalps follows Reddell & Cokendolpher (1995); cheliceral setal nomenclature follows Lawrence (1969), as modified by Villarreal *et al.* (2016); pedipalp setal terminology follows Monjaraz-Ruedas *et al.* (2017); opisthosomal setal nomenclature follows Villarreal *et al.* (2016); pygidial flagellar setal terminology follows Cokendolpher & Reddell (1992), as modified by Harvey (1992) and Monjaraz-Ruedas *et al.* (2016); and spermathecal nomenclature follows Monjaraz-Ruedas *et al.* (2019).

Selection of gene markers

Seven gene markers which evolve at different rates, and would thus be expected to provide phylogenetic resolution at different taxonomic levels (Prendini *et al.*, 2003), were initially identified as suitable candidates for the study, i.e., 18S rDNA, the D3 region of the 28S rDNA (28S), histone H3, and the internal transcribed spacer (ITS) from the nuclear genome, and 12S rDNA (12S), 16S rDNA (16S), and cytochrome *c* oxidase subunit I (*COI*) from the mitochondrial genome. As 16S was impossible to amplify consistently with the primers available, only six of these markers were assessed for phylogenetic informativeness (López-Giráldez *et al.*, 2013), on the basis of which, four, i.e. the nuclear ITS and 28S, and the mitochondrial 12S and *COI*, were selected to reconstruct the relationships of *Stenochrus sensu* Reddell & Cokendolpher (1991, 1995) (Fig. 2, Supporting Information, Appendix S3).

DNA sequencing

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. When the extraction from leg pairs II–IV failed, the entire prosoma was used, leaving the opisthosoma, along with the flagellum and spermathecae (if applicable) intact for voucher identification.

Polymerase chain reaction (PCR) amplification was performed using standard procedures (Nishiguchi *et al.*, 2002; Prendini *et al.*, 2002, 2005), with Illustra Hot Start Mix RTG beads (GE Healthcare, Little Chalfont, Buckinghamshire) in a 25 μL reaction comprising 2L μL de-ionized water, 1 μL forward and reverse primers (Supporting Information, Appendix S3), and 2 μL DNA. The PCR program involved an initial denaturing step at 94 °C for 5 min, 35 amplification cycles (94 °C for 30 s, a variable annealing temperature for 35 s, 72 °C for 30 s), and a final step of 72 °C for 7 min, in a GeneAmp PCR System 9700 thermocycler. The annealing temperature was 54 °C for ITS and 28S, 46 °C for *COI* and 42–40 °C for 12S. PCR products were purified using an AMPure Magnetic Beads Purification System (Agencourt Bioscience, La Jolla, CA) and resuspended in 40 μL de-ionized water.

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.

Double-stranded sequences were edited and assembled into consensus sequences using Sequencher v.5.4.6 (Gene Codes Corporation, Ann Arbor, MI). A total of 301 sequences were generated from 73 samples for the study (Supporting Information, Appendix S1). The data matrix representativeness was 96%: 12S sequences were absent for six samples, ITS sequences for four samples, and *COI* sequences for one sample; 28S sequences were obtained for all samples (Supporting Information, Appendix S1).

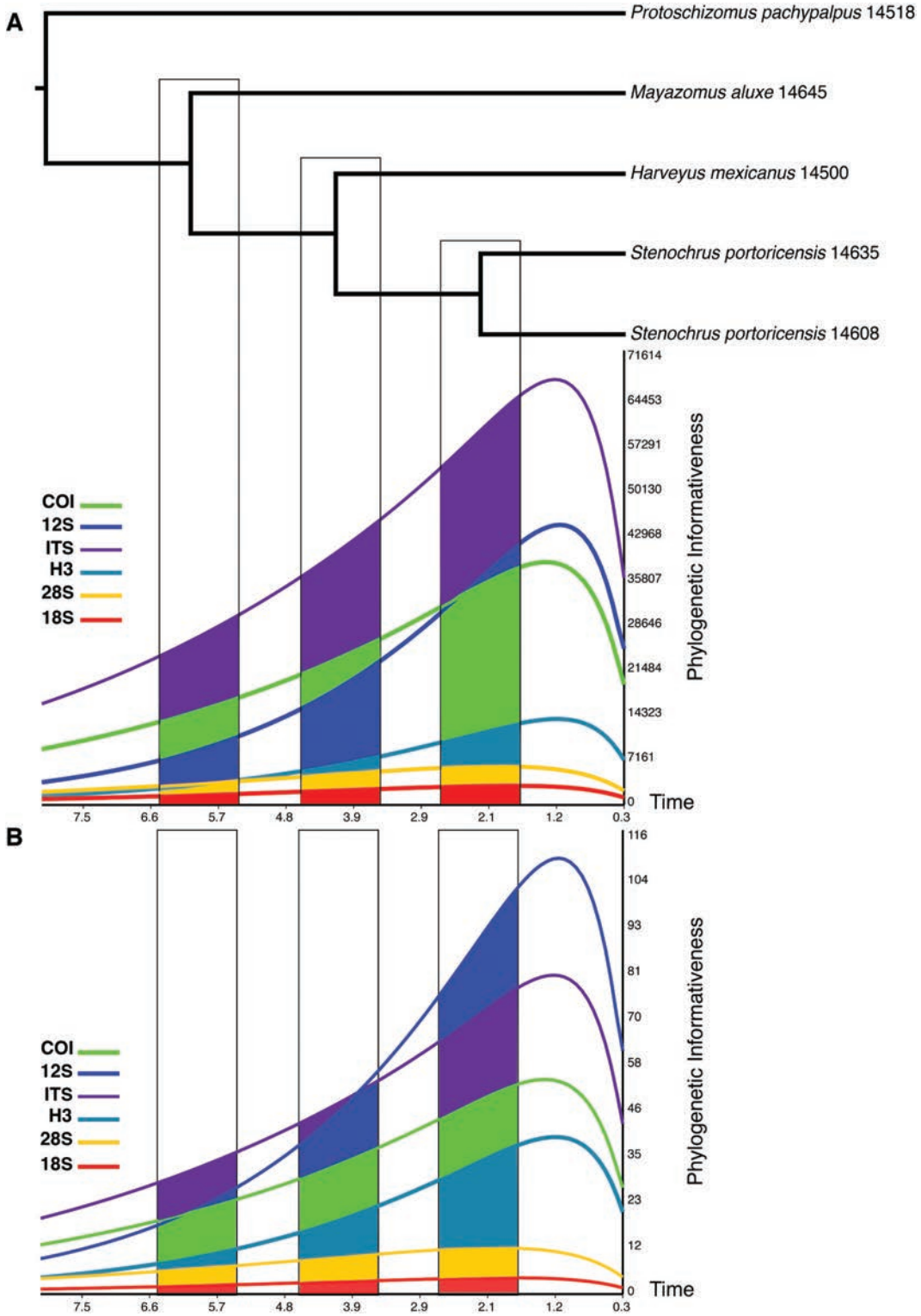

Figure 2. Phylogenetic informativeness profiles of exemplar dataset for short-tailed whipscorpion genus *Stenochrus* Chamberlin, 1922, and related genera (Schizomida: Hubbardiidae Cook, 1899). A, profile obtained for net rates (entire alignment). B, profile obtained for site rates in each gene marker. Coloured areas represent integration of the area below the curve with the highest probability of exhibiting mutations at particular levels (populations, species or genera) in the phylogeny.

Sequence alignment

Edited consensus sequences of the four gene markers were aligned using MAFFT v.6 (http://mafft.cbrc.jp/alignment/server/; Katoh *et al.* (2002, 2005, 2009)). Due to the trivial variation in sequence length and short gap openings, the G-INS-i strategy was applied for the ITS and 12S markers, and the L-INS-i strategy for the 28S and *COI* markers (Katoh *et al.*, 2005; Swain, 2018). The *COI* alignment was translated into amino acids to assess its quality by identifying stop codons in Mesquite v.3.0.4 (Maddison & Maddison, 2018).

The percentage of variable sites, conserved sites, parsimony-informative sites, nucleotide composition and transition/transversion ratios in the aligned sequences were calculated using MEGA v.7.0 (Kumar *et al.*, 2016). Calculations were conducted using the maximum composite likelihood test (mcl), applying the Tamura *et al.* (2004) substitution model (Table 1).

Phylogenetic analysis

Seven data partitions (morphology, ITS, 28S, 12S, and *COI* first, second and third codon positions) were identified with PartitionFinder v.2 (Lanfear *et al.*, 2012) using the CIPRES Science Gateway v.3.3 online portal (Miller *et al.*, 2010). jModelTest v.2.1.6 (Darriba *et al.*, 2012) was used to select the evolutionary model for each molecular partition, by comparing the Akaike Information Criterion (AIC) and the Bayesian Information Criterion (BIC) for each partition, on the basis of which, the GTR+I+G model was selected for each partition.

Phylogenetic analyses were performed using three optimality criteria, parsimony, Bayesian Inference (BI), and Maximum Likelihood (ML). Analyses were performed on four datasets, morphology and molecules analyzed separately, and two datasets in which morphology and molecules were analyzed simultaneously, one comprising 73 terminals (20 outgroup and 53 ingroup), the other comprising 79 terminals (20 outgroup and 59 ingroup), including terminals represented only by morphological data (i.e. orphans) (see Supporting Information, Appendices S1, S2).

Parsimony analyses for each data matrix were conducted with equal weighting (EW) and implied weighting (IW), applying eight values of the concavity constant (k) = 1, 5, 10, 15, 20, 30, 60 and 100 (Table 2), using TNT v.1.1 (Goloboff *et al.*, 2008). Gaps were treated as missing data. Tree search was conducted using new technology algorithms (Nixon *et al.*, 1999; Goloboff *et al.*, 2003); the command string for each search was *piwe = x; hold 80000 xmult = level 10*; where *piwe* activates the implied weighting option, *x* indicates the k value for the search, and *xmult = level 10* specifies the most stringent heuristic search strategy. Nodal support values for EW analyses were calculated using 1000 bootstrap pseudoreplicates with a removal probability of 36%, using an *xmult = level 6* search strategy. Symmetric resampling, presented as group supported/contradicted, was conducted with the post search command string *nelsen*; scores; fit*; length; resample replications 1000 sym*; for each k value, using 1000 pseudoreplicates with removal probability of 33%. IW tree selection was based on the values of Fit, Adjusted Homoplasy (AH) and Average Clade Support (ACS) (Table 2), where the tree with the best combination of values was preferred and used for comparisons with all other analyses (Table 2; Figs. 3–5).

BI analyses were conducted using MrBayes v.3.2.6 with XSEDE (Huelsenbeck & Ronquist, 2001) on the CIPRES portal. Each analysis comprised four simultaneous runs, with four chains default for 20 000 000 generations and a heat parameter of 0.10, sampling every 1000 trees. The initial 25% of sampled trees were discarded as burn-in. Effective sample size (EES > 200) for each parameter was checked in TRACER v.1.6 (Rambaut *et al.*, 2018).

ML analyses were conducted using RAxML-HPC2 v.8.2.10 with XSEDE (Stamatakis, 2014) on the CIPRES portal. Optimal trees were computed with the -f a command for rapid bootstrap analysis and search for the best scoring tree in one run, computing 1000 bootstrapping replicates, using the GTRCAT model for molecular partitions, and the MkV model, with Lewis correction for morphological data, implemented with the command *asccorr=lewis*.

Clade robustness was evaluated according to two criteria (Giribet, 2003): node or branch support, reflected as bootstrap, symmetric resampling, and posterior probability values, and clade stability or sensitivity, reflected as the recovery of clades across different optimality criteria (e.g. ML, BI and parsimony) and analytical parameters (e.g. EW and IW).

RESULTS

Sequence data

Sequences of the ribosomal 28S marker were length invariant, consisting of 511 nucleotide base pairs (bp) in all terminals. The ITS sequences varied from 639–836 bp in length, with an average of 807 bp, excluding gaps. The 12S sequences varied from 319–393 bp, with an average of 361 bp. The *COI* sequences, amplified in two fragments, were either 660 bp (one fragment) or 1077 bp (both fragments) (Table 1). Variation in the alignment length of the ITS and 12S markers had no apparent effect on topologies obtained by the separate or simultaneous analyses when different parameters, such as gap opening costs, were applied. Among the

Table 1. Genomic data statistics for aligned DNA sequences of two nuclear gene markers, 28S rDNA (28S) and internal transcribed spacer (ITS), and two mitochondrial gene markers, 12S rDNA (12S) and cytochrome *c* oxidase subunit I (*COI*) used for phylogenetic analysis of the short-tailed whipscorpion genus *Stenochrus* Chamberlin, 1922, and related genera (Schizomida: Hubbardiidae Cook, 1899): aligned length in nucleotide base-pairs (bp); number and percentage of variable positions; number and percentage of conserved (fixed) positions; number and percentage of parsimony-informative (PI) positions, including gaps; percentage nucleotide composition; percentage of transitions (ti) and transversions (tv) for each nucleotide combination and overall; transition/transversion ratio (ti:tv)

		28S	ITS	12S	*COI*	1st	2nd	3rd	Total
Length (bp)		511	971	431	1077	359	359	359	2991
Variable (%)		59 (12)	484 (50)	331 (77)	520 (48)	126 (35)	51 (14)	343 (96)	1394 (47)
Conserved (%)		452 (88)	431 (44)	83 (19)	557 (52)	233 (65)	308 (86)	16 (4)	1523 (51)
PI (%)		39 (8)	360 (37)	276 (64)	443 (41)	87 (24)	25 (7)	331 (92)	1118 (37)
A		24.8	19.7	35.6	30.9	32.9	10.1	50.21	24.8
T		18.5	18.5	41.7	37.3	26.4	45.7	39.7	18.5
C		25.0	27.4	5.5	18.9	18.2	28.8	9.4	25.0
G		31.7	34.3	17.2	12.9	22.5	15.4	0.8	31.7
ti	A↔G	29.7	22.6	52.2	7.2	11.4	16.7	31.2	29.7
	C↔T	47.7	33.7	27.8	39.4	72.5	20.6	53.5	47.7
tv	A↔C	5.6	10.3	4.1	13.2	4.1	12.2	4.6	5.6
	A↔T	4.9	8.4	7.7	18.1	4.8	17.5	6.9	4.9
	C↔G	6.4	13.5	2.3	8.4	3.3	13.9	0.8	6.4
	G↔T	5.7	11.6	5.9	13.3	3.9	19.2	3.1	5.7
ti:tv		3.3	1.2	2.9	0.8	4.7	0.8	2.2	3.3
A-T bias		43.4	38.2	77.3	68.2	59.3	55.8	89.9	43.4
C-G composition		56.7	61.8	22.7	31.8	40.7	44.1	10.1	56.7

Table 2. Tree statistics for phylogenetic analyses of the short-tailed whipscorpion genus *Stenochrus* Chamberlin, 1922, and related genera (Schizomida: Hubbardiidae Cook, 1899), using parsimony with equal weighting (EW) and implied weighting (IW): values of the concavity constant (*k*), length (L), consistency index (CI), retention index (RI), Fit, Adjusted Homoplasy (AH) and Average Clade Support (ACS) of most parsimonious trees (MPTs). Optimal tree topologies, indicated in boldface, have the highest Fit, AH and ACS values. 'Orphans' refer to simultaneous analyses including terminal taxa represented only by morphology

	k	MPTs	L	CI	RI	Fit	AH	ACS		*k*	MPTs	L	CI	RI	Fit	AH	ACS
Morphology									**Simultaneous**								
EW		8	354	0.353	0.765	30.7	–	31.5	EW		4	9301	0.267	0.574	540.4	–	58.7
IW	1	1	384	0.326	0.734	21.0	38.0	23.9	IW	1	1	9332	0.266	0.572	341.1	836.9	60.6
	3	1	357	0.350	0.762	31.6	27.4	27.4		3	1	9331	0.266	0.572	543.5	634.5	68.5
	5	1	357	0.350	0.762	37.3	21.7	31.1		5	1	9308	0.267	0.574	656.3	521.7	68.8
	10	1	354	0.353	0.765	44.6	14.4	33.3		10	1	9301	0.267	0.574	809.5	368.5	67.7
	15	2	354	0.353	0.765	48.0	11.0	33.8		**15**	**1**	**9301**	**0.267**	**0.574**	**890.6**	**287.4**	**67.7**
	20	3	351	0.356	0.768	50.3	8.7	34.1		20	1	9299	0.267	0.574	941.8	236.2	67.5
	30	3	351	0.356	0.768	52.7	6.3	34.3		30	1	9299	0.267	0.574	1003.4	174.6	67.2
	60	**3**	**351**	**0.356**	**0.768**	**55.6**	**3.4**	**34.5**		60	1	9292	0.267	0.575	1079.6	98.4	66.7
Molecules									**Orphans**								
EW		5	8872	0.266	0.567	512.4	–	59.2	EW		9	9373	0.265	0.572	535.7	–	56.0
IW	1	1	8913	0.264	0.564	324.7	794.3	59.2	IW	1	3	9406	0.264	0.57	339.2	838.8	51.1
	3	1	8894	0.265	0.565	516.4	602.6	67.9		3	3	9398	0.264	0.571	540.8	637.2	**56.2**
	5	1	8894	0.265	0.565	623.2	495.8	**69.0**		5	3	9377	0.265	0.572	653.6	524.4	57.1
	10	**1**	**8871**	**0.266**	**0.567**	**768.3**	**350.7**	**68.7**		10	4	9420	0.264	0.569	805.6	372.4	57.5
	15	1	8871	0.266	0.567	845.3	273.7	68.1		15	2	9369	0.265	0.572	888.5	289.5	57.3
	20	1	8869	0.266	0.567	894.0	225.0	67.6		20	3	9369	0.265	0.572	940.0	238.0	57.2
	30	1	8868	0.266	0.567	952.5	166.5	66.5		**30**	**2**	**9369**	**0.265**	**0.572**	**1002.8**	**175.2**	**57.1**
	60	1	8862	0.266	0.567	1026	93.9	65.0		60	3	9363	0.265	0.573	1079.2	98.8	56.6

aligned loci, 77, 50 and 48% of the sites were variable, and 64, 37 and 41% parsimony-informative, in 12S, ITS and *COI*, respectively, whereas 12% were variable, and 8% parsimony-informative in 28S (Table 1). As expected for a protein-coding gene, the third codon position of the *COI* was the most informative, with 96% of the sites variable and 92% parsimony-informative, followed by the first codon position, with 35% of the sites variable and 24% parsimony-informative (Table 1). According to the analysis of per site informativeness, 12S and ITS were the most informative markers (Fig. 2B).

The aligned ITS, 12S and *COI* sequences were concatenated together with the 28S sequences, to produce a matrix of 2991 aligned DNA nucleotides, including gaps, in which 47% of the sites were variable and 37% parsimony-informative (Table 1).

As in other arthropod taxa (DeSalle *et al.*, 1987; Prendini *et al.*, 2003; González-Santillán & Prendini, 2015) the methylation process for mitochondrial markers was evident in the schizomid sequences which demonstrated an AT-bias of 77% and 68% in the nucleotide composition of the 12S and *COI*, respectively. The nucleotide composition of the nuclear markers was more uniformly distributed with a slightly greater GC content of 61% and 56% in ITS and 28S, respectively (Table 1).

The transition/transversion ratio (ti/tv) is usually greater than two, as transitions are expected to occur more frequently than transversions (there are two kinds of transitions vs. four kinds of transversions). For example, if the value decreases to 0.5, indicating that the number of transversions is greater than expected, there is a higher probability of non-synonymous mutations, reflecting saturation of the data (DeSalle *et al.*, 1987; Wang *et al.*, 2015). The ti and tv proportions, as well as the ti/tv ratio (Table 1), confirmed a greater proportion of transitions in the ITS, 28S and 12S markers, but a more equal proportion, suggesting more saturation, in the *COI* (ti/tv ratio: 0.8). The ti/tv ratio for the different codon positions of *COI* was as expected, i.e. the first and third codon positions possessed a higher proportion of transitions, with values of 4.7 and 2.2, respectively, whereas the proportion was lower for the second codon position, with a value of 0.8 (Table 1).

Phylogenetic analysis

Stenochrus sensu Reddell & Cokendolpher (1991, 1995) was consistently polyphyletic in the analyses regardless of optimality criterion (e.g. ML, BI and parsimony), analytical parameters (e.g. EW and IW) and data combination (Figs. 3–5). Twelve clades, corresponding to four outgroup genera, *Mayazomus*, *Olmecazomus*, *Pacal* and *Sotanostenochrus*, and the eight ingroup genera defined by Monjaraz-Ruedas *et al.* (2019), i.e. *Ambulantactus*, *Baalrog*, *Harveyus*, *Heteroschizomus*, *Nahual*, *Schizophyxia*, *Stenochrus s. str.* and *Troglostenochrus*, were consistently recovered and well supported (Figs. 3, 4).

The separate morphological analyses recovered all except three of these clades, corresponding to the genera *Baalrog*, *Nahual* and *Pacal* (Fig. 4). In contrast, the separate molecular analyses with different optimality criteria recovered all except *Baalrog* and *Stenochrus s. str.*, with high support (Fig. 4).

Stenochrus s. str. was monophyletic in the analyses with ML and parsimony, albeit with weak support, but paraphyletic in the BI analyses, due to the placement of *Stenochrus alcalai* Monjaraz-Ruedas & Francke, 2018 in a polytomy comprising *Ambulantactus*, *Harveyus*, *Schizophyxia* and *Stenochrus s. str.*

Baalrog was monophyletic and highly supported in the ML and BI analyses, but rendered paraphyletic by *Schizophyxia* in the parsimony analyses. Although *Schizophyxia* was monophyletic in the ML and BI analyses, its placement, which rendered *Baalrog* paraphyletic in the parsimony analyses, was weakly supported. Whereas the topology obtained by parsimony with IW and k = 10 had the highest Fit and Adjusted Homoplasy (AH) values (Table 2), the topology obtained with k = 5, in which *Baalrog* was monophyletic and *Schizophyxia* was placed sister to *Stenochrus alcalai*, had higher Average Clade Support values.

Simultaneous analysis of the morphological and molecular data sets recovered the monophyly of all twelve clades, including the eight genera recognized by Monjaraz-Ruedas *et al.* (2019), with high support. The tree topologies obtained by the analyses with ML and BI were identical. As in the separate molecular analyses, *Baalrog* was monophyletic and highly supported in the analyses with ML and BI, but rendered paraphyletic by *Schizophyxia* in the parsimony analyses (Fig. 4). *Schizophyxia* was monophyletic and placed sister to *Harveyus* with high support in the analyses with ML and BI.

Orphans

Simultaneous analyses with the six orphan taxa included were largely congruent with the analyses from which they were excluded, and recovered the same twelve clades (Fig. 5). The tree topology obtained with ML and orphans included was otherwise identical to the topology obtained with ML and orphans excluded, except that *Ambulantactus* was placed sister to *Olmecazomus*.

Analysis with BI and orphans included recovered a similar tree topology, differing from the topology obtained with ML and orphans excluded only in the internal branches, where two polytomies were created by the unstable position of *S. bartolo* (Fig. 5). All genera

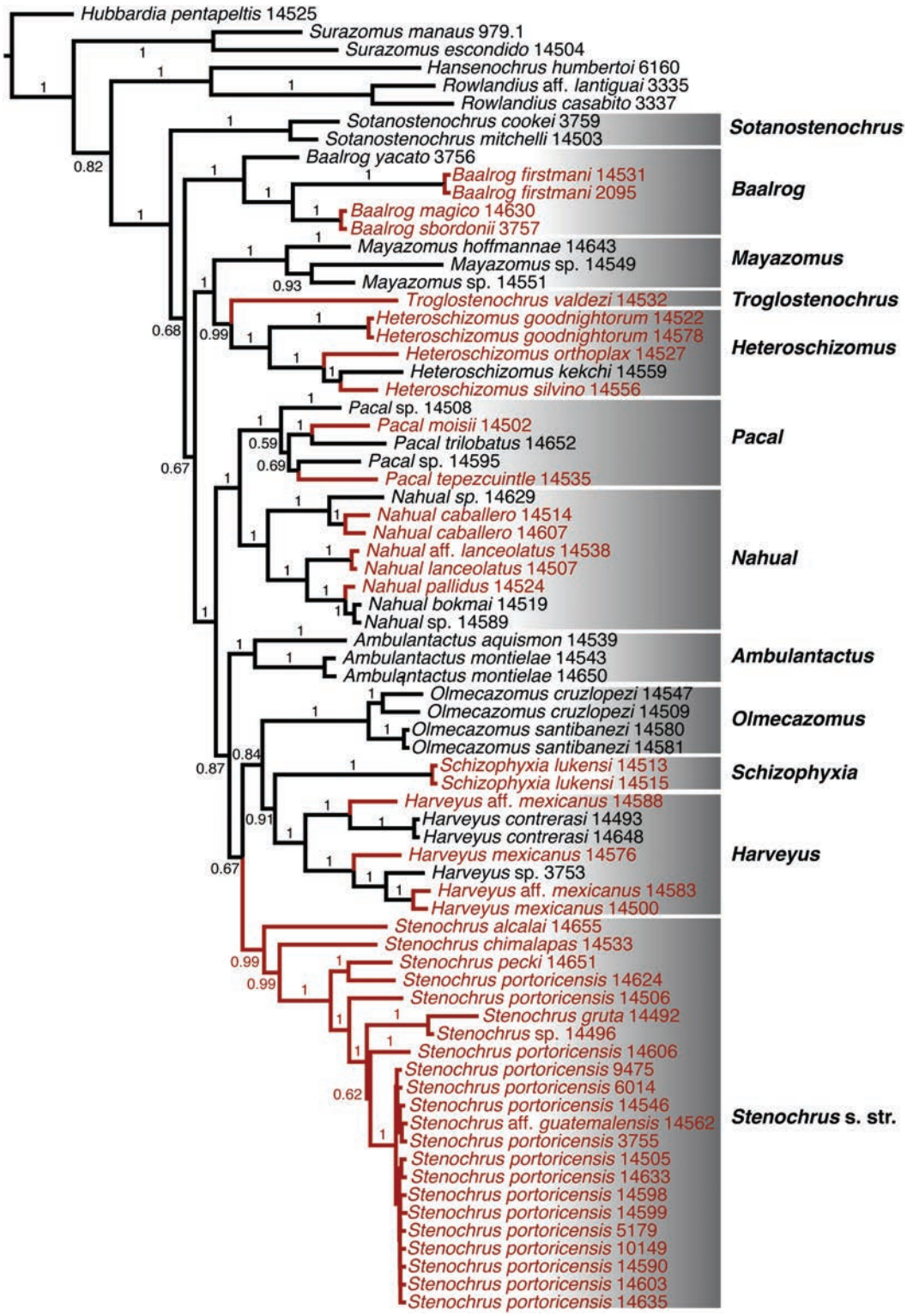

Figure 3. Phylogeny of the short-tailed whipscorpion genus *Stenochrus* Chamberlin, 1922, and related genera (Schizomida: Hubbardiidae Cook, 1899), obtained by simultaneous analysis of the morphological and molecular data with Bayesian Inference. Terminal taxa previously assigned to *Stenochrus sensu* Reddell & Cokendolpher (1991, 1995) in red. Grey areas represent clades recovered with high support values, which are congruent with the classification of Monjaraz-Ruedas *et al.* (2019). Numbers in branches represent posterior probability values.

Figure 4. Phylogeny of the short-tailed whipscorpion genus *Stenochrus* Chamberlin, 1922, and related genera (Schizomida: Hubbardiidae Cook, 1899), obtained by simultaneous analysis of the morphological and molecular data with Bayesian Inference. Nodal support and clade recovery represented as vertical bars for the Morphological (Morph), Molecular (Mol), and Simultaneous (Simul) analyses with Bayesian Inference (BI), Maximum Likelihood (ML) and parsimony with equal weighting (P). Nodal support, i.e., bootstrap (EW, ML), symmetric resampling (IW) and posterior probability (BI), for clades recovered, indicated as follows: black (> 90); dark grey (≥ 50); light grey (< 50); white (not recovered).

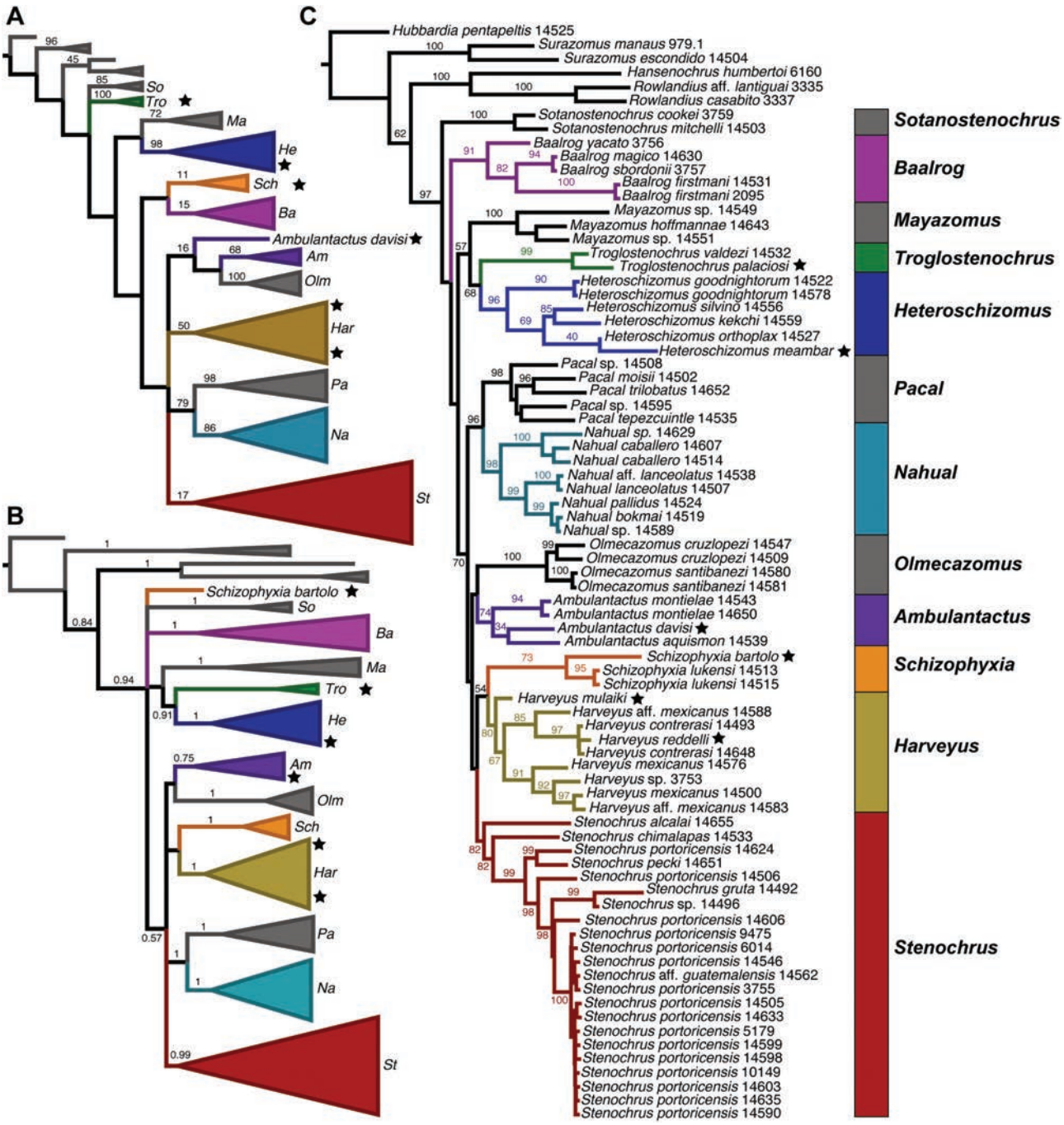

Figure 5. Phylogeny of the short-tailed whipscorpion genus *Stenochrus* Chamberlin, 1922, and related genera (Schizomida: Hubbardiidae Cook, 1899), obtained by simultaneous analysis of the morphological and molecular data including 'orphans' (i.e. terminals represented only by morphological data, indicated by stars) with parsimony (A), Bayesian Inference (B), and Maximum Likelihood (C). Nodal support values, i.e. bootstrap (parsimony, Maximum Likelihood) and posterior probability (Bayesian Inference) indicated above branches. *Am* = *Ambulantactus*; *Ba* = *Baalrog*; *Har* = *Harveyus*; *He* = *Heteroschizomus*; *Ma* = *Mayazomus*; *Na* = *Nahual*; *Olm* = *Olmecazomus*; *Pa* = *Pacal*; *Sch* = *Schizophyxia*; *So* = *Sotanostenochrus*; *St* = *Stenochrus*; *Tro* = *Troglostenochrus*.

were monophyletic with moderate or high support, except for *Schizophyxia*, rendered paraphyletic by the placement of *S. bartolo*.

Parsimony analysis with EW and orphans included recovered *Baalrog* and *Schizophyxia* monophyletic, differing from the analyses with ML and BI in the placement of *Schizophyxia* sister to *Baalrog*, rather than to *Harveyus*. Furthermore, *Troglostenochrus* was placed sister to all North American hubbardiids in the parsimony analyses, but sister to *Heteroschizomus* in the analyses with ML and BI. *Ambulantactus* was rendered paraphyletic by the orphan, *A. davisi*, placed sister to a clade comprising *Ambulantactus* and *Olmecazomus* in the parsimony analyses (Fig. 5), unlike the analyses with ML and BI, in which *Ambulantactus* was monophyletic and placed sister to *Olmecazomus*.

Parsimony analysis with IW (k = 30) and orphans included retrieved a topology largely congruent with that obtained by the analyses with ML and parsimony with EW, except for the paraphyly of *Baalrog*.

Stability and support

Separate analyses of the morphological data recovered six of the eight clades obtained by simultaneous analysis of the morphological and molecular data, regardless of optimality criterion or analytical parameters (Fig. 3), reflecting stability despite weak support. Two exceptions, which received high support, could be explained by the distinctive morphology of *Heteroschizomus* and the presence of only one of the two species of *Troglostenochrus*. Lack of resolution among the terminal branches of the tree suggests the morphological characters were insufficiently informative to resolve schizomid phylogeny at the generic level.

Clade recovery (stability) and support for terminal clades was in general high and similar in the separate analyses of the molecular data and the simultaneous analysis of the morphological and molecular data (Fig. 4). Only two genera were unstable, i.e. *Baalrog*, consistently paraphyletic in the parsimony analyses, and *Stenochrus s. str.*, paraphyletic in the separate molecular analyses with BI.

The simultaneous analyses with orphans included recovered similar topologies, but with lower support due to the missing data, than the simultaneous analyses with orphans excluded. The morphological characters were nevertheless sufficiently informative to place orphans with the most morphologically similar taxa, permitting accurate diagnoses of each genus recognized.

Phylogenetic instability was prevalent among the deeper internal nodes, especially regarding generic relationships, in all phylogenetic analyses. The tree topologies obtained using parsimony were the least congruent with those obtained using other optimality criteria.

Among the internal relationships, the clade comprising *Nahual* and *Pacal* was the most stable, recovered by all separate analyses of the molecular data and all simultaneous analyses. The clade comprising *Heteroschizomus*, *Mayazomus* and *Troglostenochrus* was also very stable, recovered by separate analyses of the molecular data and simultaneous analyses with ML, BI, and parsimony with IW, even the analysis with orphans included. Only parsimony with EW failed to recover this clade, instead placing *Troglostenochrus* sister to the clade comprising all the other New World schizomids.

One of the most unstable relationships among the analyses was the position of *Ambulantactus*. This genus was placed sister to *Stenochrus s. str.* in the separate molecular analyses with ML, sister to *Olmecazomus* in the analyses with ML, BI and parsimony with EW and orphans included, and sister to a clade comprising *Harveyus*, *Olmecazomus*, *Schizophyxia* and *Stenochrus s. str.* in the simultaneous analyses with ML and BI.

DISCUSSION

Dataset informativeness

All datasets were informative, with complex phylogenetic structure recovered by each data partition (Fig. 2). The inclusion of morphological data in a simultaneous analysis increased nodal support for all genera and facilitated the placement of orphan taxa for which molecular data were unavailable.

Unlike some arachnid taxa, in which, for example, the D3 region of the nuclear 28S rDNA gene is more conserved (Whiting *et al.*, 1997; Prendini *et al.*, 2003), this marker appears to be sufficiently variable and informative among schizomids to recover tree topologies resembling those obtained by simultaneous analyses of the morphological and molecular data.

As expected, the mitochondrial data were more informative than the nuclear data. However, the topology obtained by separate analysis of the mitochondrial data obtained a polytomy at the base of the tree, suggesting the phylogenetic signal of the mitochondrial markers was insufficient to resolve deeper relationships among these schizomids, unlike other arachnid taxa such as theraphosid spiders or harvestmen (Cruz-López & Francke, 2017; Mendoza & Francke, 2017), in which *COI* sequences alone resolve relationships at the genus level, perhaps due to the saturation (Table 1).

Optimization of the morphological characters on the various phylogenetic hypotheses (Monjaraz-Ruedas *et al.*, 2019: 15, fig. 6) assisted the identification of characters for the diagnoses of new and redefined

genera (Monjaraz-Ruedas *et al.*, 2019). Based on this assessment, characters of the female spermathecae, as well as unique character combinations recently proposed by Monjaraz-Ruedas *et al.* (2019), were found to be reasonably reliable for generic recognition.

Outgroup relationships

The present study addressed relationships within the genus *Stenochrus sensu* Reddell & Cokendolpher (1991, 1995) and focused on molecular markers considered phylogenetically informative for the terminal relationships, with predictably low support for the deeper internal nodes (Figs 2–5). A robust, well-supported phylogeny of the North American schizomid genera requires additional molecular markers with a slower mutation rate, such as 18S rDNA, additional, more conservative fragments of 28S rDNA, and/or nuclear protein-coding markers like EF-1 α and polymerase II (Fig. 2).

Despite the low support and instability of the deeper relationships in the analyses presented here, some general patterns were observed. *Surazomus* was consistently monophyletic and placed sister to all other New World schizomids, concordant with a previous phylogenetic hypothesis (Clouse *et al.*, 2017), which placed the exemplars of *Surazomus* sister to a clade comprising all New World exemplars, except for *Baalrog sbordonii* (Brignoli, 1973) and *Hubbardia pentapeltis* Cook, 1899. Caribbean and Central American species of *Hansenochrus* and *Rowlandius* included in the present analysis were also consistently separated from the clade comprising North American schizomids, as well as from the species of *Surazomus*, suggesting that the North American schizomids represent a distinct lineage from their Neotropical relatives.

Mayazomus appears to be more closely related to *Heteroschizomus* and *Troglostenochrus* than to *Pacal*, contradicting a previous morphological hypothesis (Monjaraz-Ruedas & Francke, 2017), but congruent with their distributions in southern Mexico and Central America. Additionally, *Mayazomus* shares with *Heteroschizomus* the presence of three annuli on the female pygidial flagellum. *Olmecazomus* was placed sister to *Ambulantactus*, together with *Harveyus* and *Stenochrus*, in several of the analyses presented here, again incongruent with the morphological hypothesis of Monjaraz-Ruedas & Francke (2017), according to which *Olmecazomus* was placed sister to all other Neotropical schizomids.

Ingroup relationships

Baalrog and *Sotanostenochrus* were consistently placed sister to all North American schizomids in the analyses presented here, suggesting these genera are ancient, relictual lineages restricted to cave refugia. *Baalrog* is endemic to caves in central and southern Mexico (Oaxaca and Veracruz), codistributed with species of *Nahual*, epigean representatives of a distantly related lineage. The phylogenetic position of *B. sbordonii* recovered by these analyses was incongruent with the analyses of Clouse *et al.* (2017), which placed this species (as *S. sbordonii*) sister to a clade comprising all other schizomid exemplars. Parsimony analyses of the *COI* data of Clouse *et al.* (2017) also placed *B. sbordonii* with the undetermined Mexican Hubbardiidae MCZ IZ-79707 (from Oaxaca) and MCZ IZ-79909 (from Chiapas), congruent with the analyses presented here.

A monophyletic group comprising *Nahual* and *Pacal* was among the more robust clades recovered by the simultaneous analyses and the separate analyses of the molecular data (Fig. 4). This clade is concordant with the morphology and distributions of these genera, which appear to be part of the Neotropical fauna, are mostly hypogean, and share the presence of a cheliceral lamella. The two genera did not form a monophyletic group in the separate morphological analyses, however, probably due to the extensive variation in body size, coloration and setal patterns within each genus.

The genus *Harveyus* comprises most of the species occurring in northern Mexico and the southern United States. The occurrence of several morphologically variable populations, mostly from caves along the Sierra del Abra in the Mexican state of San Luis Potosí (Rowland, 1980), suggests the existence of a species complex. Populations of *Harveyus mexicanus* (Rowland, 1971) in the Mexican state of Tamaulipas, as well as the rare *H. reddelli*, differ markedly from populations of *H. mexicanus* in the state of San Luis Potosí. *Harveyus mulaiki* and *H. reddelli* were monophyletic with respect to the other species of the genus in the simultaneous analyses with orphans included, suggesting that, despite variation in the male pygidial flagellum, the spermathecal and setal characters are informative for the diagnosis of this genus. Furthermore, some species of *Harveyus* from northern Mexico and the southern USA may need to be transferred to *Schizophyxia*, which appears to be closely related and inhabits caves across a similar distribution. However, due to the unstable position of *Schizophyxia* in the analyses presented here, the hypothesis that these genera are sister taxa awaits further testing.

Ambulantactus is also represented in Tamaulipas; however, the species of this genus differ markedly in morphology from the species of *Harveyus* and *Schizophyxia*. The disjunct distribution of this genus, along with the absence of DNA sequences of *A. davisi* could be contributing to its unstable position in the analyses. *Ambulantactus* may be related to

Harveyus and *Schizophyxia* from northern Mexico or to *Olmecazomus* and *Stenochrus s. str.* from southern Mexico.

STENOCHRUS POLYPHYLY

Stenochrus, as originally defined by Reddell & Cokendolpher (1991, 1995), was rendered polyphyletic in all analyses with different optimality criteria, analytical parameters and datasets (Figs. 3–5), by the outgroup genera *Mayazomus*, *Olmecazomus*, *Pacal* and *Sotanostenochrus*. This finding reflects the considerable disparity among North American schizomid lineages and justifies the revised classification of Monjaraz-Ruedas *et al.* (2019). The former species groups proposed by Rowland & Reddell (1980, 1981) were largely unsupported by the preferred hypotheses presented here, according to which only the previously synonymized genus, *Heteroschizomus* (i.e. the former *goodnightorum* group) was monophyletic (Figs. 3–5). The former *mexicanus* and *pecki* groups were both paraphyletic. The *mexicanus* group comprised four clades, corresponding to *Harveyus*, *Nahual*, *Schizophyxia* and *S. portoricensis* whereas the *pecki* group comprised three clades, corresponding to *Baalrog*, *Stenochrus valdezi* Monjaraz-Ruedas, 2012, recently transferred to *Troglostenochrus* by Monjaraz-Ruedas *et al.* (2019), and the remaining species of *Stenochrus*, i.e. *Stenochrus gruta*, *S. guatemalensis* and *S. pecki*. The remaining species of the *mexicanus* group, i.e. *Stenochrus moisii* (Rowland, 1973) and *Stenochrus tepezcuintle* Armas & Cruz-López, 2009, which differ morphologically from other species of *Stenochrus*, appear to be more closely related to *Pacal* (Figs. 3, 4), in which both species were tentatively placed by Monjaraz-Ruedas *et al.* (2019).

As redefined by Monjaraz-Ruedas *et al.* (2019), *Stenochrus* is a Neotropical genus, distributed from southern Mexico (Oaxaca, Chiapas and the Yucatán Peninsula) to Guatemala, Honduras and Nicaragua, with most of its species occurring in the Mexican state of Oaxaca. All Caribbean representatives of the genus form part of the *S. portoricensis* complex, which requires comprehensive sampling across its broad distribution to clarify its phylogenetic position, and determine whether it actually occurs in North America, or even in continental America, or is restricted to the Caribbean. Furthermore, the different populations of *S. portoricensis* will need to be further investigated, as preliminary results indicate deep genetic divergences (> 10%) among populations of this species from Mexico, Central America and the Caribbean. Some of the sequences identified as *S. portoricensis* in the present study appear to be more closely related to other species of the genus, e.g. *S. gruta* and *S. pecki*, than to populations from the vicinity of the type locality, Puerto Rico, suggesting they may even be different species with a conserved or homoplastic morphology. Many populations of *Stenochrus* aff. *portoricensis* appear to be parthenogenetic, and the lack of males hinders species delimitation based solely on morphology within the complex.

CONCLUSIONS

The study presented here investigated the relationships among a neglected junkyard genus of North American short-tailed whipscorpions, using different optimality criteria, analytical parameters and data sets, and assessed the usefulness of morphological characters traditionally used for schizomid systematics.

Based on the analyses presented here, the genus *Stenochrus* is polyphyletic as originally defined by Reddell & Cokendolpher (1991, 1995), comprising eight monophyletic groups supported by both morphological and molecular data, despite similar, often convergent morphology. A combination of characters permitted the diagnosis and reclassification of those clades into eight genera by Monjaraz-Ruedas *et al.* (2019). Some former species groups proposed by Rowland (1975) and Rowland & Reddell (1980, 1981) were recovered as monophyletic by the analyses, e.g. the former *goodnightorum* group, leading to the revalidation of *Heteroschizomus*; whereas others, e.g. the *mexicanus* and *pecki* groups, were not.

Despite its redefinition by Monjaraz-Ruedas *et al.* (2019), the limits of *Stenochrus s. str.* remain uncertain. The genus consists of at least two distinct lineages, in addition to the typical forms, and the wide distribution of its type species, *S. portoricensis*, and uncertain status of several of its populations merit additional data and analysis.

Further assessment, incorporating additional data, is also needed to corroborate the genus *Schizophyxia*, recognized by Monjaraz-Ruedas *et al.* (2019) based on a monophyletic group recovered by the simultaneous analyses with BI and ML. Indeed, as the present study makes clear, the systematics of the entire order is in dire need of more extensive and rigorous analysis.

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APPENDIX 1. MORPHOLOGICAL CHARACTERS USED FOR PHYLOGENETIC ANALYSIS OF THE SHORT-TAILED WHIPSCORPION GENUS *STENOCHRUS* CHAMBERLIN, 1922, AND RELATED GENERA (SCHIZOMIDA: HUBBARDIIDAE COOK, 1899). CHARACTERS OPTIMIZED WITH ACCTRAN, EXCEPT WHERE INDICATED OTHERWISE. CHARACTER MATRIX IN APPENDIX 2.

Chelicerae

0. Fixed finger, prolateral surface, G3 setae position: seta G3-4 posterior (0); setae G3-2 and G3-4 posterior (1); seta G3-3 anterior (2). [DELTRAN]

1. Fixed finger, prolateral surface, number of G5 setae: ≤ 8 (1); ≥ 9 (2).

2. Movable finger, mesal surface, margin: smooth (0); lamella (1); dentate (2). [DELTRAN]

Prosomal propeltidium

3. Anterior process setation: 1 + 1 (0); 2 + 1 (1).

4. Dorsal pairs of setae, number: 2 (0); 3 (1); 4 (2).

Prosomal metapeltidium

5. Metapeltidium: divided (0); entire (1).

Opisthosoma

6. Segments X–XII (♂): not elongate (0); elongate (1).

7. Segment XII, posterodorsal process (♂): absent (0); present (1).

8. Tergite II, number of setae: 2 (0); > 2 (1).

Pedipalps

9. Pedipalps, development (♂): homeomorphic (0); elongated (1); robust (2).

Pedipalp trochanter

10. Apical process: acute (0); acuminate (1); obtuse (2); bump (3); fan-shaped (4); digitiform (5); rounded (6).

Pedipalp femur

11. Retrolateral surface, seta Fe_1, type: acuminate (0); spiniform (1); macrosetae (2).

12. Retrolateral surface, seta Fe_5, type: acuminate (0); spiniform (1); spiniform setiferous tubercle (2); macrosetae (3).

13. Retrolateral surface, seta Fv_1, type: acuminate (0); spiniform (1); spiniform setiferous tubercle (2); macrosetae (3).

14. Retrolateral surface, seta Fv_2, type: acuminate (0); spiniform (1); spiniform setiferous tubercle (2); macrosetae (3).

15. Retrolateral surface, spiniform setiferous tubercles, position: Fv_1 and Fv_2 distal (0); Fv_1 ventral, Fv_2 distal (1); Fv_1 and Fv_2 ventral (2). [DELTRAN]

16. Prolateral surface, anterior margin, apophysis: absent (0); present (1).

17. Prolateral surface, ventral row of setae ($Fmv_{1–4}$), number: 3 (0); 4 (1).

Pedipalp patella

18. Curvature: slight (0); marked (1); none (2).

19. Retrolateral row of setae (*Pe*), count: 3 (0); 4 (1); 5 (2); 2 (3).

20. Retrolateral row of setae (*Pe*), type: acuminate (0); feathered (1); spiniform (2); macrosetae (3).

21. Prolateral row of setae (*Pm*), count: 3 (0); 4 (1); 5 (2); 6 (3).

22. Prolateral row of setae (*Pm*), type: acuminate (0); feathered (1); spiniform (2); macrosetae (3).

Pedipalp tibia

23. Spurs: absent (0); ventral (1); proventral (2).

24. Retrolateral row of setae (*Ter*), count: 3 (0); 4 (1); 5 (2); 6 (3).

25. Retrolateral row of setae (*Ter*), type: (0) acuminate (0); feathered (1); spiniform (2); macrosetae (3).

26. Medial row of setae (*Tmr*), count: 3 (0); 4 (1); 5 (2); 6 (3).

27. Medial row of setae (*Tmr*), type: acuminate (0); feathered (1); spiniform (2); macrosetae (3).

28. Prolateral row of setae (*Tir*), count: 3 (0); 4 (1); 5 (2); 6 (3).

29. Prolateral row of setae (*Tir*), type: acuminate (0); feathered (1); spiniform (2); macrosetae (3).

Male flagellum

30. Shape, dorsal view: lanceolate (0); cordate (1); spatulate (2); subrhomboidal (3); shovel shaped (4); elliptical (5); trilobed (6); bulbous or clavate (7); spear shaped (8); deltoid (9). [DELTRAN]

31. Shape, lateral view: slender (flat) (0); elliptical (1); bulbous (2).

32. Dorsal depressions: absent (0); pair of pits (1); single depression (2); pair of depressions (3); depression and pits (4).

33. Dorsal depressions, position: medial (0); submedial (1); anterior (2); posterior (3).

34. Dorsal projections: flat (0); pair of projections (1); single projection (2).

35. Dorsal projections, position: medial (0); submedial (1). [DELTRAN]

36. Seta Dm_1, position with respect to anterior margin: posterior (0); aligned with margin (1).

37. Seta Dm_4, position with respect to seta Dl_3: anterior to (0); aligned with (1).

38. Setae Dl_1, position with respect to setae Vl_1: aligned with (0); posterior to (1); anterior to (2).

39. Setae Dl_3, position with respect to setae Vl_2: aligned with (0); posterior to (1); anterior to (2).

40. Seta Vm_1, position with respect to setae Vm_2: aligned with (0); posterior to (1); anterior to (2).

Female flagellum

41. Flagellomeres, count: 3 (0); 4 (1).

42. Setae Dl_1, position with respect to setae Vl_1: aligned with (0); posterior to (1); anterior to (2).

43. Setae Dl_3, position with respect to setae Vl_2: aligned with (0); posterior to (1); anterior to (2).

Spermathecae

44. Lobes, number of pairs: 1 (0); 2 (1); 3 or more (2).

45. Median lobes, shape: linear (0); arch shaped (1); inverse J-shaped (2).

46. Median lobes, ornamentation: sclerotized (0); bulbs (1); smooth (2).

47. Median lobes, sclerotization: apically (0); half of lobe (1); entire lobe (3).

48. Median lobes, bulbs size: large (0); small (1).

49. Median lobes, apex orientation: ental (0); ectal (1); vertical (2).

50. Median lobes, base position relative to bases of lateral lobes: aligned with (0); anterior to (1); posterior to (2).

51. Lateral lobes, shape: linear (0); arch shaped (1); inverse J-shaped (2).

52. Lateral lobes, ornamentation: sclerotized (0); smooth (1).

53. Lateral lobes, apex orientation: ental (0); ectal (1); vertical (2).

54. Lateral lobes, length compared with median lobes: equal (0); 3/4 (1); 1/2 (2); 1/4 (3); longer (4).

55. Lobes, relative widths: equal (0); lateral lobes wider than median lobes (1); median lobes wider than lateral lobes (2).

56. Lobes, symmetry: symmetric (0); asymmetric (1).

57. Chitinized arch, shape: arrow shaped (0); mug shaped (1); V-shaped (2); hastate (3); bowl shaped (4); inverse arc (5); obtuse triangle (6); U-shaped (7); absent (8).

58. Chitinized arch, anterior branch: present (0); absent (1).

59. Chitinized arch, lateral tip shape: pointed (0); lobed (1); widened (2).

60. Gonopod: absent (0); present (1).

APPENDIX 2. DISTRIBUTION OF 61 MORPHOLOGICAL CHARACTERS AMONG INGROUP AND OUTGROUP TAXA USED IN PHYLOGENETIC ANALYSIS OF THE SHORT-TAILED WHIPSCORPION GENUS *STENOCHRUS* CHAMBERLIN, 1922, AND RELATED GENERA (SCHIZOMIDA: HUBBARDIIDAE COOK, 1899). TERMINALS WITH MISSING DATA (?) OR INAPPLICABLE CHARACTERS (-) WERE SCORED IN ALL ANALYSES. CHARACTER DESCRIPTIONS IN APPENDIX 1.

Ambulantactus aquismon 14539	21001 10000 20000 -0020 02101 01121 91230 -1010 0???? ????? ????? ????? ?
Ambulantactus davisi	21001 10000 00000 -0020 01101 00111 91330 -1012 00??? ????? ????? ????? ?
Ambulantactus montielae 14543 14650	01001 10000 00000 -0020 01101 01121 91230 -1011 12101 02--2 10101 00510 1
Baalrog firstmani 2095 14531	02001 10000 60000 -0020 01100 00121 720-2 00010 12111 22--1 00013 20312 1
Baalrog magico 14630	21001 10000 30000 -0021 01100 03121 720-0 -0010 12011 22--1 01011 20312 1
Baalrog sbordonii 3757	02001 10000 60000 -0021 02100 00121 ????? ????? ?2011 200-1 00113 20512 1
Baalrog yacato 3756	02001 10000 40000 -0021 02100 00121 51210 -1010 1???? ????? ????? ????? ?
Hansenochrus humbertoi 6160	21001 11100 60000 -0021 01001 01111 000-1 00021 11?21 ????? ????? ?0300 1
Harveyus aff. *mexicanus* 14583 14588	21000 10001 30000 -0021 01110 00111 31310 -1010 12101 11-11 01111 00212 1
Harveyus contrerasi 14493 14648	21000 10000 20000 -0021 01100 00111 31310 -1011 02101 11-11 00111 20512 1
Harveyus mexicanus 14500 14576	21000 10001 30000 -0021 01110 00111 31310 -1010 12101 11-11 01111 00212 1
Harveyus mulaiki	21000 10001 30000 -0021 01100 00111 71310 -1120 12??? ????? ????? ????? ?
Harveyus reddelli	21000 10000 20000 -0021 01100 00111 00310 -1011 02??1 11-11 00111 20512 1
Harveyus sp. 3753	21000 10001 30000 -0021 01110 00111 31310 -1010 12101 11-11 01111 00212 1
H. goodnightorum 14522 14578	21101 11000 00000 -0020 01100 00111 20220 -0011 02101 02--2 10100 00101 1
Heteroschizomus kekchi 14559	21102 11000 00000 -0020 01100 00111 20120 -1021 12011 02--2 10100 00101 1
Heteroschizomus meambar	??101 11100 00000 -0020 00100 00111 20220 -1022 10??1 002?0 00000 00101 1
Heteroschizomus orthoplax 14527	21102 11000 00000 -0020 00100 00111 20120 -1021 1???? ????? ????? ??1?? ?
Heteroschizomus silvino 14556	01102 11000 00000 -0020 00000 00111 20120 -1021 12011 02--2 10102 00101 1
Hubbardia pentapeltis 14525	22011 01100 01111 -0122 21102 22232 00120 -1021 01012 ----- ----- 01511 1
Mayazomus hoffmannae 14643	01000 10012 10022 11011 01021 00111 11330 -0011 11101 12--0 01113 10510 1
Mayazomus sp. 14551	01000 10012 10222 01010 01021 00101 ?1?30 ?0010 01011 12??0 01113 10710 1
Mayazomus sp. 14549	01000 10012 10222 01010 01021 00101 ?1-30 -0010 01011 12--0 01113 10710 1
Nahual aff. *lanceolatus* 14538	22101 11000 01111 -0122 02103 02131 00120 -1011 12111 002?2 00010 00001 1
Nahual bokmai 14519	11101 10000 41111 -0121 02102 02131 51110 -1001 12011 002-2 00000 00001 1
Nahual caballero 14514 14607	21201 10000 20011 -0020 01101 00121 51110 -1011 12111 02--2 00100 00001 1
Nahual lanceolatus 14507	22101 10000 01111 -0122 02103 02131 00120 -1011 12111 002-2 00010 00001 1
Nahual pallidus 14524	21101 10000 00011 -0022 02102 02131 51110 -1011 12??1 002-2 00000 00001 1
Nahual sp. 14629	21101 10000 20000 -0021 01100 00121 ????? ????? ?2??1 12--1 20010 20000 1
Olmecazomus cruzlopezi 14509 14547	12000 10002 50022 20023 21201 21112 81330 -1010 02101 101-0 20103 10611 1
Olmecazomus santibanezi 14580 14581	12000 10002 50022 20023 21201 21112 81330 -1010 02101 101-0 20103 10611 1
Pacal moisii 14502	01001 10000 60000 -0020 01100 00121 31111 11000 12011 01-12 00103 10510 1
Pacal sp. 14508	??101 10100 10000 -002? ????? ????? 62100 -1010 0???? ????? ????? ????? ?
Pacal sp. 14595	21101 10100 10000 -0020 01101 00121 51110 -1000 02111 21-01 00103 10511 1
Pacal tepezcuintle 14535	21101 10000 60000 -0021 01100 00121 31101 11010 12101 22--1 21112 00511 1
Pacal trilobatus 14652	01101 10100 10000 -0020 01100 00111 62101 01010 02210 01-02 ----- -0500 0
Rowlandius casabito 3337	22101 10101 10000 -0021 00000 30111 110-1 10000 01?21 01-02 -1124 00300 1
Rowlandius aff. *lantiguai* 3335	22101 10101 60000 -0021 00000 30111 110-1 11010 01?21 01-12 11124 00??? 1
Schizophyxia bartolo	01001 10000 01100 -0021 01100 00111 720-0 -1010 12001 001-2 02111 00610 1
Schizophyxia lukensi 14513 14515	01001 10000 01100 -0021 01100 00111 81310 -1011 12101 12--1 00121 00710 1
Sotanostenochrus cookei 3759	01001 10001 30000 -0020 01110 00122 42211 10020 12012 ----- ----- 11610 1
Sotanostenochrus mitchelli 14503	01001 10001 30000 -0020 01110 00122 42210 -0020 12012 ----- ----- 11610 1
Stenochrus alcalai 14655	21000 10000 00000 -0020 01100 00121 11410 -1010 12111 201-1 00012 00400 1
Stenochrus aff. *guatemalensis* 14562	??000 10000 ????? ????? ????? ????? ????? ????? ?2111 201-1 20112 00400 1
Stenochrus chimalapas 14533	01000 10001 60000 -0020 00100 00111 51410 -1010 12001 201-1 20112 00411 1
Stenochrus gruta 14492	01000 10001 30000 -0022 01100 00111 11410 -1010 02111 101-1 20002 00412 1
Stenochrus pecki 14651	22000 10000 61111 -0020 00100 00111 51410 -0000 12011 101-1 20012 00402 1
Stenochrus portoricensis 3755 5179 10149 14505 14506 14546 14590 14598 14599 14603 14606 14624 14633 14635 9475 6014	01000 10000 60000 -0020 01100 00111 51400 -1000 12111 201-1 20012 00401 1
Stenochrus portoricensis 14624	01000 10001 60000 -0020 00100 00111 51400 -1000 12111 201-1 20012 00401 1
Stenochrus sp. 14496	01000 10001 60000 -0022 01100 00111 11410 -1010 02111 22--1 20102 00510 1
Surazomus manaus	??001 10102 50000 -000?? ??0?? ???? 62302 10121 12??1 01-12 00100 008-- 0
Surazomus sp. 14504	01001 00102 50000 -0000 00100 00010 62302 10011 02011 01-12 00100 008-- 0
Troglostenochrus palaciosi	01201 10000 40011 -0021 21100 01121 620-1 10110 12001 02--1 00111 00300 1
Troglostenochrus valdezi 14532	21201 10000 40011 -0121 21100 00111 620-1 11100 12011 22--1 10111 10300 1

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Tissue samples 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 genus *Stenochrus* Chamberlin, 1922, and related genera (Schizomida: Hubbardiidae Cook, 1899). Samples deposited in the Ambrose Monell Cryocollection (AMCC) at the American Museum of Natural History (AMNH), New York, and Colección Nacional de Arácnidos at Instituto de Biología, Universidad Nacional Autónoma de México (IBUNAM), Mexico.

Appendix S2. Material examined for phylogenetic analysis of the short-tailed whipscorpion genus *Stenochrus* Chamberlin, 1922, and related genera (Schizomida: Hubbardiidae Cook, 1899), deposited in the AMCC and the Division of Invertebrate Zoology at the AMNH, New York, and the Colección Nacional de Arácnidos at IBUNAM.

Appendix S3. Primers used to amplify DNA sequences of two nuclear gene markers, the internal transcribed spacer (ITS) and 28S rDNA (28S), and two mitochondrial gene markers, 12S rDNA (12S) and cytochrome *c* oxidase subunit I *(COI)*, for phylogenetic analysis of the short-tailed whipscorpion genus *Stenochrus* Chamberlin, 1922, and related genera (Schizomida: Hubbardiidae Cook, 1899).