Molecular systematics of the *Dichopetala* genus group (Orthoptera: Phaneropteridae)

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ABSTRACT. Introduction: The *Dichopetala* genus group was proposed recently after revision of the genus *Dichopetala* Brunner von Wattenwyl, 1878. Currently, the group consists of eight genera and 44 species distributed from Southern United States to Southern Mexico. This generic arrangement was based solely on morphological evidence, and was accompanied by discussions on new erected genera, for which their monophyly was not tested. **Objective:** To assess the phylogenetic relationships among representative species of the eight genera of the *Dichopetala* group. **Methods:** We generated DNA sequences for one mitochondrial (Cytochrome oxidase I: COI) and two nuclear (28S, Histone III: H3) gene markers, and included species of other Phaneropterinae genera to test the monophyly of the ingroup; Bayesian and maximum likelihood evolutionary models were used. **Results:** The monophyly of the *Dichopetala* group and the monophyly of genera *Dichopetala, Obolopteryx, Planipollex, Mactruchus* and *Rhabdocerca* is supported. In addition, *Acanthorintes* and *Pterodichopetala* were recovered as paraphyletic. The mitochondrial markers also suggest that the widely distributed genera *Rhabdocerca* and *Acanthorintes* may actually contain various overlooked species. **Conclusions:** The first contribution on the Phylogeny of the *Dichopetala* group, and a more robust phylogenetic and morphological definition of some of the genera involved are provided.

Key words: Dichopetaline genera, molecular analyses, monophyly, morphology.

The subfamily Phaneropterinae (Orthoptera: Tettigonioidea) was described by Burmeister (1838); currently, it comprises 33 tribes and *c.*315 *genera* distributed worldwide (Cigliano, Braun, Eades, & Otte, 2018). Among Tettigonioidea, Phaneropterinae is perhaps the subfamily with the highest abundance and species richness in Mexico, with records for the country ranging from 156 to 163 species (Barrientos-Lozano, 2004; Fontana, Buzzetti, & Mariño-Pérez, 2008; Barrientos-Lozano, Rocha-Sánchez, Buzzetti, Méndez-Gómez, & Horta-Vega, 2013b). For more than a century, classification of Phaneropterinae relied exclusively on morphological features (Brunner von Wattenwyl, 1878; Rehn & Hebard, 1914; Cohn, Swanson, & Fontana, 2014). However, as in other orthopteran taxa (*e.g.* De Jesús-Bonilla,

Barrientos-Lozano, & Zaldívar-Riverón, 2017; Sanabria-Urbán, Song, Oyama, González-Rodríguez, & Cueva Del Castillo, 2017), species delimitation and recognition of supraspecific groups in this subfamily have been considerably problematic. The latter due to the existence of species complexes in an apparent initial state of differentiation, and the recent discovery of several new species and genera for the subfamily (Arce-Pérez & Morón, 2000; Walker, Forrest, & Spooner, 2003; Rocha-Sánchez, Barrientos-Lozano, Zaldívar-Riverón, & Almaguer-Sierra, 2016; Heller et al., 2017).

Dichopetala Brunner von Wattenwyl, 1878 is a genus of Phaneropterinae that was largely neglected in taxonomic studies. Until few years ago, it comprised 19 described species but only six were closely related according to morphological evidence (Cohn et al., 2014). Recently, Cohn et al. (2014), carried out a taxonomic revision for members of the genus *Dichopetala*, confirming the presence of various morphologically distinct groups. The authors pointed out that "if all the *Dichopetala* species were included in that genus, there would only be a single diagnostic character, the females' subgenital plate". They also indicated that male genital structures (*e.g.*, cerci, supranal plate, epiproct, phallic complex, and subgenital plate) exhibited extensive variation, and thus they could not be used to define the genus. Based on this, the above authors proposed the *Dichopetala* genus group (temporary name), to include two previously recognized genera, *Dichopetala* and *Pterodichopetala*, plus six new erected genera: *Obolopteryx, Planipollex, Rhabdocerca, Gymnocerca, Mactruchus,* and *Acanthorintes* (Cohn et al., 2014) (Table 1). Currently, the *Dichopetala* group includes eight genera and 44 species (Cigliano et al., 2018).

The use of molecular markers, both nuclear and mitochondrial, have shed light on the phylogenetic relationships of a large number of insect groups at different taxonomic levels, including orthopterans (*e.g.* Mugleston, Song, & Whiting, 2013; Song, 2015; De Jesús-Bonilla et al., 2017; Kensinger, Schwemm, & Luttberg, 2017; Sanabria-Urbán et al., 2017;

Grzywacz, Lehmann, Chobanov, & Lehmann, 2018). In this study we aimed to investigate the phylogenetic relationships among species of the eight representative genera that belong to the *Dichopetala* group to test both the monophyly of the group as well as of its genera. We generated sequences of one mitochondrial (Cytochrome oxidase I: COI) and two nuclear (28S, Histone III: H3) markers. We also included representative specimens from various populations of some species with wide geographic distribution in order to explore whether they actually represent composite taxa.

MATERIALS AND METHODS

Examined material: Specimens of the *Dichopetala* group and other Phaneropterinae were collected in different localities along the Mexican territory from 2000 to 2018 (Table 2). Determination to species level was carried out using relevant literature and resources online (Fontana et al., 2008; Barrientos-Lozano, Ramírez-Núñez, Rocha-Sánchez, Horta-Vega, & Almaguer-Sierra, 2013a; Barrientos-Lozano et al., 2013b; Cohn et al., 2014; Cigliano et al., 2018). We generated sequences for 60 specimens of the *Dichopetala* group. These specimens were assigned to 25 taxa out of the 44 currently described species. The remaining sequences belong to 35 morphospecies that could not be assigned to any of the described species. These morphospecies correspond to the genera that have the widest geographic distribution (*Acanthorintes, Obolopteryx, Planipollex, Pterodichopetala* and *Rhabdocerca*).

We also included 36 specimens belonging to ten additional genera of Phaneropterinae to test the monophyly of the *Dichopetala* group (Table 2). These genera are as follows: *Amblycorypha* Stål, 1873, *Stilpnochlora* Stål, 1873, *Philophyllia* Stål, 1873, *Petaloptera* Saussure, 1859, *Microcentrum* Scudder, 1862, *Pycnopalpa* Serville, 1838, *Turpiliodes* Hebard, 1932, *Scudderia* Stål, 1873, *Insara* Walker, 1869 and *Vellea* Walker, 1869. Published Sequences of *Conocephalus* sp., of the Conocephalinae subfamily, were used to root

TABLE 1

Taxonomic organization of the *Dichopetala* group

Genus	Species
Acanthorintes Cohn, Swanson and Fontana, 2014	*A. erythrephaptor* Cohn, Swanson and Fontana, 2014
	A. neomexicanus (Barrientos-Lozano and Ramírez-Núñez, 2013)
	A. tauriformis (Rehn and Hebard, 1914)
	A. thenarocercus Cohn, Swanson and Fontana, 2014
	A. xanthephaptor Cohn, Swanson and Fontana, 2014
	A. zeuglaius Cohn, Swanson and Fontana, 2014
Dichopetala Brunner von Wattenwyl, 1878	*D. mexicana* Brunner von Wattenwyl, 1878
Gymnocerca Cohn, Swanson and Fontana, 2014	*G. cycloprista* Cohn, Swanson and Fontana, 2014
	G. enaulites Cohn, Swanson and Fontana, 2014
	G. falcata (Rehn and Hebard, 1914)
Mactruchus Cohn, Swanson and Fontana, 2014	*M. cryothermastris* Cohn, Swanson and Fontana, 2014
	M. durangensis (Rehn and Hebard, 1914)
	M. ischnodus Cohn, Swanson and Fontana, 2014
	M. megasynactor Cohn, Swanson and Fontana, 2014
	M. serrifer (Rehn and Hebard, 1914)
Obolopteryx Cohn, Swanson and Fontana, 2014	*O. brevihastata* (Morse, 1902)
	O. castanea (Rehn and Hebard, 1914)
	O. catinata (Rehn and Hebard, 1914)
	O. *emarginata* (Brunner von Wattenwyl, 1878)
	O. eurycerca Barrientos-Lozano and Rocha-Sánchez, 2016
	O. gladiator (Rehn and Hebard, 1914)
	O. huastecana Barrientos-Lozano and Rocha-Sánchez, 2016
	O. nigra Barrientos-Lozano and Rocha-Sánchez, 2016
	O. oreoeca (Rehn and Hebard, 1914)
	O. poecila (Hebard, 1932)
	O. seeversi (Strohecker, 1941)
	O. tamaholipana Barrientos-Lozano and Rocha-Sánchez, 2016
	O. tanchipae Barrientos-Lozano and Rocha-Sánchez, 2016
	O. truncoangulata Barrientos-Lozano and Rocha-Sánchez, 2015
Planipollex Cohn, Swanson and Fontana, 2014	*P. pollicifer* (Rehn and Hebard, 1914)
Pterodichopetala Buzzetti, Barrientos-Lozano and Rocha-Sánchez, 2010	*P. alfredoi* Barrientos-Lozano and Rocha-Sánchez, 2013
	P. cieloi Buzzetti, Barrientos-Lozano and Rocha-Sánchez, 2010
	P. cultricerca (Strohecker, 1945)
	P. geovanyi Barrientos-Lozano and Rocha-Sánchez, 2015
	P. hypsibates Cohn, Swanson and Fontana, 2014
	P. monternach Barrientos-Lozano and Zaldívar-Riverón, 2015
	P. padrisima Cohn, Swanson and Fontana, 2014
	P. pityophila Cohn, Swanson and Fontana, 2014
	P. robertoi Barrientos-Lozano and Rocha-Sánchez, 2015
	P. strepsidactyla Cohn, Swanson and Fontana, 2014
	P. tuliensis Barrientos-Lozano and Rocha-Sánchez, 2015
Rhabdocerca Cohn, Swanson and Fontana, 2014	*R. caudelli* (Rehn and Hebard, 1914)
	R. tridactyla (Rehn and Hebard, 1914)
	R. zanclophora Cohn, Swanson and Fontana, 2014

Cohn et al., 2014.

 Rev. Biol. Trop. (Int. J. Trop. Biol. ISSN-0034-7744) Vol. 67(6): 1431-1448, December 2019

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TABLE 2

Taxa of the *Dichopetala* group and other genera of Phaneropterinae included in the phylogenetic analyses

Tribe	Group	Genus	species	Collection locality	Voucher
Odonturini	*Dichopetala*	*Acanthorintes*	xanthephaptor	San José de Iturbide, Guanajuato	P006
			zeuglaius	Tula, Tamaulipas	P013
			sp. 1	San José de Iturbide, Guanajuato	P030
			sp. 2	Guadalcazar, San Luis Potosí	P050
			sp. 3	Apaseo el Alto, Guanajuato	P051
			sp. 4	Querétaro, Querétaro	P061
			sp. 5	Guadalcazar, San Luis Potosí	P062
			sp. 6	Polotitlán, Estado de México	P064
			sp. 7	El Marques, Querétaro	P065
			neomexicanus	Soto La Marina, Tamaulipas	P208
		Dichopetala	mexicana	Teloloapan, Guerrero	P004
			mexicana	Tepexi de Rodríguez, Puebla	P049
		Gymnocerca	enaulites	Mártir de Cuilapan, Guerrero	P007
		Mactruchus	serrifer	Jalisco, Guadalajara	P008
			serrifer	Ixtlán del Rio, Nayarit	P066
		Obolopteryx	castanea	Hidalgo, Tamaulipas	P001
			truncoangulata	Palmillas, Tamaulipas	P002
			nigra	Tula, Tamaulipas	P026
			poecila	Tula, Tamaulipas	P027
			sp. 1	Victoria, Tamaulipas	P079
			sp. 2	Victoria, Tamaulipas	P080
			sp. 3	Mante, Tamaulipas	P081
			sp. 4	Valles, San Luis Potosí	P082
			eurycerca	Hidalgo, Tamaulipas	P173
		Planipollex	chirura*	Antiguo Morelos, Tamaulipas	P012
			pollicifer	Gómez Farías, Tamaulipas	P003
			sp. 1	Huejutla de Reyes, Hidalgo	P029
			sp. 2	Naranjos Amatlán, Veracruz	P202
		Pterodichopetala	cieloi	Gómez Farías, Tamaulipas	P016
			hypsibates	Galeana, Nuevo León	P023
			monternach	Guadalcazar, San Luis Potosí	P020
			padrisima	Arteaga, Coahuila	P195
			robertoi	Galeana, Nuevo León	P186
			robertoi	Galeana, Nuevo León	P188
			tuliensis	Tula, Tamaulipas	P018
			sp. 1	Galeana, Nuevo León	P025
		Rhabdocerca	caudelli	Tula, Tamaulipas	P009
			zanclophora	Guadalcazar, San Luis Potosí	P043
			sp. 1	Concepción del Oro, Zacatecas	P010
			sp. 2	Arteaga, Coahuila	P031
			sp. 3	San Pedro, Nuevo León	P032
			sp. 4	Santiago, Nuevo León	P033
			sp. 5	San Luis Potosí, San Luis Potosí	P036
			sp. 6	Cedral, San Luis Potosí	P037
			sp. 7	Victoria, Tamaulipas	P039
			sp. 8	Arteaga, Coahuila	P041
			sp. 9	Cerritos, San Luis Potosí	P042

Tribe	Group	Genus	species	Collection locality	Voucher
			sp. 10	Iturbide, Nuevo León	P045
			sp. 11	Santiago, Nuevo León	P057
			sp. 12	Palmillas, Tamaulipas	P058
			sp. 13	Cerritos, San Luis Potosí	P059
			sp. 14	Galeana, Nuevo León	P060
			sp. 15	Galeana, Nuevo León	P068
			sp. 16	Iturbide, Nuevo León	P174
			sp. 17	Iturbide, Nuevo León	P176
			sp. 18	Iturbide, Nuevo León	P177
			sp. 19	Iturbide, Nuevo León	P178
			sp. 20	Galeana, Nuevo León	P182
Amblycoryphini		*Amblycorypha*	sp. 4	Guadalcazar, San Luis Potosí	P111
			sp. 5	Victoria, Tamaulipas	P114
			sp. 6	Ocampo, Tamaulipas	P115
			sp. 8	Ocampo, Tamaulipas	P110
			sp. 10	Huejutla de Reyes, Hidalgo	P098
			sp. 11	Gómez Farías, Tamaulipas	P099
			sp. 12	Gómez Farías, Tamaulipas	P100
Insarini		*Insara*	*abbreviata*	Teloloapan, Guerrero	P053
			covilleae	Cedral, San Luis Potosí	P076
			prasina	Jalisco, Guadalajara	P094
			tolteca	Xilitla, San Luis Potosí	P075
Microcentrini		*Microcentrum*	*rhombifolium*	Gómez Farías, Tamaulipas	P117
			rhombifolium	San Felipe Orizatlán, Hidalgo	P198
			stylatum	Jalisco, Guadalajara	P093
			stylatum	Huejutla de Reyes, Hidalgo	P197
			syntechnoides	Gómez Farías, Tamaulipas	P073
			syntechnoides	Gómez Farías, Tamaulipas	P116
			syntechnoides	Gómez Farías, Tamaulipas	P168
			sp. 1	Jalisco, Guadalajara	P086
			sp. 2	Guadalcazar, San Luis Potosí	P118
		Petaloptera	*zendala*	Gómez Farías, Tamaulipas	P105
		Philophyllia	*guttulata*	Gómez Farías, Tamaulipas	P104
Pycnopalpini		*Pycnopalpa (P.)*	*bicordata*	San Andrés Tuxtla, Veracruz	P055
Scudderiini		*Scudderia*	*fasciata*	Antiguo Morelos, Tamaulipas	P121
			furcata furcata	Galeana, Nuevo León	P184
			furcata furcifera	Jalisco, Guadalajara	P088
			mexicana	Hidalgo, Tamaulipas	P170
			sp. 1	San Luis Potosí	P120
			sp. 2	Gómez Farías, Tamaulipas	P122
			sp. 3	Victoria, Tamaulipas	P119
		Vellea	*mexicana*	San Felipe Orizatlán, Hidalgo	P200
Steirodontini		*Stilpnochlora*	*azteca*	Arroyo Seco, Querétaro	P194
			quadrata	Gómez Farías, Tamaulipas	P106
			thoracica	Huejutla de Reyes, Hidalgo	P107
			sp. 1	Gómez Farías, Tamaulipas	P103
			sp. 2	Gómez Farías, Tamaulipas	P169
	Turpiliae	*Turpiliodes*	*mexicanum*	San Felipe Orizatlán, Hidalgo	P199

*Species under discussion.

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the trees (28S: KX429798, H3: KX429889, COI: HQ609222). This subfamily has been recovered as sister to Phaneropterinae (Mugleston et al., 2016).

The examined material is deposited in the Orthoptera collection of the Tecnológico Nacional de México, Instituto Tecnológico de Ciudad Victoria (TecNM-ITCV) and the Colección Nacional de Insectos (CNIN), Instituto de Biología, Universidad Nacional Autónoma de México (IB-UNAM). Specimens were photographed in the field and in the laboratory to confirm their identification. Diagnostic characters taken into account to assign specimens to the different taxa are as follows: in both males and females, shape and size of fastigium of vertex and fastigium frontalis, pronotum dorsal and lateral view; in males, the stridulatory file, cerci in dorsal and ventral view, subgenital plate, and internal genitalia (titillators and phallic complex); in females, subgenital plate, ovipositor and the ovipositor's lobe (Buzzetti, Barrientos-Lozano, & Rocha-Sánchez, 2010; Barrientos-Lozano et al., 2013a; Barrientos-Lozano & Rocha-Sánchez, 2013; Cohn et al., 2014; Barrientos-Lozano, Rocha-Sánchez, & Correa-Sandoval, 2015; Rocha-Sánchez, Barrientos-Lozano, & Zaldívar-Riverón, 2015; Barrientos-Lozano, Rocha-Sánchez, Zaldívar-Riverón, & Correa-Sandoval, 2016; Cigliano et al., 2018). Photographs taken in the field were made using a professional camera Nikon D3000. Photographs of diagnostic characters were taken in the laboratory with a MOTIC stereomicroscope SMZ-168 equipped with a 10 mp digital camera.

Laboratory protocol: Genomic DNA was extracted for each specimen from muscle tissue of the hind leg. DNA was extracted with Chelex (Chelex®-100 Bio-Rad) following the protocol mentioned in Zaldívar-Riverón et al. (2006). We also used the DNeasy Bloody Tissue kit (QIAGEN®: Austin, USA) to extract DNA from dry specimens following the protocol mentioned by the manufacturer. Genomic DNA was preserved at -4 °C until it was employed for amplification.

We sequenced two nuclear markers: 330 bp of the protein coding H3 and 725 bp of the ribosomal 28S DNA genes. We also obtained 384 bp of the mitochondrial cytochrome oxidase I (COI) DNA gene. The primers employed to amplify the above gene fragments were: H3: H3F (5'-ATGGCTCGTACCAAGCA-GACVGC-3') and H3R (5'-ATATCCTTRGG-CATRATRGTGAC-3') (Colgan et al., 1998); 28S: 28SFwd (5'-GCGAACAAGTAAC-CGTGAGGG-3') (Belshaw & Quicke, 1997) and 28SRev (5'-GGAGTGCGGAGGCCGC-CGCCCMC-3') (De Jesús-Bonilla et al., 2017); COI: LCO (5'-GTCAACAAATCATAAAGA-TATTGG-3') and HCO (5'-TAAACTTCA-GGGTGACCAAAAAATCA-3') (Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994).

Amplification of the selected markers was carried out using the programs described in previous studies (H3: Colgan et al., 1998; 28S: Whiting, 2002; COI: Svenson & Whiting, 2004). PCRs were carried out in 15 μL of total volume containing 10x PCR Buffer (1.5 μL), MgCl2 (0.75 μL), deoxyribonucleotides (dNTP) (0.3 μL), primers (10 μM) (0.24 μL each), Taq DNA polymerase (Bioline) (0.12 μL), 1 μL of DNA and ddH2O (10.85 μL). PCR products were sequenced at the Genomic Sequencing Laboratory of Biodiversity and Health of the IB-UNAM. Sequences were edited with Sequencher 4.1.4 (Genecodes Corporation, 2011). The COI and H3 alignment were carried out manually with the program PAUP* (Swofford, 2002) and inspected translating them to amino acids with the program Mesquite version 2.75 (Maddison & Maddison, 2011). The 28S marker was aligned manually.

Phylogenetic analyses: Bayesian and maximum likelihood (ML) phylogenetic analyses were performed with the programs MrBayes version 3.2.6 (Ronquist & Huelsenbeck, 2003) and RAxMLversion 8 (Stamatakis, 2014). Thirty-four specimens could not be sequenced for COI. We therefore analyzed the following two concatenated matrices: *(i)* 28S + H3, with 94 terminal taxa (*CONC94*); and *(ii)* 28S + H3 + COI, with 60 terminal taxa (*CONC60*). One

partition was considered for 28S and three for each coding protein genes according to their codon positions. The following models were selected for each partition with the program Partition Finder version 2.1.1 (Lanfear et al., 2012): 28S, GTR + G; H3_post1, GTR + I; H3_post2, JC + I; H3_post3, GTR + I + G; COI_post1, GTR + I + G; COI_post2, SYM + G; COI_post3, F81 + I.

The Bayesian analyses had two simultaneous runs and were carried out for 50 million generations each, using four independent Markov Chains, sampling trees every 1 000 generations and with a burnin of 0.25. The resulting trees were used to build a phylogram with posterior probabilities of clades, considering the clades as significantly supported if they had a posterior probability (PP) value 0.95 (e. g. De Jesús-Bonilla et al., 2017). For the ML method, the best tree and its associated bootstrap values were carried out simultaneously. The bootstrap support values were calculated from 1 000 replicates, with the estimation of the fixed rate model, using the GTRGAMMA model and random trees as seed. Clades were considered as significantly supported if they had a Bootstrap value (BTP) ≥ 70 (Hillis & Bull, 1993).

RESULTS

Phylogenetic relationships: The Bayesian and ML phylograms recovered similar topologies (Fig. 1, Fig. 2). The topologies derived from all analyses recovered two major clades, one with all members of the *Dichopetala* group with strong support (PP = 1.0, BTP = 84-85); the second containing the remaining Phaneropterinae genera (PP = 0.7-1, BTP = 43-56) (Fig. 3, Fig. 4), which was significantly supported by the Bayesian analyses but had a low BTP value.

The Bayesian and ML phylograms derived from the *CONC60* matrix recovered the highest number of significantly supported clades (36 and 25 nodes with PP and BTP ≥ 0.95 and 70, respectively). The Bayesian and ML phylogram obtained with the *CONC94* matrix had on the other hand 26 and 19 significantly supported clades (PP ≥ 0.95, BTP ≥ 70). In this phylogram, *Dichopetala*, *Obolopteryx*, *Planipollex* and *Mactruchus* were recovered as monophyletic in the latter two analyses (PP ≥ 0.90, BTP = 62-91). *Obolopteryx* was recovered as sister to *Planipollex* (PP = 0.99, BTP = 48). *Mactruchus* appeared as sister to *Obolopteryx* and *Planipollex*, though with low support (PP = 0.35, BTP = 12). *P. pollicifer* and *P. chirura*, appeared nested in two different clades though they are apparently morphologically similar. Nonetheless, cerci, subgenital plate, supranal plate and stridulatory file show consistent inter-specific differences (Fig. 5, Fig. 6). *Pterodichopetala* was not recovered as monophyletic in this analysis with the *CONC94* matrix, since two of its species appeared nested as sister to the remaining members of the *Dichopetala* group, though the relationships involved were weakly supported.

The Bayesian and ML phylograms with both data sets recovered *Acanthorintes* as paraphyletic, by having *A. neomexicanus* (*CONC60*) and *A. xanthephaptor* (*CONC60* and *CONC94*) nested separately. *Rhabdocerca* was recovered as monophyletic in the Bayesian and ML phylogram with the *CONC60* matrix (PP= 0.92, BTP= 1), with its taxa being divided into three subclades. The first incorporates *R. caudelli*, *R.* sp. 9, *R.* sp. 13 and *R. zanclophora*, which are distributed in the Central Mexican Plateau (PP= 1, BTP= 71). The second accommodates species that occur in the Mexican Altiplano and the eastern portion of the Sierra Madre Oriental (SMO) (*Rhabdocerca* spp. 5, 7, 6, 15 and 14, PP= 1, BTP= 61). The third subclade encompasses species from the northernmost portion of the SMO (*R.* sp. 16, *Rhabdocerca* spp. 19 and 18, *Rhabdocerca* spp. 17 and 10, PP= 1, BTP= 99) (Fig. 7). In the Bayesian and ML phylogram with the *CONC94* matrix (PP= 0.40, BTP= 21), *Rhabdocerca* was recovered as paraphyletic, with *A. neomexicanus* deeply nested into this clade.

All phylograms recovered a clade with seven morphospecies originally assigned to *Acanthorin tes*. The latter represents various
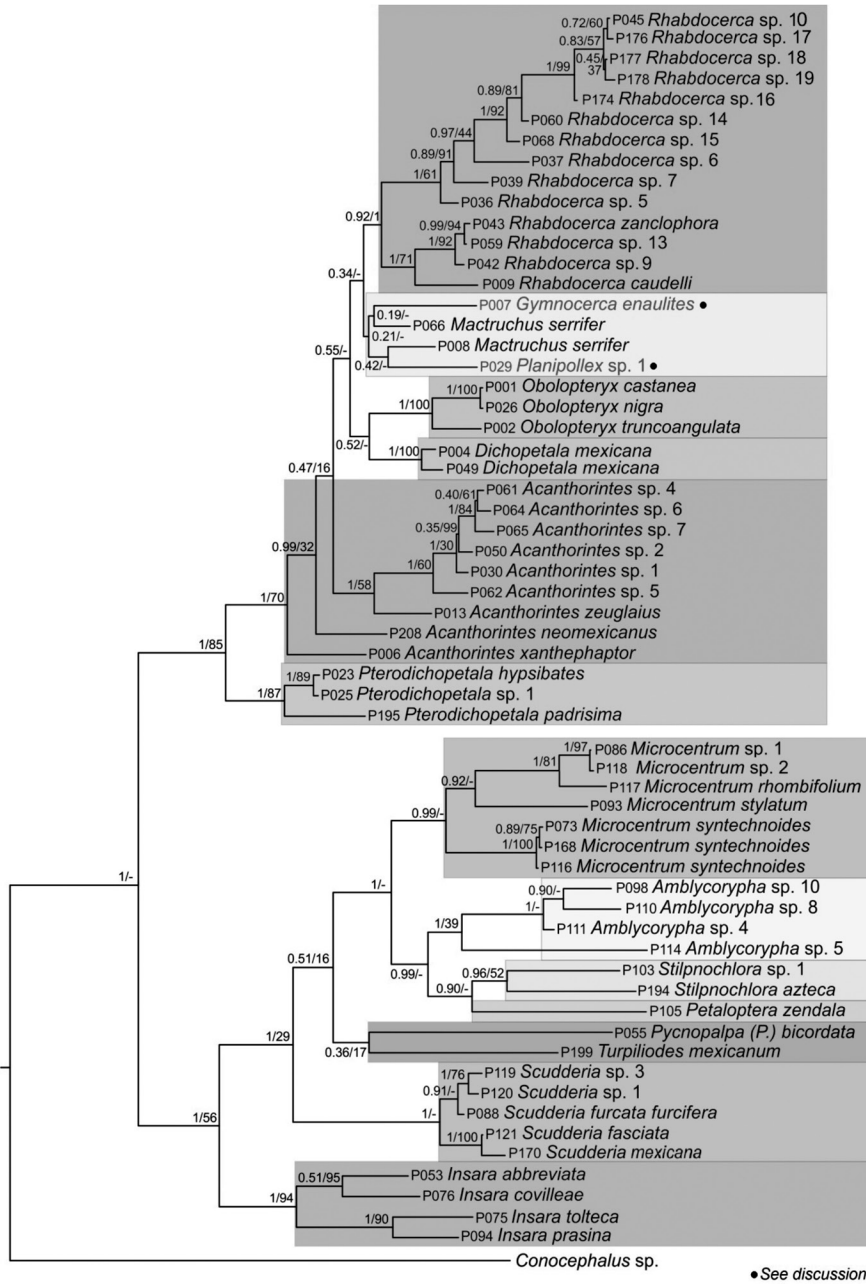
Fig. 1. Bayesian phylogram derived from the *CONC94* matrix. The two values on each branch represent Bayesian posterior probability / ML bootstrap values, respectively. Species name in red indicates that its position in the clade does not agree with what is reported taxonomically.

Fig. 2. Bayesian phylogram derived from the *CONC60* matrix. The two values on each branch represent Bayesian posterior probability / ML bootstrap values. Species name in red indicates that its position in the clade does not agree with what is reported taxonomically.

Rev. Biol. Trop. (Int. J. Trop. Biol. ISSN-0034-7744) Vol. 67(6): 1431-1448, December 2019

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Fig. 3. Genera of the *Dichopetala* group. A. *Acanthorintes zeuglaius* Cohn, Swanson & Fontana, 2014. B. *Planipollex pollicifer* (Rehn & Hebard, 1914). C. *Pterodichopetala tuliensis* Barrientos-Lozano & Rocha-Sánchez, 2015. D. *Obolopteryx castanea* (Rehn & Hebard, 1914). E. *Rhabdocerca caudelli* (Rehn & Hebard, 1914). F. *Mactruchus serrifer* (Rehn & Hebard, 1914).

separate species based on both their morphological features (Fig. 8) and sequence based tree topologies (*Acanthorintes* spp. 5, 2, 1, 6, 4, 7, 3). The followings relationships are well supported (*CONC60* matrix): *A.* sp. 5, San Luis Potosí; *A.* sp. 1, Guanajuato; *A.* sp. 7, Querétaro (PP = 1, BTP = 30-84). The Bayesian and ML phylogram (*CONC94* matrix), recovered *Acanthorintes* spp. 5, 2, 1, as closely related to *A. zeuglaius* (PP = 0.89, BTP = 52). *Acanthorintes* sp. 4 was recuperated as sister to

A. sp. 6 (PP = 0.91, BTP = 60) and *A.* sp. 3 as sister to *Acanthorintes* sp. 7 (PP = 0.65, BTP = 38). In all phylograms *Acanthorintes* was closely related to the *Pterodichopetala* clade (*CONC60:* PP = 1, BTP = 85; *CONC94:* PP = 67, BTP = 19). Regarding the Phaneropterinae clade *Insara, Scudderia, Microcentrum,* and *Amblycorypha* were recovered as monophyletic in all phylograms (PP ≥ 0.92; BTP = 40-97), with *Insara* as sister to the remaining Phaneropterinae genera (PP ≥ 0.93; BTP ≥ 45).
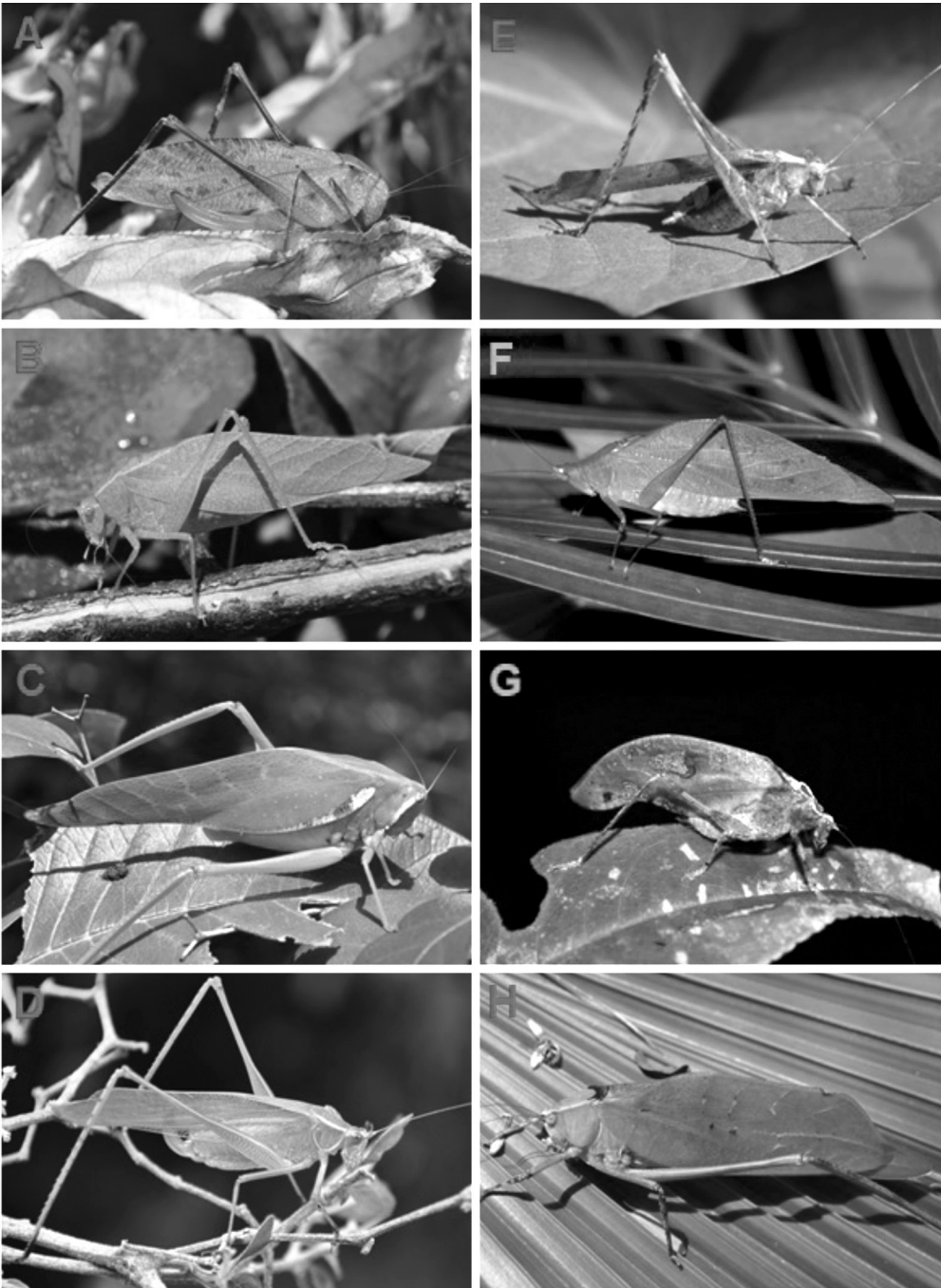
Fig. 4. Other Phaneropterinae genera included in the analyses. A. *Amblycorypha huasteca* (Saussure, 1859). B. *Microcentrum rhombifolium* (Saussure, 1859). C. *Philophyllia guttulata* Stål, 1873. D. *Scudderia furcata furcata* Brunner von Wattenwyl, 1878. E. *Insara tolteca* (Saussure, 1859). F. *Petaloptera zendala* (Saussure, 1859). G. *Pycnopalpa* (*Pycnopalpa*) *bicordata* (Saint-Fargeauy Serville, 1825). H. *Stilpnochlora thoracica* (Serville, 1831).
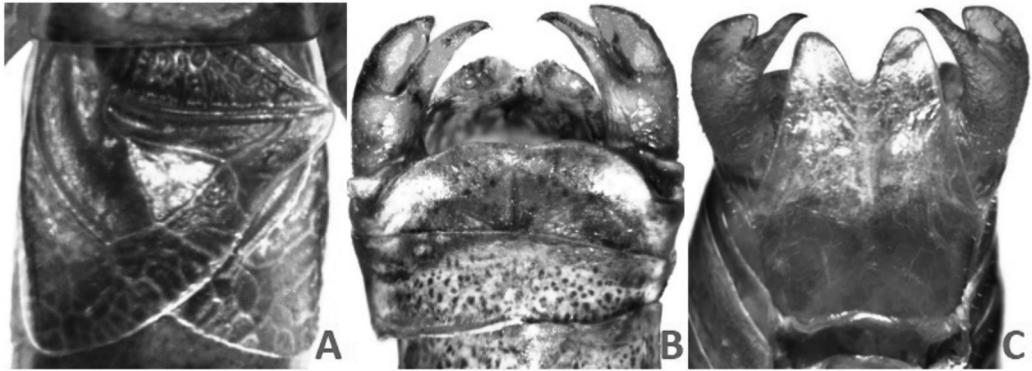
Fig. 5. *Planipollex pollicifer* male. **A.** stridulatory area; **B.** cerci and terminal tergite, dorsal view; **C.** cerci and subgenital plate, ventral view.

Fig. 6. *Planipollex chirura* male. **A.** stridulatory area; **B.** cerci and terminal tergite, dorsal view; **C.** cerci and subgenital plate, ventral view.

Amblycorypha and *Stilpnochlora* were also recovered as sister taxa (PP ≥ 0.99; BTP ≥ 51).

DISCUSSION

This work represents the first contribution on the phylogeny of the *Dichopetala* group members. Our phylogenetic analyses confirmed the monophyly of this genus group with respect to the rest of the Phaneropterinae taxa included in the analyses and support its generic classification as proposed by Cohn et al. (2014). These authors also consider that the *Dichopetala* group may represent an independent tribe from the rest of the tribe Odonturini. Although, in this study we did not include other taxa of the Odonturini, our results herein obtained on the monophyly of the *Dichopetala* group suggest its separation from other Odonturini as proposed by Cohn et al. (2014) based on a single morphological feature, the females' subgenital plate. This feature is divided into two separated sclerotized lobes by a medial-longitudinal membrane. According to Cohn et al. (2014), this character is shared by all genera of the *Dichopetala* group and none of other Phaneropterinae studied possesses it. They also consider that the females' subgenital plate represents an apomorphic character since it is unique to members of the *Dichopetala* group. Accordingly, other genera of Odonturini possess a simple triangular female subgenital
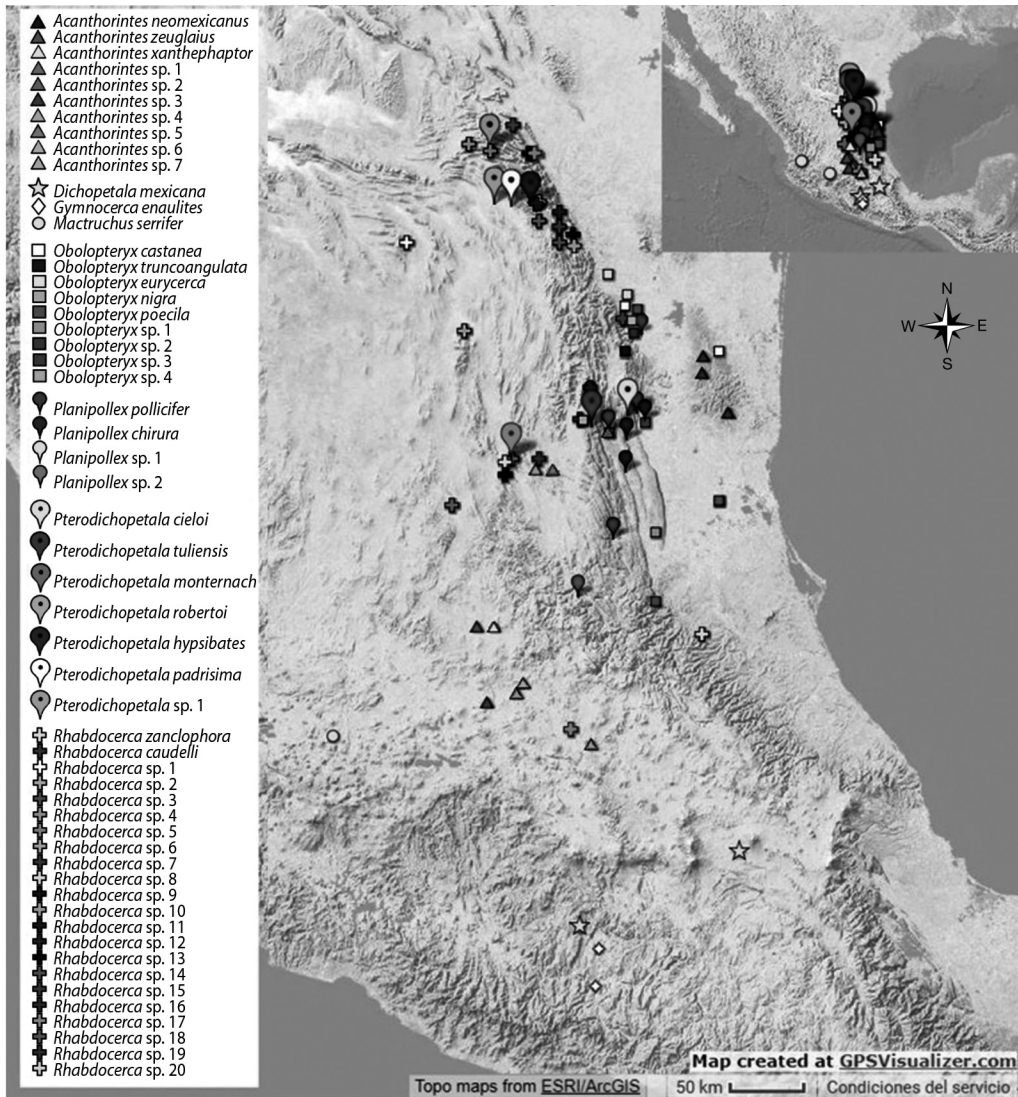
Fig. 7. Geographic distribution of the *Dichopetala* group members.

plate completely sclerotized, considering this as a primitive condition. Therefore, further phylogenetic studies considering additional taxa of Odonturini may be necessary to confirm whether the *Dichopetala* group could be raised to a tribe category.

The results obtained in our phylogenetic analyses are congruent with the taxa delimitation based on morphological traits, *i.e.,* males' genitalic characters (cerci, epiproct, subgenital plate, internal genitalia) are useful to separate

genera and species of the *Dichopetala* group. This also agrees with Cohn et al. (2014) and Kensinger et al. (2017) studies, the latter being a molecular study on the genus *Obolopteryx* which found that morphological characteristics such as the shape of cerci are a diagnostic character that serves for delimitation of that genus of the *Dichopetala* group. Several authors have demonstrated that male and female genitalic characters, external and internal, are subject to intense sexual selection pressure
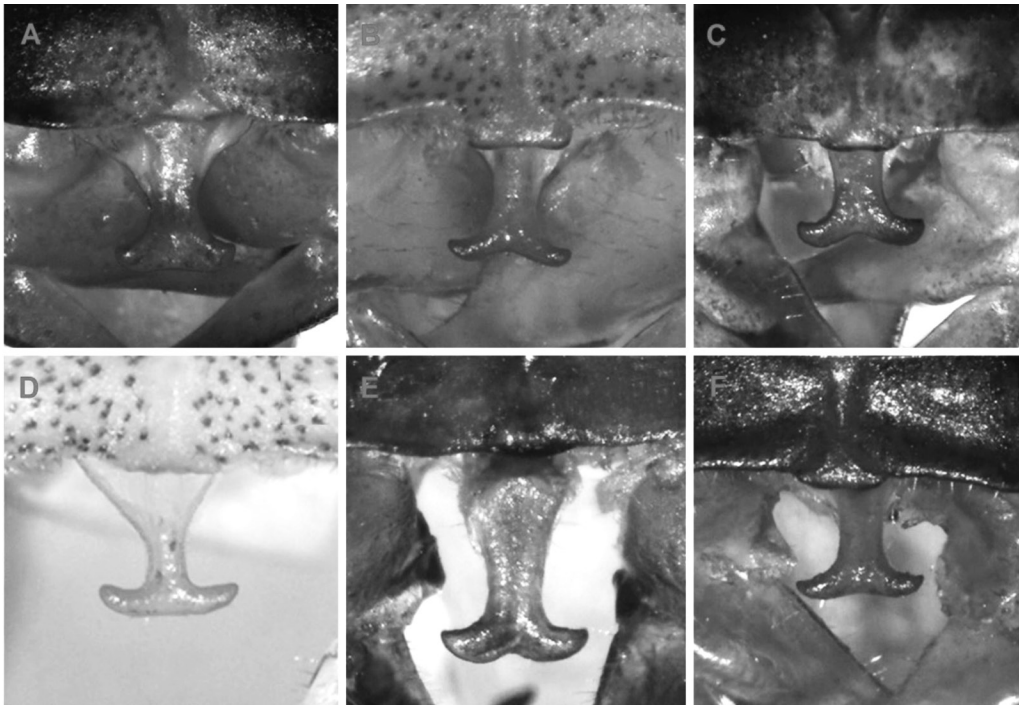
Fig. 8. *Acanthorintes* males' epiproct variation, frontal view. **A.** *Acanthorintes* sp. 1; **B.** *Acanthorintes* sp. 2; **C.** *Acanthorintes* sp. 4; **D.** *Acanthorintes* sp. 5; **E.** *Acanthorintes* sp. 6; **F.** *Acanthorintes* sp. 7.

and evolve more rapidly than other morphological traits, and thus they vary at specific, generic and suprageneric levels (Eberhard, 2004; Chamorro-Rengifo & Lopes-Andrade, 2014). Regarding the generic relationships, the rearrangement proposed by Cohn et al. (2014) for the genus *Dichopetala sensu latu* into six additional genera was mostly supported by our phylogenetic analyses, with *Dichopetala*, *Obolopteryx*, *Planipollex, Mactruchus* and *Rhabdocerca* confirmed as monophyletic.

The placement of the species that were reassigned to the new genera erected by Cohn et al. (2014) [*O. castanea* (Rehn & Hebard, 1914), *O. poecila* (Hebard, 1932), *P. pollicifer* (Rehn & Hebard, 1914), *R. caudelli* (Rehn & Hebard, 1914), *M. serrifer* (Rehn & Hebard, 1914) and *A. neomexicanus* (Barrientos-Lozano & Ramírez-Núñez, 2013)] is also supported in our study. The grouping obtained in our phylograms agrees with the classification reported by Cohn et al. (2014) based on morphological features. *Pterodichopetala* was recovered in our analyses as the basal lineage of the *Dichopetala* group, followed by *Acanthorintes*. Cohn et al. (2014) pointed out that *Acanthorintes* is morphologically similar to *Pterodichopetala* due to their similarity in the last abdominal tergite, which has two acute projections of medium size. This feature is only present in members of these two genera within the *Dichopetala* group and possibly represents a symplesiomorphy for these two genera. Members of *Pterodichopetala* also have other distinctive morphological features not present in the remaining genera of the *Dichopetala* group, such as tegmina and pronotum with different shape and size and more complex external and internal male genitalia (Cohn et al., 2014; Rocha-Sánchez et al., 2015; Barrientos-Lozano et al., 2015, 2016).

Phylogenetic relationships among species of *Pterodichopetala* in the *CONC94* Bayesian phylogram are concordant with the results of Cohn et al. (2014) based on morphology.

Accordingly, morphological features of male genitalia are taxonomically informative to delimit this genus and its members. *Pterodichopetala tuliensis* and *P. monternach* are in the most basal sub-clade, with these two species having cerci with two branches (Buzzetti et al., 2010; Barrientos-Lozano & Rocha-Sánchez, 2013; Barrientos-Lozano et al., 2013b; Rocha-Sánchez et al., 2015). *Pterodichopetala robertoi, P. padrisima* and *P.* sp. 1 on the other hand are grouped in an additional sub-clade, and are morphologically characterized by having cerci with three branches (Buzzetti et al., 2010; Barrientos-Lozano & Rocha-Sánchez, 2013; Barrientos-Lozano et al., 2013b; Rocha-Sánchez et al., 2015). Therefore, taking into account this molecular and morphological evidence, we suggest splitting *Pterodichopetala* into two genera.

All specimens assigned to *Obolopteryx* based on morphological characters were grouped into a single clade in our molecular analyses. This result agrees with Kensinger et al. (2017), who performed a molecular phylogenetic study for this genus considering seven species (*O. emarginata, O. brevihastata, O. gladiator, O. seeversi, O. catinata, O. castanea* and *O. oreoeca*). These authors recovered this genus as monophyletic. Our phylograms show *O. nigra* closely related to *O. castanea,* which agrees with Barrientos-Lozano et al. (2016) taxonomic study. They pointed out that these two species are morphologically similar, though they differ in cerci, subgenital plate of males and females, epiproct, stridulatory file, acoustic signal and internal genitalia (titillators). *Obolopteryx eurycerca* appears as the most basal lineage within the genus clade, followed above by *O. truncoangulata*. Barrientos-Lozano et al. (2016) considered *O. eurycerca* morphologically similar to *O. castanea*, but this relationship is not sustained in this study.

Cohn et al. (2014) synonymized *P. chirura* with *P. pollicifer*, since they did not find consistent morphological features in the topotypic material of *P. pollicifer* from Brownsville, Texas, and specimens of *P. chirura* from Ciudad Valles, San Luis Potosí, Mexico. However,

Strohecker (1945) had mentioned previously that there are differences between males' cerci of *P. pollicifer* and *P. chirura*. Cohn et al. (2014) regarded cerci differences between males of these two taxa "as minor", and difference in length of the ovipositor between females "as not a constant character". We included sequences of four populations of this genus in our study that were collected at different localities. In one of our Bayesian phylograms (*CONC94* matrix), this genus was divided into two sub-clades, with a sub-clade containing *P. chirura* (Antiguo Morelos, Tamaulipas) and *P.* sp. 2 (Naranjos Amatlán, Veracruz), and the other *P. pollicifer* (Gómez Farías, Tamaulipas) and *P.* sp. 1 (Huejutla de Reyes, Hidalgo). Accordingly, *P. pollicifer* and *P. chirura*, although morphologically similar, actually may represent distinct species. Therefore, it is proposed to reestablish the specific status of *P. chirura*. Males of these two taxa may be separated by morphological features such as the cerci, subgenital plate, pronotum, and stridulatory file (Fig. 5, Fig. 6) (Barrientos-Lozano et al., in prep.).

Some widely distributed species of the *Dichopetala* group may represent species complexes. This may be the case of genera *Rhabdocerca* and *Acanthorintes,* included in this study. The genus *Rhabdocerca* currently contains three species (*R. caudelli, R. tridactyla* and *R. zanclophora*) that are distributed along central and Northern Mexico (Cohn et al., 2014; Cigliano et al., 2018). In the *CONC60* Bayesian phylogram, out of 20 morphospecies assigned to *Rhabdocerca* 14 were grouped into three main clades whose relationship is consistent with their geographic distribution and their specific status assignment. Each of these clades is distributed along the Mexican Plateau, Mexican Altiplano and Eastern SMO, and Northern most portion of the SMO. Our molecular results support morphological differences, accordingly these populations may be considered as different taxa (Barrientos-Lozano et al., in prep).

We examined sequences of seven morphospecies assigned to the genus *Acanthorintes* collected in various localities. Males exhibited

Rev. Biol. Trop. (Int. J. Trop. Biol. ISSN-0034-7744) Vol. 67(6): 1431-1448, December 2019

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morphological variation in the epiproct (Fig. 8). *Acanthorintes* spp. 2 and 5 (Fig. 8), which were collected in San Luis Potosí, are characterized by having a slimmer epiproct. On the other hand, in specimens from locations further South the epiproct is more robust (*Acanthorintes* spp. 1 and 3, from Guanajuato; *Acanthorintes* spp., 4 and 7, from Querétaro; *Acanthorintes* sp. 6, Estado de México) (Fig. 8). All phylograms recovered a clade that may contain various independent evolutionary lineages based on COI + H3 + 28S tree topologies. Cohn et al. (2014) recognized five species for *Acanthorintes* and pointed out considerable variation in epiproct's projection. Members of *Acanthorintes* show variation not only in epiproct's projection, but also in other diagnostic morphological characters such as cerci, subgenital plate, stridulatory file and internal genitalia (titillators) (Barrientos-Lozano et al., in prep.). Our results suggest an overlooked species diversification in this genus, which could be explained by its wide geographic distribution in central and Northeastern Mexico. A rapid evolution of genital structures in *Acanthorintes*, driven by sexual selection mechanisms and ecological factors (biotic and abiotic), probably acted as an effective mean of reproductive isolation (Eberhard, 2004; Hosken & Stockley, 2004; Rowe & Arnqvist, 2012; Masly, 2012; Barrientos-Lozano et al.. 2013a; Simmons, 2014).

In the Bayesian phylogram derived from the *CONC94* matrix, sequences of specimens assigned to *A. neomexicanus* and *Gymnocerca enaulites* exhibited an incongruent arrangement. A similar situation occurred for *Gymnocerca enaulites* and *Planipollex* sp. 1 in the Bayesian phylogram derived from the *CONC60* matrix. These incongruences may be artifacts caused by the high amount of missing data in their sequence.

Ethical statement: authors declare that they all agree with this publication and made significant contributions; that there is no conflict of interest of any kind; and that we followed all pertinent ethical and legal procedures and requirements. All financial sources are fully and clearly stated in the acknowledgements section. A signed document has been filed in the journal archives.

RESUMEN

Sistemática molecular del grupo de géneros *Dichopelata* (Orthoptera: Phaneropteridae). Introducción: El grupo de géneros *Dichopetala* se propuso recientemente después de una revisión del género *Dichopetala* Brunner von Wattenwyl, 1878. Actualmente, el grupo consta de ocho géneros y 44 especies distribuidas desde el sur de los Estados Unidos hasta el sur de México. Este acuerdo genérico, se basó únicamente en evidencia morfológica y se acompañó por discusiones sobre nuevos géneros erigidos, para los cuales no se probó su monofilia. **Objetivo:** Evaluar las relaciones filogenéticas entre especies representativas de los ocho géneros del grupo *Dichopetala*. **Métodos:** Generamos secuencias de ADN para un gen mitocondrial (Citocromo oxidasa I: COI) y dos marcadores de genes nucleares (28S, Histona III: H3), e incluimos especies de otros géneros de Phaneropterinae para probar la monofilia del grupo en estudio. Utilizamos modelos evolutivos bayesianos y de máxima verosimilitud. **Resultados:** Se respalda la monofilia del grupo *Dichopetala* y la monofilia de los géneros *Dichopetala, Obolopteryx*, *Planipollex, Mactruchus* y *Rhabdocerca*. Además, los géneros *Acanthorintes* y *Pterodichopetala* como parafiléticos. Los marcadores mitocondriales también sugieren que los géneros *Rhabdocerca* y *Acanthorintes* ampliamente distribuidos, pueden en realidad contener varias especies no vistas previamente. **Conclusión:** Se proporciona la primera contribución a la filogenia del grupo de *Dichopetala* y una definición filogenética y morfológica más robusta de algunos de los géneros involucrados.

Palabras clave: Dichopetaline, análisis molecular, mono-filia, morfología.

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