



Phylogeny and biogeography of the unique snakefly genus *Alena* Navás, 1916 (Raphidioptera: Raphidiidae)

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Abstract

The genus *Alena* Navás, 1916, is considered the most distinct genus of Raphidiidae, because of the uncommon shape of its male genital sclerites and its geographic distribution restricted to the southwestern U.S.A. and western Mexico. Herein, we present a new species of the subgenus *Aztekorphidia* U. Aspöck and H. Aspöck, 1970, – *Alena* (*Aztekorphidia*) *alanae* **sp. nov.** Based on this discovery we present a detailed morphological study and the first morphological phylogeny of *Alena*. Our results recover this genus as monophyletic, including the subgenus *Aztekorphidia* as sister to a clade composed by the other two monotypic subgenera, *Alena* s.s. Navás, 1916, and *Mexicorphidia* U. Aspöck and H. Aspöck, 1970. We also provide a hypothesis about the biogeographic history of the group, which advocates that species of *Alena* are strongly associated with central Mexico and their ancestors were probably widely distributed through western North America in the past, of which only a few small groups survived in glacial refuges.

Keywords

Biodiversity, central Mexico, glacial refuges, morphology, western North America, Neuropterida.

1. Introduction

Alena Navás, 1916, is a small snakefly genus of the family Raphidiidae, which is restricted to southernmost North America, especially western Mexico (U. Aspöck and H. Aspöck 2013). Despite the molecular phylogeny of Raphidiidae by Haring et al. (2011) that recovered *Alena* as sister to all Palaearctic Raphidiidae, several previous studies have considered this genus to be sister to all Raphidiidae (H. Aspöck et al. 1991; U. Aspöck and H. Aspöck 1996; H. Aspöck et al. 2001; H. Aspöck and U. Aspöck 2007), following its unique genitalic traits

among raphidiid genera: males with tergite and sternite of the ninth abdominal segment separated, instead of forming a ring; sternite IX poorly-developed, inconspicuous in most species; fusion (or amalgamation) of the complex of gonocoxites X (“parameres”) with the gonapophyses IX (“hypovalva”), thus seeming apices of the hypovalva; and females with adhesion or fusion of atrium bursae and receptaculum seminis (H. Aspöck et al. 1991; Haring et al. 2011; U. Aspöck and H. Aspöck 2013).

The genus *Alena* was described by Navás in 1916, and originally included the type species, *Alena distincta* (Banks, 1911), and *Al. minuta* (Banks, 1903), both previously included in *Raphidia* Linnaeus, 1758. However, its oldest known species is actually *Alena* (*Az.*) *australis* (Banks, 1895), which implies more than 120 years of history of a group with only ten described species up to now. When compared with other genera of Raphidiidae, *Alena* is still poorly known, although its latest study was published only a few years ago by U. Aspöck and H. Aspöck (2013), who stated: “snakeflies are among the rarest insects in Mexico, and, in particular, every record of a specimen of *Alena* merits attention ... the finding of any new species of *Alena* was and will always remain an exciting event”. In response to the paucity of studies on this genus, aiming to contribute to the general phylogenetic understanding of Raphidiidae, herein we describe *Alena* (*Az.*) *alanae* **sp. nov.**, increasing the number of species of this genus to 11. We also present the first morphological phylogenetic analysis of *Alena*, with a hypothesis about its biogeographic history. A review of the Mexican snakeflies is nearly concluded and will be published elsewhere.

2. Material and methods

2.1. Taxonomic and morphological study

Adult specimens of *Alena*, *Agulla* Navás, 1914, and *Indianoinocellia* U. Aspöck and H. Aspöck, 1970, species had their external morphology and its genital structures compared in detail. For the morphological study of genitalia, the last five abdominal segments were dissected in a Petri dish with glycerin under a Leica M16 stereomicroscope, then they were cleared with a 10% potassium hydroxide (KOH) solution, washed in sequence with distilled water, 10% acetic acid, and 70% ethyl alcohol. Posteriorly, the genital structures were observed and stored in micro-vials with glycerin, and pinned below the respective specimen. High-resolution images from the new species of *Alena* were produced with a Leica DC500 digital camera attached to a Leica M16 stereomicroscope; the photographs were stacked and processed with the software Auto-Montage Pro v5.02® (Leica Microsystems, Wetzlar, Germany), and the final image was edited in the software Photoshop CS®. Drawings were made using a Zeiss Stemi SV11 stereomicroscope, and then scanned and vectored in the software Adobe Illustrator CS®. The studied specimens are deposited in the Colección Nacional de Insectos of Instituto de Biología, Universidad Nacional Autónoma de México, Mexico City (CNIN-UNAM).

Wing venation terminology follows H. Aspöck et al. (1991), but we used RP instead of Rs to mention the posterior branch of the Radius. We use the interpretation that the MA is fused with RP, originating as the basal branch of RP, i.e. the “sigmoidal vein” on hind wing represents the MA base. For the terminology of genital structures –

and the colors utilized in the drawings to represent them – we followed U. Aspöck and H. Aspöck (2008, 2013), i.e. gx corresponds to gonocoxites; gst to gonostyli; and gph to gonapophyses. Herein, we prefer to use gonapophyses IX instead of “pseudostyli” (Inocelliidae) and “hypovalva” (Raphidiidae); complex of gonocoxite X (gonocoxites + gonapophyses + gonostyli of segment X) and not “arcessus” (Inocelliidae) and “parameres” (Raphidiidae); and gonocoxites XI rather than “gonarcus”; and maintain the use of hypandrium. For females, we maintain the use of atrium bursae for the base of bursa copulatrix; ductus sacculi for the ductus leading from the atrium bursae to the sacculus bursae; sacculus bursae for the sac-like proximal part of the bursa copulatrix; ductus receptaculi for the ductus leading from sacculus bursae to the receptaculum seminis; receptaculum seminis for the sac-like seminal receptacle that is connected to sacculus bursae by the ductus receptaculi, to which the paired glandulae receptaculi open; and glandulae receptaculi for the paired tubes with glandular function opening into the receptaculum seminis.

2.2. Phylogenetic analysis

For the phylogenetic analysis of *Alena* Navás, 1916, all 11 species were included as the ingroup, with *Indianoinocellia mayana* U. Aspöck et al., 1992 (Inocelliidae) and *Agulla* (*Ag.*) *bicolor* (Albarda, 1891) as outgroups, while the Inocelliidae species was used to root the tree under rooting procedures of Nixon and Carpenter (1993). *Alena* sp. from U. Aspöck and H. Aspöck (1996), which is known only by females, was not included in the phylogenetic analysis because of the large number of missing data generated by the absence of males.

The characters used for the phylogenetic analysis were determined through a detailed comparative study of the morphology of *Indianoinocellia*, *Agulla*, and *Alena* species. A matrix (Table 1) with 36 adult morphological characters (see APPENDIX 1) and 13 terminals, was built using WinClada (Nixon 2002), then it was exported as Nexus file to perform a parsimony analysis in TNT® version 1.5 (Tree Analysis using New Technology) (Goloboff and Catalano 2016). The most parsimonious trees were obtained through heuristic algorithms using the tree bisection and reconnection method, using as parameters: random seed = 0, hold = 3000, hold / = 50 in a TBR (“Tree Bisection Reconnection”) of 60 replications; all characters were treated as unordered. Posteriorly, the study of optimization and evolution of characters was made with WinClada (Nixon 2002).

Analyses were first performed with characters treated with equal weight; subsequent analyses were performed using implied weighting with different values of constant k (3–10), prioritizing character sets with a greater congruence reflecting in the reduction of information loss on phylogenetic relationships in the consensus (Goloboff 1993).

Bremer support or BS (Bremer 1988, 1994), which calculates the number of extra steps needed for a consensus branch to collapse, was used to calculate the branch

Table 1. Data matrix of adult morphological characters for the genus *Alena* Navás, 1916 (Raphidioptera: Raphidiidae) phylogenetic analysis (? , missing data; –, inapplicable character).

Species	Characters and states																																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	34	25	26	27	28	29	30	31	32	33	34	35	36	
<i>Indianoiocellia mayana</i>	0	0	0	0	0	0	0	0	0	0	0	0	0	0	–	0	0	0	0	0	0	–	–	–	–	0	0	0	0	0	0	0	0	0	0	0	0
<i>Agulla (Ag.) bicolor</i>	1	0	1	1	0	0	0	0	–	0	0	0	0	0	–	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0
<i>Al. (Al.) distincta</i>	0	1	2	1	1	1	1	1	1	1	1	0	0	1	0	0	1	1	0	1	1	0	1	1	1	1	1	–	1	1	2	1	0	0	1	2	2
<i>Al. (Mx.) americana</i>	0	1	2	2	1	1	1	0	1	1	1	0	0	1	0	0	1	1	0	1	1	0	0	1	1	1	1	–	1	1	1	1	1	1	1	2	0
<i>Al. (Az.) alanae sp.nov.</i>	0	1	2	1	1	1	1	0	1	1	1	0	0	2	0	0	1	1	0	0	1	0	0	1	1	1	1	–	1	1	1	1	0	0	2	1	1
<i>Al. (Az.) michoacana</i>	0	0	1	1	1	1	1	1	1	1	1	0	0	2	0	0	2	2	1	0	1	1	1	1	0	1	2	1	1	1	1	?	?	?	?	?	
<i>Al. (Az.) horstaspoecki</i>	0	1	2	2	1	1	1	1	1	1	1	0	0	2	0	0	2	2	2	1	0	1	1	1	0	1	2	1	1	1	0	0	0	2	1	1	
<i>Al. (Az.) schremmeri</i>	0	1	2	2	1	1	1	0	1	2	1	0	0	2	1	1	1	2	1	0	1	0	0	0	0	1	1	–	1	1	1	0	0	0	2	1	1
<i>Al. (Az.) caudata</i>	0	1	2	2	1	1	0	1	1	2	1	0	0	2	1	1	1	2	1	0	1	0	1	1	1	1	1	–	1	1	1	1	0	0	2	1	1
<i>Al. (Az.) infundibulata</i>	0	1	2	2	1	1	0	0	1	2	1	0	0	2	1	2	1	2	1	0	1	0	1	1	1	1	?	–	1	1	1	1	0	1	2	1	1
<i>Al. (Az.) minuta</i>	1	0	1	1	1	1	0	0	1	2	2	1	0	2	1	?	1	2	1	0	1	0	1	1	1	1	1	–	2	2	3	1	0	0	2	1	1
<i>Al. (Az.) australis</i>	1	0	1	1	2	1	1	1	1	2	2	1	1	2	0	0	1	2	1	0	1	0	1	1	1	1	1	–	2	2	3	1	2	1	2	1	1
<i>Al. (Az.) tenochtitlana</i>	1	0	1	1	2	1	1	1	1	2	2	1	1	2	0	0	1	2	1	0	1	0	1	1	1	1	1	2	0	2	2	3	1	2	1	2	1

support values. This analysis was carried out using TNT® 1.5 (Goloboff and Catalano, 2016), employing suboptimal trees of 1–10 extra steps with TBR.

2.3. Biogeographical analysis

To generate a hypothesis for the biogeographical history of *Alena* we used the most parsimonious tree topology recovered in the phylogenetic analysis to perform a statistical dispersal–vicariance analysis (S-DIVA) in RASP software (Ronquist 1997; Yu et al. 2015). Each species was coded as present or absent in each of the ten provinces used in the present study, which were based in Morrone (2001, 2019) and Escalante et al. (2013, 2021), namely: (a) Californian, (b) Mohavian, (c) Navahonian, (d) Baja Californian, (e) Sonoran, (f) Sierra Madre Occidental, (g) Chihuahuan, (h) Transmexican Volcanic Belt, (i) Balsas Basin, (j) Sierra Madre del Sur.

3. Results

3.1. Taxonomy of *Alena* Navás, 1916

Genus *Alena* Navás, 1916

Alena Navás, 1916.

Type species. *Raphidia distincta* Banks, 1911 (by original designation). — Subgenera included: *Alena* s.s. Navás, 1916; *Aztekorphidia* U. Aspöck and H. Aspöck, 1970; *Mexicorphidia* U. Aspöck and H. Aspöck, 1970. — Species included: *Alena (Alena) distincta* (Banks, 1911), *Alena (Mexicorphidia) americana* Carpenter, 1958, *Alena (Aztekorphidia) alanae* **sp. nov.**, *Al. (Az.) australis* (Banks, 1895), *Al. (Az.) caudata* (Navás, 1914), *Al. (Az.) horstaspoecki* U. Aspöck and Contreras-Ramos, 2014; *Al. (Az.) infundibulata* U. Aspöck et al., 1994; *Al. (Az.) michoacana* U. Aspöck and H. Aspöck, 2013; *Al. (Az.) minuta* (Banks, 1903); *Al. (Az.) schremmeri* U. Aspöck et al., 1994; *Al. (Az.) tenochtitlana* (U. Aspöck and H. Aspöck, 1978).

Distribution. Areas of southwestern U.S.A. and western Mexico (Fig. 1).

Alena (Aztekorphidia) alanae Martins, H. Aspöck, U. Aspöck, and Contreras-Ramos, **sp. nov.**

<http://zoobank.org/D4B156D5-DEE8-4756-BD67-5ACF991-8EEBE>

Figs 1–3

Material examined. **Holotype.** ♂ (pinned), “México: Estado de México: Nanchititla, Parque Sierra de Nanchititla, 18°51'42.5"N,

100°25'38.1"W, 1758 m, 14.VIII.2018, trampa de Luz, F. Villagomez" (CNIN). **Allotype.** ♀ (pinned), same data as holotype. **Paratypes.** 3♂♂ (1 pinned and 2 in alcohol), same data as holotype.

Diagnosis. Habitus mostly blackish-brown, with some pale yellow elements (pedicel, scape, clypeus, pterostigma) and abdominal apex amber (Fig. 2A); abdomen with lateral yellow stripe, which possesses a blackish-brown stripe in its median region (Fig. 2A); pterostigma in both pairs of wings with one apical vein; hind wing with base of MA as crossvein (Fig. 2B). Male (Figs 3A–C) with gx IX apex as digitiform projection above the origin of gst IX; gst IX large, subquadrangular, and strongly curved dorsad and inward; apex of gph IX surrounded by a semi-membranous structure with apical tooth and tiny setae arranged in rows. Female (Figs 3D–F) median region of tergite VIII anterior margin possessing a triangular projection; glandulae receptaculi elongated, and club-like apically.

Description. Male measurements (n=4). Body length: 8.6–8.9 mm; forewing length: 7.4–7.7 mm; hind wing length: 6.3–6.7 mm. **Female measurements** (n=1): Body length: 10.3 mm; forewing length: 9.1 mm; hind wing length: 7.3 mm. — **Head** (Fig. 2C): Subtriangular; blackish-brown with yellow elements; with fine sculpture. Vertex rugose, blackish-brown. Ocelli black, without peripheral mark; internal region of ocellar triangle asetose. Antennae filiform, scapus pale yellow, cylindrical, with fine, pale setae; pedicellus pale yellow, cylindrical, with fine, pale setae; flagellum with base pale yellow, darkening towards apex with a blackish-brown color, with 36–37 segments twice as long as wide, all possessing apices with long pale setae and pale microtrichia. Compound eyes blackish, sub-spherical, as wide as 0.3 of the interocular distance at antenna insertion level. Frons blackish-brown, with short pale setae. Clypeus pale yellow, with short pale setae. Labrum rectangular, dark brown, with long, pale setae. Gena dark brown to pale yellow, postgena blackish-brown. Maxilla dark brown to pale yellow color, maxillary palpus dark brown. Labium and labial palpus dark brown. Occiput blackish brown with a median dark brown stripe. — **Thorax** (Figs 2A, C): Prothorax elongate, pronotum sub-rectangular, ca. 3.2 times as long as wide, blackish brown, laterally with broad yellow margins, covered with small thin setae. Ventral region of prothorax blackish-brown, with anterior margin yellow. Meso- and metanotum subrectangular, blackish-brown. Pteropleura blackish-brown, yellow colored on some sutures and on insertion of coxae. Legs with coxae and trochanter blackish-brown; femora and tibiae amber; with short spinous brown setae; basal tarsomeres amber; apical tarsomeres brown; pretarsal claws dark brown. — **Wings** (Fig. 2B): Elongate, ovoid, with posterodistal margin convex; membrane hyaline, venation predominantly ochre brown. Forewing: costal area short; costal crossveins simple. Subcosta short, entering at anterior wing margin on 2/3 of its length; basal subcostal vein present at 1/3 of wing length. Pterostigma yellow, with a straight vein running through it; apical vein

present. RA running until wing apex, weakly curved at base. RP+MA originating from R at 1/2 length of wing; MA with apex three-branched; RP four-branched. MP1+2 and MP3+4 bifurcated at apex. CuA 2-branched; CuP simple. Anal veins short and simple. Hind wing. Similar venation to that of forewing, but RP three-branched; base of MA as crossvein. — **Abdomen** (Fig. 2A): Tergites blackish-brown, surface with some fine and brown setae. Pleural membrane yellow with a median blackish-brown inner stripe. Sternites blackish-brown, with some fine and brown setae; intersternites region yellow. — **Male genitalia** (Figs 3A–C): Sternite of segment VIII subtriangular in ventral view; only slightly shorter than tergite in lateral view. Tergite IX trapezoidal in lateral view, sharply elongated in the lateroanterior margin, with several short brown setae; trapezoidal in dorsal view, with median suture at basal 2/3; basal and apical margins with incisions. Sternite IX subtriangular, plate-like. Basal sclerite of gx IX large, broadly fused with the gx IX, but clearly delimited by a suture. Gx IX forming huge reddish plates with a digitiform brownish apex located above the origin of gst IX; surface covered with several long brown setae. Gst IX large, possessing several long, thick brown setae; subquadrangular in lateral view, especially in its broad base; strongly curved dorsad and inward; acute apex. Gph IX paired, with two broad basal sclerotized rods, connected to basal sclerites of gx IX by a membrane; apices parallel and slightly curved dorsally; terminally the gph IX is surrounded by a semi-membranous structure containing small teeth apically, and tiny setae arranged in rows. Complex of gx X and gx XI absent. Hypandrium internum small. Ectoproct lacking distinct trichobothria, nevertheless possessing several thick, long, and brown setae; consisting in a plate subtriangular in lateral view; subquadrangular in dorsal view, with weak incision on anterior margin, and strong incision on posterior margin generating two subtriangular lateroposterior processes. — **Female genitalia** (Figs 3D–F): Sternite VII subrectangular in lateral view, with posterior margin convex; subrectangular in ventral view, with anterior margin straight and posterior margin slightly convex. Spiracle VII free. Area between segments VII and VIII small, membranous. Tergite VIII subrectangular in lateral view, reaching the ventral part of segment, with anterior margin possessing a subtriangular projection in the median region. Subgenital absent. Atrium bursae with wide base, a medial and apical region elongated, tube-like. Ductus sacculi narrow, short. Sacculus bursae large, membranous. Receptaculum seminis membranous, closely adhered with atrium bursae, hard to see. Ductus receptaculi inconspicuous. Glandulae receptaculi elongate, apically club-like enlarged.

Immatures. Unknown.

Etymology. This new species is dedicated to the first author's wife, Alana C. D. Brunini, who has been an unconditional source of support in all his academic and non-academic ventures during these 16 years together.

Distribution. Mexico (Estado de México) (Fig. 1).

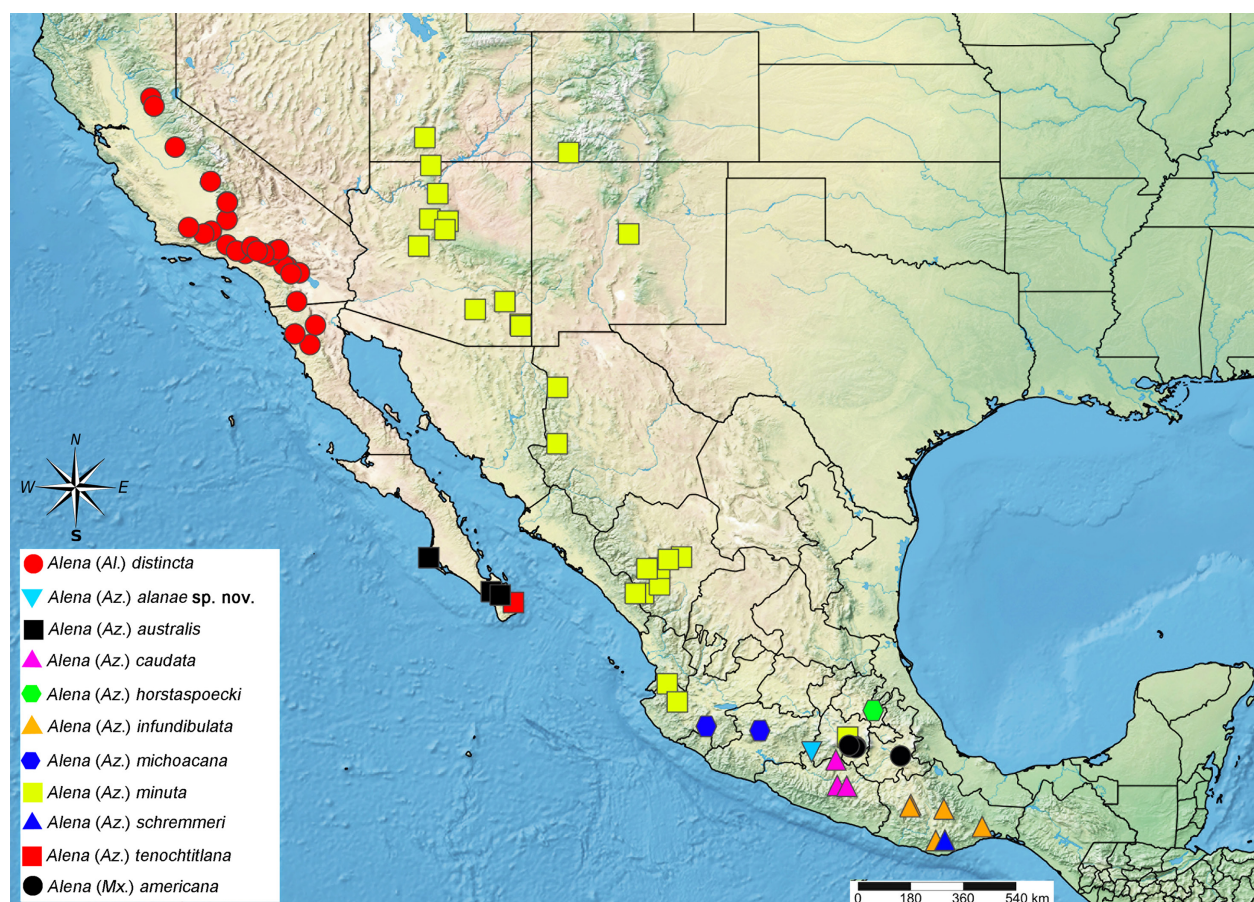


Figure 1. Distribution of *Alena* Navás, 1916 (Raphidioptera: Raphidiidae).

Ecology and biology. The new species was collected at an altitude of 1758 m at light trap. The region where *Alena* (Az.) *alanae* sp. nov. was encountered is a large mountainous area, with pine-oak vegetation within an extensive part of low deciduous forest, especially *Pinus* and *Quercus*. This region is considered temperate sub-humid, with average annual temperature greater than 18 °C – coldest month temperature less than 18 °C, hot-test month temperature greater than 22 °C –, and annual precipitation between 500 and 2,500 mm (CONABIO 2021).

Remarks. Because of the displacement of the gx IX apex above the origin of the gst IX in males, plus females with atrium-bursae tube-like, receptaculum seminis adhered to atrium-bursae, and glandulae receptaculi elongated, *Alena alanae* sp. nov. clearly belongs to the subgenus *Aztekorphidia*. This new species has the base of MA on the hind wing as a crossvein, which differentiates it from other *Alena* species with base of MA as a longitudinal vein, i.e., *Al.* (Mx.) *americana*, *Al.* (Az.) *infundibulata*, *Al.* (Az.) *caudata*, *Al.* (Az.) *horstaspoecki*, and *Al.* (Az.) *schremmeri*. *Alena* (Az.) *alanae* sp. nov. possesses an apical vein on pterostigma, separating it from *Al.* (Az.) *australis*, *Al.* (Az.) *michoacana*, *Al.* (Az.) *minuta* (Banks, 1903), and *Al.* (Az.) *tenochtitlana*, species without such vein.

Alena (Az.) *alanae* sp. nov. has no evident relationship to any of the other *Aztekorphidia* species, and may be separated from all other *Alena* species by the shape of the

distinctly male large gst IX, which is subquadrangular in lateral view, especially in its broad base, and curved dorsad and inward. All other species of *Aztekorphidia* have gst IX subcylindrically, arm-like, and only in *Al.* *tenochtitlana* these structures are weakly curved inward. *Alena* (Al.) *distincta* and *Al.* (Mx.) *americana* have male gst IX bifurcated apically which clearly separates these species from *Al.* (Az.) *alanae*. Females of the new species have the glandulae receptaculi apically club-like, which is not observable in other *Aztekorphidia* species; only *Al.* (Az.) *horstaspoecki* has the apex of glandulae receptaculi enlarged, but it is smaller when compared with that from the new species.

3.2. New records of *Alena* Navás, 1916 species

Fig. 1

Alena (*Aztekorphidia*) *michoacana* U. Aspöck and H. Aspöck, 2013

Distribution. Mexico (Michoacán; Jalisco (**new record**))

Material examined. “México: Jalisco: Venustiano Carranza, 20 KM W(est) Cd. Guzmán, 19°38.6'N, 103°40.1'W, 1955 m, 14.VI.2013, Bosque pino, L. Cervantes and D. Brzoska”, 1♂ (alcohol) (CNIN).

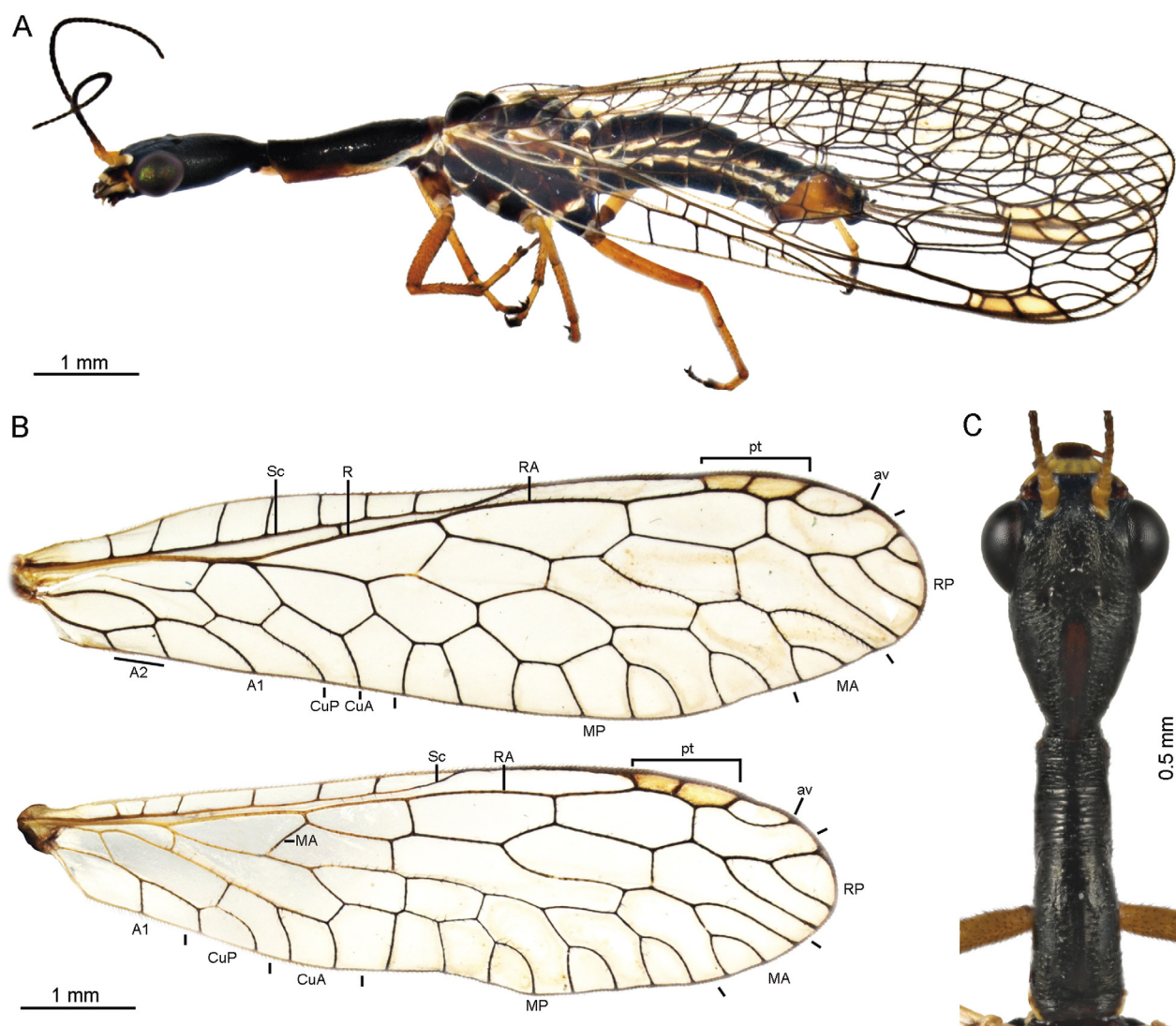


Figure 2. *Alena (Aztekoraphidia) alanae* sp. nov., holotype male. **A:** habitus, lateral view; **B:** wings; **C:** head, dorsal view. A1–A2, anal veins; av, apical vein; CuA, cubitus anterior; CuP, cubitus posterior; MA, media anterior; MP, media posterior; pt, pterostigma; RA, radius anterior; RP, radius posterior; Sc, subcosta.

Alena (Aztekoraphidia) minuta (Banks, 1903)

Distribution. U.S.A. (Arizona; Colorado; New Mexico; Utah); Mexico (Chihuahua; Durango; Estado de México (new record); Jalisco (new record)).

Material examined. “México: Jalisco: San Sebastián del Oeste, Camino a Santiago de Pinos, BP, 1500 m, 9.VII.1997, ex. Luz, J. L. Navarrete”, 1 ♀ (pinned) (CNIN); “26 Km E(ast) Talpa, 1580 m, 16.VII.1993, A. Rodríguez and F. A. Noguera”, 1 ♀ (pinned) (CNIN). “Distrito Federal: Del(egación) Cuajimalpa, Parque Nacional Desierto de los Leones, Río San Borja, 19°18'27"N, 99°18'45"W, 2971 m, 16.VI.2007, R. Juaréz and M. Razo”, 1 ♀ (pinned) (CNIN).

Alena (Mexicoraphidia) americana Carpenter, 1958

Distribution. Mexico (Morelos; Puebla (new record)).

Material examined. “México: Puebla: 3 Km al N(orth)W(est) de Tecalzingo, 18°43'91.3"N, 97°41'02.1"W, 3-4.VI.1995, E. Barrera”, 1 ♀ (pinned) (CNIN).

3.3. Phylogeny of *Alena* Navás, 1916

All the cladistic analyses performed, either using equal weighting or using implicit weighting with different constant *k* values (3–10), recovered the same most parsimonious tree (77 steps in length (L), consistence index (CI) of 70, and retention index (RI) of 73) (Fig. 4). In the cladistics analysis we used a single species of Inocelliidae to root the tree, so it would be expected that the family Raphidiidae, represented here by *Agulla* and *Alena*, be recovered as monophyletic and with high support (BS > 10). In our analysis this family has four synapomorphies: hind wing with base of MA as cross or oblique vein (char. 4:1); males with the ridge of the gst IX well developed

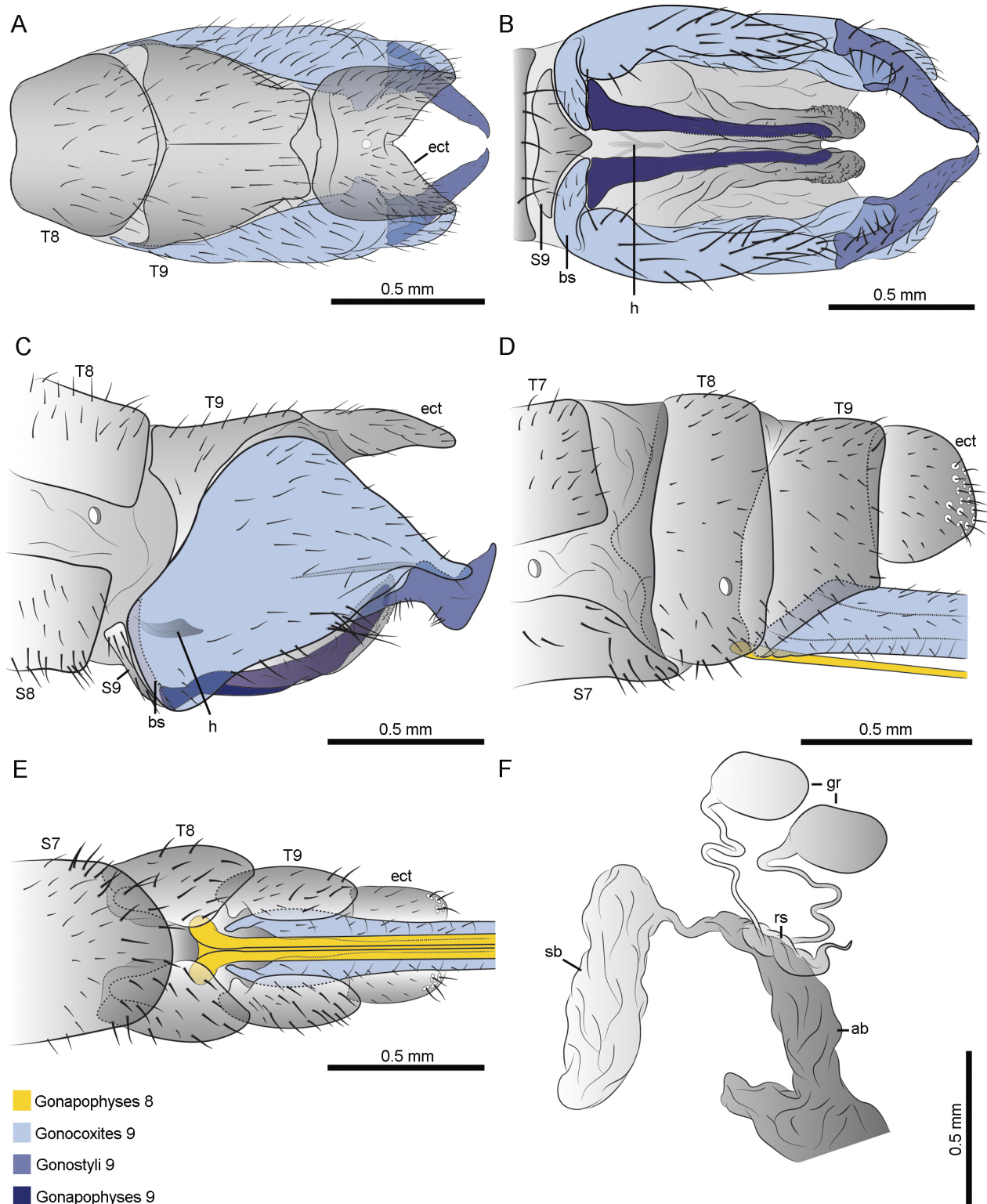


Figure 3. *Alena (Aztekoraphidia) alanae* sp. nov. Male genitalia, **A**: dorsal view; **B**: ventral view; **C**: lateral view. Female genitalia, **D**: lateral view; **E**: ventral view; **F**: complex of bursa copulatrix, lateral view. ab, atrium bursae; bs, basal sclerite of gonocoxite 9; ect, ectoproct; gr, glandulae receptaculi; rs, receptaculum seminis; S7–S9, seventh-ninth sternites; sb, sacculus bursae; T7–T9, seventh-ninth tergites.

(char. 17:1) and gph IX composing a structure with two elongated rods, commonly known as “hypovalva” (char. 21:1); and females with atrium bursae large (char. 34:1).

The monophyly of *Alena* is well-supported (BS = 9) by the following 16 autapomorphies: wings with apical vein on pterostigma (char. 2:1), forewing with second ra-rp

crossvein in the level of the median region of pterostigma (char. 3:2); males with the length of sternite VIII corresponding to $3/4$ – $1/2$ of the tergite VIII length (char. 5:1), tergite and sternite of abdominal segment IX separated (char. 6:1), tergite IX with median suture on dorsal region (char. 7:1); gx IX 1.2 – $1.5\times$ longer than wide (char. 10:1),

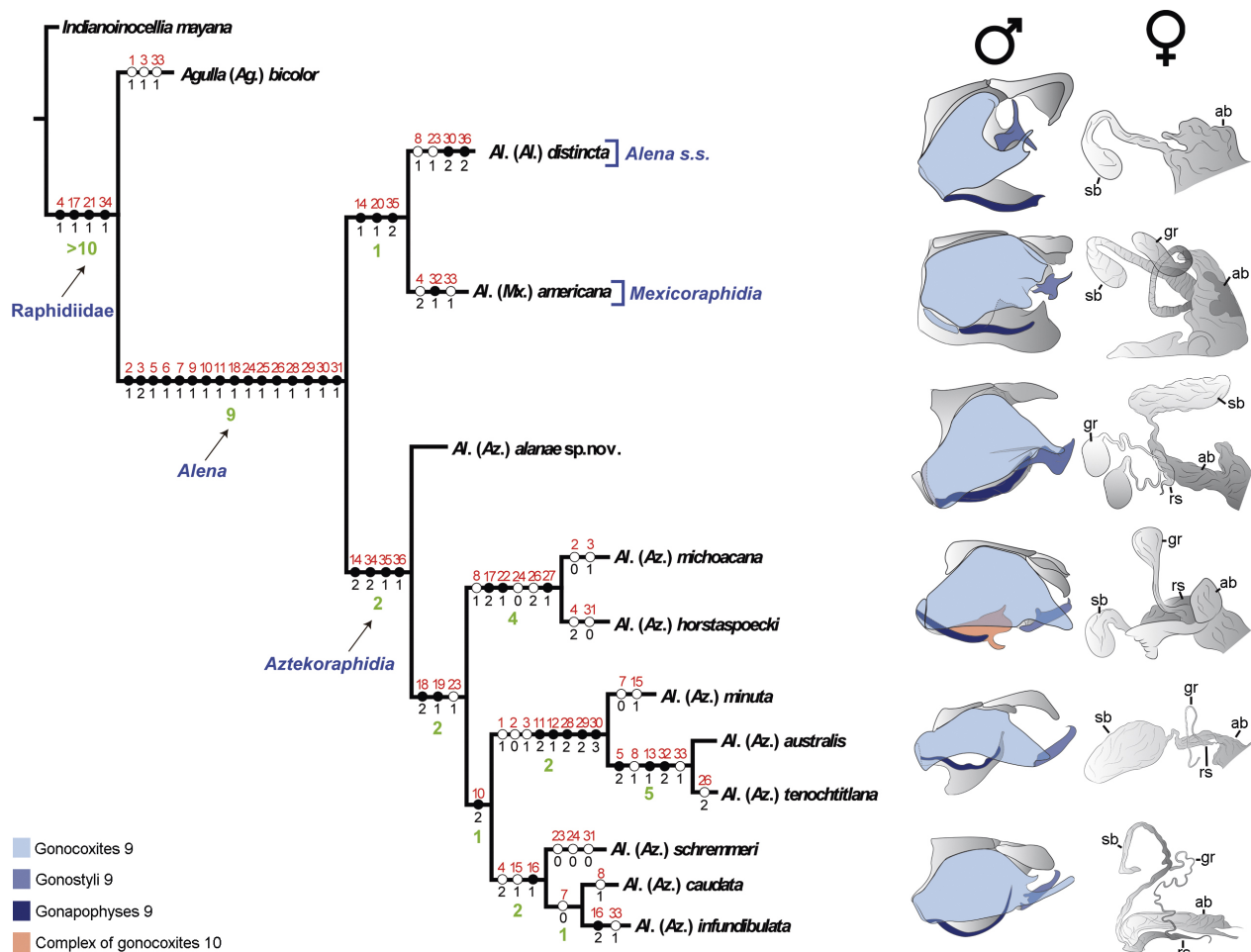


Figure 4. Phylogenetic relationship of *Alena* Navás, 1916 (Raphidioptera: Raphidiidae) and its genitalic evolution. Most parsimonious and unique tree (number of steps = 77, consistency index (CI) = 70, retention index (RI) = 73) of equal and implied weight analyses using constant $k = 3-10$. Character changes are marked on branches, red above = character numbers, and black below = character state. Forward unequivocal changes (black circles) and reversed or multiple changes (white circles) are shown. Bremer values of branch support are presented below nodes in green. Male figures from top to bottom, *Al. (Al.) distincta*, *Al. (Mx.) americana*, *Al. (Az.) alanae sp. nov.*, *Al. (Az.) michoacana*, *Al. (Az.) tenochtitlana*, and *Al. (Az.) caudata*. Female figures from top to bottom *Al. (Al.) distincta*, *Al. (Mx.) americana*, *Al. (Az.) alanae sp. nov.*, *Al. (Az.) horstaspoecki*, *Al. (Az.) minuta*, and *Al. (Az.) caudata* (Figures modified from H. Aspöck et al. 1991; U. Aspöck et al. 1994b; U. Aspöck et al. 2014; U. Aspöck and Contreras-Ramos 2004; U. Aspöck and H. Aspöck 2013). ab, atrium bursae; gr, glandulae receptaculi; rs, receptaculum seminis; sb, sacculus bursae.

with distinct basal sclerites (char. 9:1), and basal third approximately 2/3 as wide as its median area (char. 11:1); gst IX subquadrangular (char. 18:1); gph IX (when composing the “hypovalva”) with tapered apex (char. 24:1), and broadly inserted on the basal sclerites of gph IX (char. 25:1); complex of gph X completely amalgamated with gph IX (char. 26:1); ectoproct rectangular in dorsal view (char. 28:1), and subtriangular in lateral view (char. 30:1), with median V-shaped incision on posterior margin (char. 29:1), and trichobothria reduced or absent (char. 31:1).

Mexicoraphidia and *Alena s.s.*, both monotypic subgenera of *Alena*, were recovered in a clade (BS = 1) sister to the species belonging to the third subgenus, *Aztekorphidia*. The clade composed of *Mexicoraphidia* and *Alena s.s.* is supported by males with apex of gph IX located below or before the gst IX base (char. 14:1), gst IX with bifurcated apex (char. 20:1), and females with receptaculum seminis absent or strongly reduced (char. 35:2), all of them synapomorphies. *Aztekorphidia* (BS = 2) has its

monophyly supported by males with apex of gph IX located above or in front of gst IX (char. 14:2), and females with tube-like atrium bursae (char. 34:2), receptaculum seminis adhered or fused with the atrium bursae (char. 35:1), and glandulae receptaculi elongated (char. 36:1).

Among the species belonging to *Aztekorphidia*, *Al. (Az.) alanae sp. nov.* is considered sister to the remaining eight species, which share males with arm-like gst IX (char. 18:2), that have its base approximately as large as its apex (char. 19:1), as well as gph IX with divergent apex (char. 23:1). *Alena (Az.) michoacana* was recovered as sister to *Al. (Az.) horstaspoecki* in a clade supported by three synapomorphies, males with ridge of gst IX poorly developed (char. 17:2), apex of gph IX located before the tergite IX apex (char. 22:1), and complex of gph X (when not amalgamated with gph IX) bearing projections (char. 27:1). This last clade is also supported by males with sternite IX indistinct (char. 8:1), apex of gph IX rhomboid (char. 24:0), and complex of gph X located apically to gph

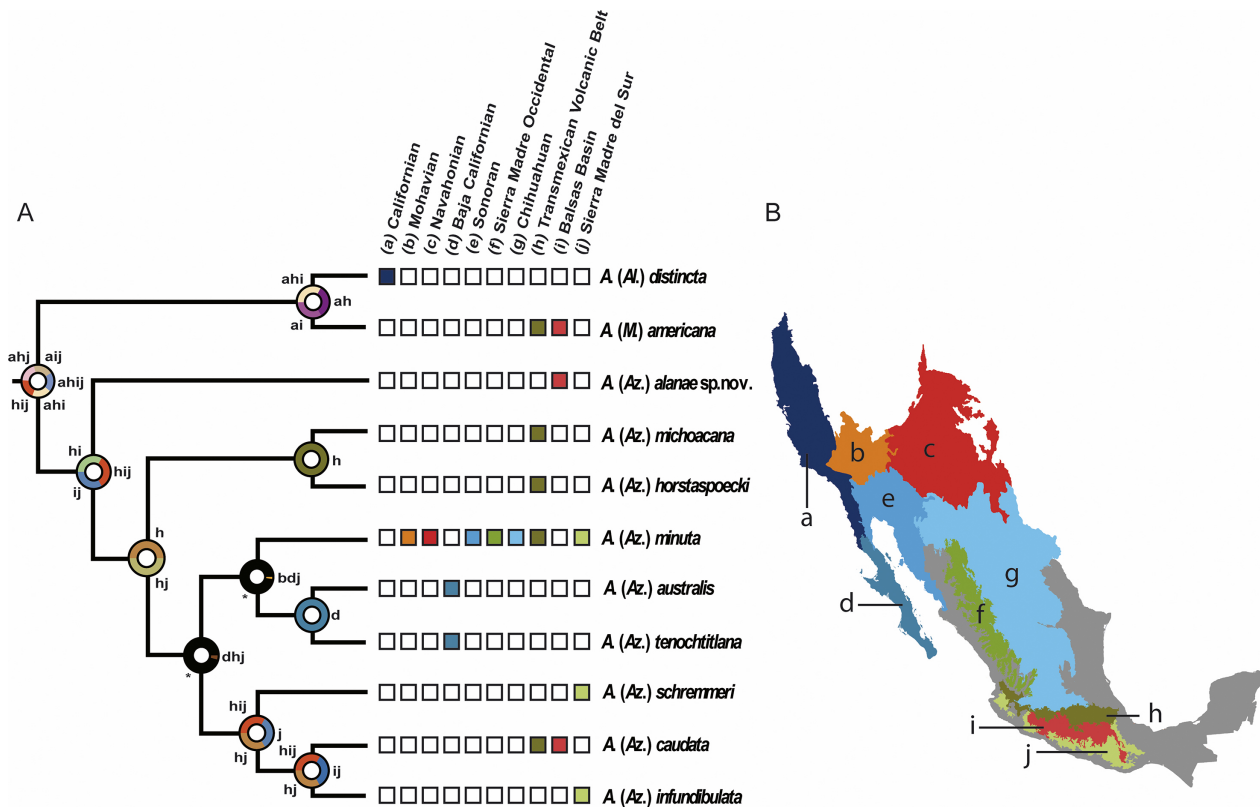


Figure 5. Historical biogeography of *Alena* Navás, 1916 (Neuroptera: Raphidiidae). **A:** chronogram showing intergeneric relationships within *Alena* Navás, 1916 (based on the topology results recovered in the equal and implied weight analysis using constant k values of 3–10), reconstructed ancestral areas mapped for each node. **B:** biogeographic provinces of Mexico and southwestern U.S.A., after Escalante et al. (2013, 2021) and Morrone (2019).

IX (char. 26:2), which are independently present in other species.

The remaining six species of *Aztekorphidia* share males with gx IX at least 1.7× longer than wide (char. 10:2), and are divided in two clades with three species each. The first clade is composed of *Al. (Az.) minuta* + (*Al. (Az.) australis* + *Al. (Az.) tenochtitlana*). These three species have as main characteristics, both wings with two-colored pterostigma (char. 1:1), and lacking apical vein (char. 2:1), forewing with second ra-rp crossvein located apically to pterostigma (char. 3:1); males with gx IX presenting the basal third elongated (char. 12:1), and about 1/2 as wide as its median region (char. 11:2), as well as the ectoproct trapezoidal in dorsal view (char. 28:2), with median region of posterior margin convex, in this same view (char. 29:2), and arm-like in lateral view (char. 30:3). *Alena (Az.) australis* and *Al. (Az.) tenochtitlana* share three synapomorphies – males with sternite VIII with 1/3 of the tergite VIII length (char. 5:2), gx IX with ventral margin concave on median region (char. 13:1), and female with tergite VIII fused ventrally, forming a ring (char. 32:2) –, and two homoplastic characters also present in other *Alena* species, males with sternite IX indistinct (char. 8:1), and females with base of tergite VIII clearly larger than its median region, in lateral view (char. 33:1). The second clade has *Al. (Az.) caudata* sister to *Al. (Az.) infundibulata* – sharing a reversion, male tergite IX without median suture (char. 7:0) –, and both species sister to *Al. (Az.) schremmeri*, making a group supported

by base of MA on hind wing present as longitudinal vein (char. 4:2), and males with gx IX with dorsoposterior tapered process (char. 16:1), and with elongated apex (char. 15:1).

3.4. Biogeography of *Alena* Navás, 1916

The S-DIVA analysis for the biogeographical history of *Alena* (Fig. 5) yielded a hypothesis with 15 dispersal events, four vicariance events, and two extinction events, which resulted in ambiguous ancestral areas for some nodes. The dispersal events occurred once in the node of *Alena*; once in the node of *Al. (Al.) distincta* + *Al. (Mx.) americana*; once in the node of *Aztekorphidia*; twice in the node of *Aztekorphidia* minus *Al. (Az.) alanae* sp. nov.; twice in the node of (*Al. (Az.) minuta* + (*Al. (Az.) australis* + *Al. (Az.) tenochtitlana*)) + (*Alena (Az.) schremmeri* + (*Al. (Az.) caudata* + *Al. (Az.) infundibulata*)); twice in the node of *Alena (Az.) schremmeri* + (*Al. (Az.) caudata* + *Al. (Az.) infundibulata*); once in the node of *Al. (Az.) caudata* + *Al. (Az.) infundibulata*; and five times in the node *Al. (Az.) minuta* + (*Al. (Az.) australis* + *Al. (Az.) tenochtitlana*).

Concerning the vicariance events, they occurred once in the nodes of *Al. (Al.) distincta* + *Al. (Mx.) americana*; *Aztekorphidia*; *Al. (Az.) minuta* + (*Al. (Az.) australis* + *Al. (Az.) tenochtitlana*); and *Al. (Az.) caudata* + *Al. (Az.)*

infundibulata. The extinction events occurred once in the nodes of *Alena*, and *Al. (Az.) schremmeri* + (*Al. (Az.) caudata* + *Al. (Az.) infundibulata*).

The ancestral area of *Alena* is composed of the Californian (southwest U.S.A) and Transmexican Volcanic Belt, Balsas Basin, and Sierra Madre del Sur provinces from central Mexico.

Alena (Al.) distincta, the only species of the subgenus *Alena* s.s., occurs in the California province, extreme north region of the genus distribution; while its sister species and only representative of *Mexicoraphidia* – *Al. (Mx.) americana* –, occurs in the Transmexican Volcanic Belt and Balsas Basin provinces, in central Mexico. The disjunctive distribution between these species recovered an ancestral area composed of northern and southern provinces.

The biogeographic history of the subgenus *Aztekorphidia* is strongly associated with central Mexico, involving the Transmexican Volcanic Belt, Balsas Basin, and the Sierra Madre del Sur provinces. The species of *Aztekorphidia* except *Al. (Az.) alanae* **sp. nov.** are also associated with this general region, however, their biogeographic ancestral area does not include the Balsas Basin province.

Alena (Az.) horstaspoecki and *Al. (Az.) michoacana* occur in the same biogeographic province, the Transmexican Volcanic Belt, hypothesized as the area of occurrence of their stem species. The other clade of *Aztekorphidia*, composed of six species, presented a doubtful ancestral area. *Alena (Az.) schremmeri*, *Al. (Az.) caudata*, and *Al. (Az.) infundibulata* compose a clade strongly associated with the Transmexican Volcanic Belt, Balsas Basin, and Sierra Madre del Sur provinces, all of them from central Mexico.

The ancestral area for *Al. (Az.) minuta* + (*Al. (Az.) australis* + *Al. (Az.) tenochtitlana*) was inconclusive, probably because of the large distribution of *Al. (Az.) minuta*, which is the species with largest distribution in the genus, from provinces of the central Mexican region to those in the southwestern U.S.A: Mohavian, Navahonian, Sonoran, Sierra Madre Occidental, Chihuahuan, Transmexican Volcanic Belt, and Sierra Madre del Sur. *Alena (Az.) tenochtitlana*, and *Al. (Az.) australis* are sympatric in the Baja Californian province, and share this province as their possible ancestral area.

4. Discussion

4.1. Morphology and phylogeny of *Alena* Navás, 1916

The genus *Alena* was considered for a long time the sister group of the remaining extant Raphidiidae by several studies (H. Aspöck et al. 1991; U. Aspöck and H. Aspöck, 1996; H. Aspöck et al. 2001; H. Aspöck and U. Aspöck 2007). This previous notion was based on its highly derived morphology, especially the structure of the male

ninth abdominal segment, which is different to the common and plesiomorphic condition present in other Raphidiidae genera, i.e. tergite and sternite of ninth segment forming a ring, gx IX short in lateral view, gph IX weakly or not inserted on the basal sclerites of gx IX, and the complex of gx X located at the base or laterally to gph IX. The morphology of *Alena* precluded a possible hypothesis about its relationship with other genera of Raphidiidae, resulting in the choice of H. Aspöck et al. (1991) to keep this genus as sister to the remaining Raphidiidae. Nevertheless, Haring et al. (2011) performed a molecular phylogeny on the Raphidiidae, and recovered *Agulla* as sister to all remaining taxa of the family, while *Alena* was recovered as sister only to the Palearctic genera sharing the presence of basal sclerites of gx IX, considered as a primitive element of Raphidiidae by U. Aspöck and H. Aspöck (2013).

Based on our results, *Alena* is a monophyletic genus with several autapomorphies. Despite in our analysis this genus is supported by 16 autapomorphies, only five – all of them related to male genitalia – are exclusively to *Alena*. (1) *Alena* males have the tergite and sternite of the ninth abdominal segment separated, instead of forming a ring as in the other Raphidioptera representatives. The other two orders of insects closely related to Raphidioptera, i.e. Megaloptera and Neuroptera, also possess the ninth segment of the abdomen with separated tergite and sternite (U. Aspöck and H. Aspöck 2008), but these sclerites compounding a ring represent an autapomorphic character to Raphidioptera (Haring et al. 2011). Although genital characters are hardly visible in compression fossils, recently described amber specimens provided important details allowing to note that the ninth tergite and sternite compounding a ring are present in fossil families of Raphidioptera as well (see examples in Liu et al. 2016), so the separation of both sclerites in *Alena* is of secondary origin, besides being a useful character to identify its representatives. (2) Males of *Alena* have the gx IX elongate on the longitudinal axis, whereas the plesiomorphic condition, wider than long, or as long as wide is present in remaining Raphidiidae. In the genus *Alena*, the gx IX is at least 1.2 times longer than wide, and can be up to twice as long as wide in *Al. (Az.) minuta*, *Al. (Az.) australis*, and *Al. (Az.) tenochtitlana*. (3) The basal third of gx IX is narrower than its median region in males of *Alena*, while in other Raphidiidae this region is generally wider; most species of *Alena* have male gx IX basal third with approximately 2/3 as wide as the median area of gx IX, but in *Al. (Az.) minuta* + (*Al. (Az.) australis* + *Al. (Az.) tenochtitlana*) this region is approximately 1/2 as wide as the median area of gx IX; probably the narrowing of this gx IX region is related to the elongation on the longitudinal axis of such genital structure. (4) As already highlighted by Haring et al. (2011), *Alena* males have gph IX broadly inserted on the basal sclerites of gx IX, whereas in other Raphidiidae the gph IX are weakly or not inserted on the basal sclerites of gx IX. (5) The last exclusive autapomorphy of *Alena* is related to the amalgamation of the complex of gx X (“parameres”) and the gph IX (“hypovalva”), giving the appearance that the first structure is

the apex of the second (Haring et al. 2011). The species *Al. (Az.) horstaspoecki*, *Al. (Az.) michoacana*, *Al. (Az.) tenochtitlana* have the complex of gx X located as a separated structure, apically to the apex of gph IX, and they confirmed the hypothesis of U. Aspöck (2002) that hypovalva of some Raphidiidae species (especially *Alena*) are composed of the gph IX plus the complex of gx X.

Concerning the other 11 autamorphies of *Alena*, two are related to wing venation and nine are related to male genitalia. Although all of these characters are present in some degree in other Raphidioptera groups, they remain of great importance for understanding the genus morphology. Most of *Alena* species have the presence of the apical vein on pterostigma – which probably is a modification of the RA apex –, however *Al. (Az.) michoacana*, and the clade *Al. (Az.) minuta* + (*Al. (Az.) australis* + *Al. (Az.) tenochtitlana*) secondarily lacks such vein; the presence of this vein is considered an autapomorphy of *Alena*, but it is also present in other species of Raphidiidae, e.g. *Atlantoraphidia maculicollis* (Stephens, 1836), and *Ohmella postulata* (H. Aspöck and U. Aspöck, 1977). Another characteristic related to wing venation and considered an autapomorphy of *Alena* is the second ra-rp crossvein of forewing located on the median region of the pterostigma, but *Al. (Az.) michoacana* and the clade *Al. (Az.) minuta* + (*Al. (Az.) australis* + *Al. (Az.) tenochtitlana*) have this crossvein located apically to pterostigma. It is interesting to note that only the species of *Alena* with apical vein have the second ra-rp crossvein located in the median region of pterostigma, whereas the species which lack the apical vein possess this crossvein located apically on pterostigma. The location of the second ra-rp crossvein on forewing is variable in other Raphidioptera species, although there is a tendency for this crossvein to be apical to the pterostigma; contrary to what is observed in *Alena*, this crossvein can be located in the median (e.g. *Mongoloraphidia tshimganica* (H. Aspöck, U. Aspöck and Martynova, 1968), see fig. 254 of H. Aspöck et al. (1991)) or apical region of the pterostigma (e.g. *At. maculicollis*, and *O. postulata*, see figs 244–245 of H. Aspöck et al. (1991)) in species with apical vein.

All *Alena* species have the male sternite VIII in lateral view, shorter than tergite VIII, which reaches at most 1/3 of the tergite length in the two sister species *Al. (Az.) australis* and *Al. (Az.) tenochtitlana*; this character is variable between Raphidioptera groups. The presence of the dorsal median suture on the tergite IX is also autapomorphic to *Alena*, with secondary loss in *Al. (Az.) minuta*, and *Al. (Az.) caudata* + *Al. (Az.) infundibulata*. The male gx IX have a distinct basal sclerite, and despite the clear presence of this structure is herein considered as an autapomorphy of *Alena*, it is actually considered synapomorphic for the clade *Alena* + Palearctic Raphidiidae by Haring et al. (2011); *Agulla* has this structure indistinct (considered here as absent), and since this genus is considered sister to remaining Raphidiidae (Haring et al. 2011) the question whether this sclerite is an ancestral element lost in *Agulla* or is it a novelty of *Alena* + Palearctic Raphidiidae arose. U. Aspöck and H. Aspöck (2013) discussed about it and concluded that the basal sclerite of gx IX is an an-

cient element belonging to the ground pattern of the stem species of Raphidiidae. The male gst IX shape is highly variable in snakeflies, but it is of great importance helping in the differentiation of *Alena* subgenera; despite most of the species (*Aztekorphidia* minus *Al. (Az.) alanae* sp. nov.) have this structure arm-like in shape, the subquad-rangular gst IX is considered as autapomorphic of the genus. Male gph IX with tapered apex is also considered as autapomorphy of *Alena*, but *Al. (Az.) michoacana* + *Al. (Az.) horstaspoecki* and *Al. (Az.) schremmeri* have gph IX with a rhomboid apex, which is considered a reversion to the state present in *Ag. (Ag.) bicolor*.

An important structure, with several significant characteristics, of *Alena* is the ectoproct. Most species of the genus have the ectoproct rectangular in dorsal view, and subtriangular in lateral view, with a median V-shaped incision on the posterior margin, and lacking trichobothria. The exceptions are as follows: *Al. (Az.) minuta* + (*Al. (Az.) australis* + *Al. (Az.) tenochtitlana*), which have the ectoproct trapezoidal – with anterior margin narrower than posterior margin – in dorsal view, arm-like (elongate and narrow) in lateral view, and with posterior margin convex; *Al. (Al.) distincta* having a C-shaped ectoproct, in lateral view, with its apex ventrally folded; and *Al. (Az.) horstaspoecki* and *Al. (Az.) schremmeri*, which have trichobothria, considered a reversion in our analysis.

Regarding the three *Alena* subgenera, the monotypic *Mexicorphidia* and *Alena* s.s. are recovered herein as sister groups, comprising a clade sister to the monophyletic *Aztekorphidia*, confirming the hypothesis about their relationships presented by H. Aspöck et al. (1991). *Mexicorphidia* and *Alena* s.s. share males with apex of gx IX located below or before the gst IX base (located above or in front of gst IX in *Aztekorphidia*) and gst IX with bifurcated apex (simple in *Aztekorphidia*), and females with receptaculum seminis absent or strongly reduced (adhered or fused with atrium bursae in *Aztekorphidia*). In addition to the differences mentioned above, *Aztekorphidia* females have tube-like atrium bursae and elongate glandulae receptaculi, while females of *Mexicorphidia* and *Alena* s.s. have large and robust atrium bursae, and inconspicuous (*Alena* s.s.) or short (*Mexicorphidia*) glandulae receptaculi.

Based on our phylogenetic analysis, *Alena* s.s. and *Mexicorphidia* could be considered as parts of one subgenus, but we decided to maintain them as two monotypic subgenera established in other differences. *Alena* (*Al.*) *distincta*, the only species of the subgenus *Alena* s.s., has the base of MA on the hind wing as an oblique vein, whereas the only species of *Mexicorphidia* – *Al. (Mx.) americana* – possesses this vein as longitudinal. The male of *Al. (Al.) distincta* has the sternite IX indistinct, the gph IX with divergent apex, and a C-shaped ectoproct (in lateral view), with an apex ventrally folded, whereas the male of *Al. (Mx.) americana* has the sternite IX distinct, the gph IX with parallel apex, and a subtriangular ectoproct in lateral view. Apart from the differences related to the glandulae receptaculi mentioned above, the females of both subgenera have other important differences. Whereas females of *Alena* s.s. have the tergite VIII

ventrally separated, with its base narrower than its median region (in lateral view), those of *Mexicoraphidia* have the tergite VIII with a strong extension ventrally, with its base larger than its median region (in lateral view).

Alena (*Az.*) *alanae* **sp. nov.** is a remarkable species of *Aztekoraphidia*, and it is considered as sister to the remaining species of the subgenus. Despite this species shares several characteristics – mentioned above – with the other species of *Aztekoraphidia*, *Al.* (*Az.*) *alanae* is easily separated from them by the male *gst* IX and apex of *gph* IX. This new species has a large and subquadrangular *gst* IX, with a large base, whereas the remaining species of *Aztekoraphidia* have arm-like *gst* IX, with a narrow base. It is important to highlight that the *gst* IX of the new species is subquadrangular, but it is much longer when compared to those of *Mexicoraphidia* and *Alena* s.s. H. Aspöck et al. (1991) interpreted the arm-like male *gst* IX as an autapomorphy of *Aztekoraphidia*, however, it counts only for *Aztekoraphidia* minus *Al.* (*Az.*) *alanae* **sp. nov.**

The remaining eight species of *Aztekoraphidia* are recovered in three small clades. In its original description made by U. Aspöck and H. Aspöck (2013), *Al.* (*Az.*) *michoacana* was not related to any other species. Nevertheless, this species is herein deemed as closely related to *Al.* (*Az.*) *horstaspoecki*, sharing several characters among which we can highlight males with ridge of *gst* IX poorly-developed (well developed in other *Alena* species), *gph* IX with apex before the tergite IX apex in lateral view (apex located beyond the tergite IX apex in other *Alena* species), and complex of *gx* X located apically to *gph* IX as a clearly separated structure (also present in *Al.* (*Az.*) *tenochtitlana*, and completely amalgamated with *gph* IX in other *Alena* species), with projections (absent in other *Alena* species).

The second clade of *Aztekoraphidia* is composed of *Al.* (*Az.*) *minuta* + (*Al.* (*Az.*) *australis* + *Al.* (*Az.*) *tenochtitlana*). In the original description made by U. Aspöck and H. Aspöck (1978), *Al.* (*Az.*) *tenochtitlana* was considered closely related to *Al.* (*Az.*) *australis*, which is confirmed by our results. Herein, is the first time that the *Al.* (*Az.*) *minuta* is clearly related to other species. These three species share several synapomorphies, including the basal third of male *gx* IX elongate (short in other *Alena* species), approximately 1/2 as wide as its median region (approximately 2/3 as wide as its median region in other *Alena* species), and ectoproct trapezoidal in dorsal view (rectangular in other *Alena* species), and arm-like in lateral view (subtriangular or C-shaped in other *Alena* species), with median region of posterior margin convex (with V-shaped incision on other *Alena* species).

The third clade of *Aztekoraphidia*, which is sister to the group noted above, is composed of *Al.* (*Az.*) *schremmeri* + (*Al.* (*Az.*) *caudata* + *Al.* (*Az.*) *infundibulata*). This is not a surprise, as since the original description of *Al.* (*Az.*) *schremmeri* and *Al.* (*Az.*) *infundibulata* made by U. Aspöck et al. (1994a) it is known that these three species are closely related. This group shares hind wings with MA base present as a longitudinal vein (also observed in *Al.* (*Az.*) *horstaspoecki* and *Al.* (*Mx.*) *americana*), and

male *gx* IX with elongated apex (independently originated in *Al.* (*Az.*) *minuta*, and short in other *Alena* species), and bearing a dorsoposterior tapered process (absent in other *Alena* species).

4.2. Biogeography of *Alena* Navás, 1916

The genus *Alena* is closely related to mountainous regions, and its species are recorded in high altitudes of provinces belonging to: (1) the Western subregion, which is composed of the Californian and Rocky mountain dominions, representing the western coastal and western central regions of southwest of U.S.A., respectively (Escalante et al. 2013, 2021), and (2) the Mexican Transition Zone, a varied and complex area where Nearctic and Neotropical biotas overlap (Halffter and Morrone 2017; Morrone 2019) (Fig. 5). Except for *Al.* (*Al.*) *distincta*, which has the lowest record for the genus (900–1200 m), *Alena* species are found from 1270 to 2970 m.

Apart from *Al.* (*Al.*) *distincta* and *Al.* (*Az.*) *minuta*, which also occur in the southwestern region of the U.S.A. – in the states of California, Arizona, Colorado, New Mexico, and Utah –, *Alena* species are present in restricted mountainous areas from western Mexico (Fig. 1) (U. Aspöck and H. Aspöck 1996; 2013). The genus diversity is probably much greater than what is known so far, since Mexico has 65% of its surface considered mountainous, exceeding 500 m (Ferrusquía-Villafranca 1998; Morrone 2019), areas that are scarcely explored and may host other unknown endemic *Alena* species.

North American snakeflies occur only in the western region of this subcontinent, which according to U. Aspöck et al. (2012) could be due to the epicontinental sea crossing North America in the Cretaceous (Skelton 2006; Morrone 2019) having place as an effective barrier preventing the dispersal of the Raphidioptera to the eastern region of North America, plus the low expansion capacity of these insects preventing their dispersion to the northern parts of the subcontinent.

Alena species are also known only from the western region of North America, nevertheless this distribution is probably related to the low expansion capacity of these insects preventing their dispersion, plus the high mountain systems present in this subcontinent acting as a geographic barrier and the necessity for low temperatures for its correct development.

At present, in its northernmost portion, the eastern limit of *Alena*'s distribution is essentially the Rocky Mountains, which is connected to the Sierra Madre Occidental (the western limit of *Alena* in Mexico) in its southern portion. In the Mexican area, the eastern limit of *Alena* is the western part of the Mexican Plateau, the Sierra Madre Oriental, the Transmexican Volcanic Belt, the Sierra Norte de Oaxaca, and south of the Sierra Madre del Sur. All these mountains are connected and form a large barrier to *Alena* species in its eastern limit. The southeast part of the Mexican Plateau has connection to the Sierra Madre Oriental, which is connected in its

southern region to the Transmexican Volcanic Belt – a mountain range which crosses the country in an east-west direction, approximately between the parallels of 19 and 21° N (Morrone 2019) –; the north of the Sierra Norte de Oaxaca is connected to the eastern part of the Transmexican Volcanic Belt, and in its southwest portion this sierra has connection to the eastern part of the Sierra Madre del Sur, locality where the southernmost records of *Alena* are present.

The western limits for *Alena*, from north to south, are composed of the Coastal Range Mountains, and the Sierra Nevada, which connect to the Cordillera of Baja California in the Baja California Peninsula. The western limit of the genus in the remaining Mexico is the Sierra Madre Occidental, which is connected to the Rocky Mountains in the northern part, and in the southern part with the Transmexican Volcanic Belt, which has its western part connected to the Sierra Madre del Sur (Morrone 2019), forming a western barrier to *Alena*.

U. Aspöck et al. (2012) primarily argued that the biogeographic pattern of Raphidiidae may be explained by the existence of a stem species (by the end of the Cretaceous but prior to the K-T boundary) with an extensive northern – possibly circumpolar – distribution, which originated the *Agulla* lineage (Nearctic region) and the stem species of remaining Raphidiidae, which was separated in the course of the continental drift originating the Nearctic *Alena* and the Palearctic lineage with the remaining taxa of the family. A subsequent hypothesis to explain the *Alena* biogeographic pattern was presented by U. Aspöck and H. Aspöck (2013), who argued that an ancestral stem species of *Alena* reached Mexico when it was orographically much more homogeneous than at present. A dramatic radiation caused by volcanism plus tectonic events, raising of mountains, flooding and climatic changes catalyzed the rate of evolution of *Alena*. These hypotheses fit with the results herein presented, with the stem species of *Alena* widely distributed, from the areas that now form the southwest of the U.S.A. (especially Californian Province) and reaching until central Mexico, in the Transmexican Volcanic Belt, the Sierra Madre del Sur, and the Balsas Basin provinces.

Alena is the Raphidiidae group closest to the tropical region in distribution, and although their species are present in provinces considered as part of the Mexican Transition or Neotropical Zones, this genus also needs a cool period (winter) for proper development, and occurs only in high altitudes where the temperatures are low. Nowadays, Mexican *Alena* species represent a snakefly fauna “imprisoned” in its former glacial refuge areas. According to this hypothesis, *Alena* species could extend their distribution to and in lower lands during the glacial periods, nevertheless in interglacial periods (including to date), the lower elevations lack *Alena* species when the rise of temperature began. This probably occurred during the Pleistocene, which is considered a series of glacial periods interposed by interglacial ones that began 2.58 Ma, and strongly affected North America, among other northern regions of the planet (Ehlers and Gibbard 2011).

All the main lineages of *Alena* have some affiliation with the Transmexican Volcanic Belt, the Sierra Madre del Sur, and the Balsas Basin provinces, which are biogeographically related and share a great amount of species with one other (Halffter and Morrone 2017; Morrone 2001, 2019).

The Transmexican Volcanic Belt province corresponds to the mountainous system of the Transmexican Volcanic Belt, a set of mountain ranges of volcanoes that crosses the country from west to east, and have a complex geological history and biotic connections to other biogeographic provinces resulting in one of the most complex and heterogeneous Mexican provinces, with two well-defined subprovinces (Gámez et al. 2012; Morrone 2019). The Sierra Madre del Sur province is composed of the mountain system of the Sierra Madre del Sur, which runs parallel to the coast of the Pacific Ocean from Jalisco to the Isthmus of Tehuantepec, in a northwest-southeast direction (Hernández-Cerda et al. 2016; Morrone 2019). This province possesses a variety of valleys and rivers, which together with great climatic diversity, results in a region with large diversity of flora and fauna, as well as three well-delimited subprovinces (Hernández-Cerda et al. 2016; Morrone 2019). The Balsas Basin province corresponds to the Balsas River Basin, which is located between the former two provinces and also has a wide diversity of climates, resulting in two districts (Gámez et al. 2012; Morrone 2019).

The genus *Alena* possesses two main lineages, the smallest of them is composed of two disjunctively distributed monotypic subgenera, *Mexicoraphidia*, recorded in central Mexico (provinces of the Transmexican Volcanic Belt and the Balsas Basin), and *Alena* s.s., located in the southwestern U.S.A. (Californian province). Although the biogeographic pattern of this clade is unclear, it is possible to assume based on our biogeographic analysis that its stem species was probably present in a large area of distribution – similar to the stem species of the genus *Alena* – and a vicariance event resulted in this disjunctive pattern. The second lineage of *Alena* is composed of *Aztekoraphidia* species with its stem species associated with the Transmexican Volcanic Belt, the Balsas Basin, and the Sierra Madre del Sur provinces, in central Mexico. Possibly, the changes of the distribution of *Alena* populations in glacial and interglacial periods provided the separation of the stem species of *Aztekoraphidia* from the stem species of *Alena* s.s. + *Mexicoraphidia* by dispersal and extinction events, as highlighted in our analysis.

Alena (*Az.*) *alanae* **sp. nov.**, sister to other *Aztekoraphidia*, occurs in the Upper Balsas District located in the eastern area of the Balsas Basin province, and presents the higher altitudes of it, as well as a higher proportion of temperate climate species (Castro-Torreblanca et al. 2014; Morrone 2019). The remaining species of the subgenus share a biogeographic ancestral area on the Transmexican Volcanic Belt and the Sierra Madre del Sur provinces. Based on our results, a dispersal event of the stem species of *Aztekoraphidia* from the Balsas Basin and Sierra Madre del Sur provinces to the Transmexican Volcanic Belt province, followed by a vicariance event

between the Balsas Basin and the two other provinces separated *Al. (Az.) alanae* **sp. nov.** from the stem species of the remaining *Aztekoraphidia* species. This result fits with the possible effect of glacial and interglacial periods in the *Alena* populations; a dispersal event may have occurred in a glacial period allowed by the drop of temperature, followed by a vicariance event and the temperature rise in the interglacial period isolating the new species from the stem species of the other *Aztekoraphidia*.

The group composed of *Aztekoraphidia* minus *Al. (Az.) alanae* **sp. nov.** possesses two lineages. The smaller of them is composed of *Al. (Az.) horstaspoecki* and *Al. (Az.) michoacana* occurring in the Transmexican Volcanic Belt province and sharing a biogeographic ancestral area in this same locality. Nevertheless, the former species (*Al. (Az.) horstaspoecki*) is recorded to the east subprovince (areas between 2000 and 3000 m, vegetation of Pine-Encino and xerophytic scrub forests), whereas the latter species (*Al. (Az.) michoacana*) is present in the west subprovince (areas lower than 2000 m, deciduous tropical forests) (Torres-Miranda and Luna 2007; Morrone 2019). The second lineage is composed of the remaining six *Aztekoraphidia* species – divided in two clades –, and has a doubtful ancestral area. Based on the distribution of these two lineages, their ancestral area, and the two dispersal events related to this node, it may be hypothesized that the stem species of both clades separated in central Mexico, with the stem species of *Al. (Az.) horstaspoecki* and *Al. (Az.) michoacana* dispersing through the Transmexican Volcanic Belt province and the stem species of the larger clade (composed of six *Aztekoraphidia* species) dispersed to the north occupying a large distribution area.

The biogeographic ancestral area of *Al. (Az.) minuta* + (*Al. (Az.) tenochtitlana* + *Al. (Az.) australis*) was recovered as inconclusive. *Alena (Az.) minuta* is a highly variable species with wide distribution, occurring in several southern and northern provinces. *Alena (Az.) tenochtitlana* and *Al. (Az.) australis* are sympatric in the Baja Californian province, sharing a biogeographic ancestral area in this same locality. Our S-DIVA analysis recovered five dispersal events of the stem species of *Al. (Az.) minuta* + (*Al. (Az.) tenochtitlana* + *Al. (Az.) australis*) resulting in its presence in eight provinces, and a posterior vicariance event separating *Al. (Az.) minuta* from the stem species of *Alena (Az.) tenochtitlana* and *Al. (Az.) australis* of the Baja Californian province. Such events, similar to that from the *Alena (Az.) alanae* **sp. nov.** and the stem species of other *Aztekoraphidia*, also can be explained by the variation of temperature provided by the glacial and interglacial periods.

Alena (Az.) schremmeri + (*Al. (Az.) infundibulata* + *Al. (Az.) caudata*) have their biogeographical history closely related to the provinces of the central Mexican region, i.e. the Transmexican Volcanic Belt, the Balsas Basin, and the Sierra Madre del Sur. The stem species of the clade probably was present in these three provinces, and posteriorly *Al. (Az.) schremmeri* (currently located in the occidental subprovince *sensu* Morrone (2019) of the Sierra Madre del Sur province) separated from the stem species of *Al. (Az.) infundibulata* + *Al. (Az.) caudata* that

occurred in the three provinces cited above. *Alena (Az.) infundibulata* is located in the occidental subprovince of the Sierra Madre del Sur province, whereas *Al. (Az.) caudata* has records to high altitudes on the Upper Balsas District (east of the Balsas Basin province), and in the east subprovince of the Transmexican Volcanic Belt.

Lastly, we want to connect the present analyses with previous approaches concerning the biogeography of *Alena* based on the concept of de Lattin (1967). This master work published in German has largely been overlooked in biogeographic circles of English-speaking authors (see U. Aspöck et al. 1992), although the “Zones” favored by Noonan (1988) show an amazing congruence with the concept of de Lattin. The “biogeographic glossary” in the monograph of the Raphidioptera (H. Aspöck et al. 1991) is based on the concept of glacial refuge centers defined by de Lattin (1967). The background is the hypothesis that extant distribution patterns of species are only partly due to the ecological valences of the single species but additionally have an historical biogeographical background, e.g. glacial refuge centers. These centers may concern many diverse organisms, creating general patterns for postglacial dispersal, which are, however, superimposed by ecological parameters of the concrete single species.

The huge Mexican glacial refuge center in the sense of de Lattin (1967), extended by H. Aspöck et al. (1991), concerns the arboreal biome and awaits the structuring into biogeographic sub-centers reflecting the rich orographic scenario of Mexico.

The distribution areas of Mexican snakeflies are still poorly known (Fig. 1) and it is not known whether those states in which neither *Alena* nor other Raphidioptera have been found so far really lack them. The historical background of recent distribution patterns of snakeflies of Mexico is extremely complex. Fluctuations of species / populations caused by pre-glacial, glacial, and post-glacial climate changes, volcanic events and other factors, are not yet sufficiently understood and thus detract analyses with respect to reconstruction of extant distribution areas of *Alena*.

The structuring of the huge Mexican glacial refuge center into sub-centers deduced from the records of single species and characterizing these species as faunal elements of these sub-centers, has been a challenge from the very beginning of our studies on Mexican snakeflies (U. Aspöck and H. Aspöck 1970; U. Aspöck 1974), and kept us energized (H. Aspöck et al. 1991.; U. Aspöck et al. 1992, 1994; U. Aspöck and H. Aspöck 1996, 2013; U. Aspöck and Contreras-Ramos 2004).

4.3. Biology and ecology of *Alena* Navás, 1916

In addition to its uncommon morphology, *Alena* also has a remarkable biology, differing from other Raphidiidae species. This genus possesses larvae that pupate in the summer and autumn, and develop to imago (about within two weeks) also in these periods, whereas the most

part of other Raphidiidae genera have its larvae pupating in spring after hibernation of the last instar (H. Aspöck 2002; U. Aspöck and H. Aspöck 2013). According to Aspöck (2002), the Raphidioptera genera have their biology cataloged into three types, which are related to the period of time between the end of hibernation of larvae and emergence of adults, and the last hibernating instar; based in this study *Alena* represents “Type III”, which highlights that the low temperature is important for the mature larvae to pupate (H. Aspöck 2002).

The known larvae of *Alena* are considered to be corticolous, developing under bark of trees, feeding on a great variety of soft-bodied arthropods; while the adults' food is similar to all other Raphidiidae genera, and is especially composed of insects with preference for aphids and other Sternorrhyncha, but also predating other arthropods, and occasionally feeding on pollen (H. Aspöck 2002; U. Aspöck and H. Aspöck 2013). Concerning the mating behavior, *Alena* uses the “wrecking position” to copulate, similar to other Raphidiidae. However, the couple marches together and then the male lands in the back of the female, which is not observed in other genera (U. Aspöck et al. 1994a; H. Aspöck 2002). In general, snakefly species are considered active during the daytime (H. Aspöck et al. 1991; Oswald and Machado 2018), nevertheless, several specimens of *Alena* – including all specimens of *Al. (Az.) alanae* **sp. nov.** – have been collected on light traps at night, which raises the doubt if this group has adults with also crepuscular or nocturnal activity.

Representatives of *Alena* are found in high areas, between 900 and 2970 m, and occur especially in *Calliandra*, cypresses trees, and especially in pine-oak forests, particularly in species of *Arctostaphylos*, *Juniperus*, *Pinus*, *Quercus* (H. Aspöck et al. 1991; U. Aspöck and H. Aspöck 2013). It is interesting to note that although there is a large number of parasites, parasitoids, and hyperparasites that have snakeflies as host (see the table of H. Aspöck 2002), none are known to affect *Alena* species.

5. Conclusion

The genus *Alena* is monophyletic, as well as its subgenus *Aztekorphidia*, which is sister of a clade composed of the other two monotypic genera, *Alena* s.s. and *Mexicorphidia*. This genus has a biogeographic history strongly associated with central Mexico, especially the mountains of the Transmexican Volcanic Belt, the Balsas Basin, and the Sierra Madre del Sur provinces. Probably the stem species of the genus was originally (in preglacial periods) distributed across the central and southern regions of western North America. Its current diversity is only composed of small groups that survived imprisoned in their former glacial refuge areas in the mountains from central and western Mexico, and southwestern U.S.A. Nevertheless, the recent finding of the new Mexican species – *Al. (Az.) alanae* **sp. nov.** – demonstrates that the knowledge on actual diversity of *Alena* remains incomplete.

6. Competing interests

The authors have declared that no competing interests exist.

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Appendix 1

List of characters (coded in Table 1), with notes on issues of homology, distribution of the characters states and corresponding hypotheses of autapomorphy and synapomorphy, multiple origin and/or secondary loss.

Wings

1. Color of pterostigma: (0) one color (usually brown or yellow); (1) two colors (usually with brownish base and yellowish apex).

Pterostigma color is also variable in Neuropterida, however it is constant in each species of *Alena*, what enabled its use as a reliable character in our analysis. Most of the *Alena* species possess the plesiomorphic state, pterostigma with one color, while this region with two colors is synapomorphic to *Al. (Az.) minuta* + (*Al. (Az.) australis* + *Al. (Az.) tenochtitlana*), and has an independent origin in *Ag. (Ag.) bicolor*.

2. Apical vein: (0) absent; (1) present.

Apical vein is an oblique vein located at the apical region of pterostigma and is present in some representatives of Raphidiidae, including most part of *Alena*. Its presence is considered an autapomorphy of the genus, with two independent secondary losses, in *Al. (Az.) michoacana* and *Al. (Az.) minuta* + (*Al. (Az.) australis* + *Al. (Az.) tenochtitlana*).

3. Second ra-rp crossvein on forewing: (0) on the base of pterostigma; (1) apically to pterostigma; (2) on the median region of pterostigma.

The location of the forewing second ra-rp crossvein is also variable in Raphidioptera. The presence of this crossvein on the median region of pterostigma is considered autapomorphic to *Alena*, with two independent displacements to the most apical region of pterostigma in *Al. (Az.) michoacana*, and *Al. (Az.) minuta* + (*Al. (Az.) australis* + *Al. (Az.) tenochtitlana*), also present in *Ag. (Ag.) bicolor*.

4. Base of MA on hind wing: (0) absent; (1) as cross or oblique vein; (2) as longitudinal vein.

Indianoinocellia mayana has no visible base of MA on the hind wings, so its presence is synapomorphic to the remaining species of the analysis. Hind wing with base of MA as cross/ oblique vein is synapomorphic to *Ag. (Ag.) bicolor* and *Alena*, while MA base as longitudinal vein is present in *Al. (Mx.) americana*, *Al. (Az.) horstaspoecki*, and *Al. (Az.) schremmeri* + (*Al. (Az.) caudata* + *Al. (Az.) infundibulata*).

Male abdomen and genitalia

5. Sternite VIII length, lateral view: (0) as long as tergite VIII; (1) 3/4–1/2 of the tergite VIII length; (2) 1/3 of the tergite VIII length.

Despite the length of the sternite VIII is variable among Raphidiidae genera, a shorter male sternite VIII is synapomorphic to *Alena* representatives, with *Al. (Az.) australis* and *Al. (Az.) tenochtitlana* sharing an even more extreme condition of this state, i.e. sternite VIII length corresponding to 1/3 of the tergite VIII length.

6. Tergite and sternite of segment IX: (0) forming a ring; (1) separated.

Male sclerites of ninth abdominal segment forming a ring is autapomorphic to Raphidioptera (Haring et al. 2011), but they are separated in *Alena*, which has secondary origin and is autapomorphic to the genus.

7. Tergite IX, dorsal view: (0) without median suture; (1) with median suture.

Most *Alena* species have a median suture in the dorsal region of male tergite IX, herein considered autapomorphic to the genus. *Alena (Az.) minuta*, and *Al. (Az.)*

caudata + *Al. (Az.) infundibulata* have independently secondary losses of such suture.

8. Sternite IX: (0) distinct; (1) indistinct.

The plesiomorphic state is widely distributed in the *Alena* lineages. The apomorphic state originated four times: *Al. (Al.) distincta*, *Al. (Az.) michoacana* + *Al. (Az.) hostaspoecki*, *Al. (Az.) australis* + *Al. (Az.) tenochtitlana*, and *Al. (Az.) caudata*.

9. Basal sclerites of gx IX: (0) amalgamated; (1) distinct.

Agulla species have males with no basal sclerites, which is apomorphic or plesiomorphic depending from the hypothesis of Raphidiidae phylogenetic relationships (Haring et al. 2011). The clearly presence of basal sclerites of gx IX is synapomorphic to *Alena* + Palearctic Raphidiidae taxa (Haring et al. 2011), herein we did not use Palearctic Raphidiidae as outgroups, resulting in this characteristic as *Alena*'s autapomorphy.

10. Shape of gx IX (length/width): (0) gx IX wider than long; (1) gx IX 1.2–1.5× longer than wide; (2) gx IX at least 1.7× longer than wider.

The length of gx IX was measured as the distance between its base and the base of its apex, and the width was measured in the median region of gx IX.

The ratio between length and width of gx IX varies between Raphidioptera species, but *Alena* species have this structure elongated on the longitudinal axis, at least 1.2× longer than wide. In the species *Al. (Az.) schremmeri*, *Al. (Az.) caudata*, *Al. (Az.) infundibulata*, *Al. (Az.) minuta*, *Al. (Az.) australis*, and *Al. (Az.) tenochtitlana* this elongation is even more remarkable, with gx IX at least 1.7× longer than wide.

11. Width of basal third of gx IX: (0) wider than the median region of gx IX; (1) approximately 2/3 as wide as the median region of gx IX; (2) approximately 1/2 as wide as the median region of gx IX.

Male gx IX with basal third narrower than its median region is autapomorphic to *Alena*. In *Al. (Az.) minuta*, *Al. (Az.) australis*, and *Al. (Az.) tenochtitlana* it is more emphasized, with the basal third having 1/2 of the median region of gx IX.

12. Length of basal third of gx IX: (0) short; (1) elongated.

The elongation of the basal third of male gx IX clearly occurred once in the *Alena* evolutionary history, with the apomorphic state being a synapomorphy of the clade composed of *Al. (Az.) minuta*, *Al. (Az.) australis*, and *Al. (Az.) tenochtitlana*.

13. Ventral margin of gx IX: (0) slightly convex or straight; (1) concave on median region.

Alena (Az.) australis and *Al. (Az.) tenochtitlana* possess the ventral margin of male gx IX slightly curved up, resulting in a concave median region, synapomorphic to this clade.

14. Apex of gx IX: (0) absent; (1) located below or before the gst IX base; (2) located above or in front of gst IX.

All the male *Alena* species have the gx IX with a distinct apex. It can be located below or before the gst IX base, synapomorphic to *Alena* s.s. + *Mexicoraphidia*, or located above or in front of gst IX, autapomorphy of *Aztekorphidia*.

15. Apex of gx IX (when present): (0) short, and digitiform; (1) elongated, and arm-like or rod-shaped.

The apomorphic state originated independently twice, in *Al. (Az.) minuta*, and synapomorphic to *Al. (Az.) schremmeri* + *Al. (Az.) caudata* + *Al. (Az.) infundibulata*.

16. Dorsoposterior tapered process of gx IX: (0) absent; (1) present, not serrated; (2) present, serrated.

The presence of the dorsoposterior tapered process on male gx IX is synapomorphic to *Al. (Az.) schremmeri* +

Al. (Az.) caudata + *Al. (Az.) infundibulata*. Different from the other two species, males of *Al. (Az.) infundibulata* have the dorsal margins of such process serrated. Some of the *Al. (Az.) minuta* males possess this process, while others not, so this species was coded as missing data “?”.

17. Ridge of gst IX: (0) absent; (1) well developed, elongate; (2) poorly developed.

The ridge of male gst IX is a sclerotized region of the gx IX which serves as insertion point to gst IX muscles, it is located at the base of gst IX, and is easily observed in Raphidiidae representatives (H. Aspöck et al. 1991). All the *Alena* males have this structure well developed, except *Al. (Az.) michoacana* and *Al. (Az.) horstaspoecki*, which share poorly developed gst IX ridge as synapomorphy.

18. Shape of gst IX: (0) subtriangular; (1) subquadrangular; (2) arm-like, elongated.

The outgroup males utilized in our analysis have a subtriangular gst IX. The subquadrangular gst IX was recovered as an autapomorphy of *Alena*, however it is only present in *Al. (Al.) distincta*, *Al. (Mx.) americana*, and *Al. (Az.) alanae* **sp. nov.** The remaining species of *Alena* share an arm-like gst IX as synapomorphy, characteristic already considered as autapomorphy of *Aztekorphidia* by H. Aspöck et al. (1991).

19. Shape of base of gst IX, lateral view: (0) large, approximately two times as large as its apex; (1) narrow, approximately as large as its apex.

Males with large base of gst IX are present in *Al. (Al.) distincta*, *Al. (Mx.) americana*, and *Al. (Az.) alanae* **sp. nov.**, while the remaining species of *Alena* shares gst IX with narrow base.

20. Apex of gst IX: (0) simple; (1) bifurcated.

Most Raphidiidae species, including all *Aztekorphidia*, have males with simple gst IX apex. The other two subgenera of *Alena*, i.e. *Alena* s.s. and *Mexicoraphidia*, share male gst IX with bifurcated apex, synapomorphy of this clade.

21. Gph IX: (0) composing paired short structures (“pseudostyli”); (1) composing a structure with two elongated rods (“hypovalva”).

According with U. Aspöck and H. Aspöck (2004), the male gonapophyses IX composing the “hypovalva” is autapomorphic to Raphidiinae. In our analysis, this characteristic is synapomorphic to *Agulla* and *Alena*, with *I. mayana* presenting the plesiomorphic state.

22. Position of gph IX apex (when composing the “hypovalva”), lateral view: (0) located beyond the tergite IX apex (generally reaching at least 3/4 of gx IX length); (1) located before the tergite IX apex (generally reaching 1/4–1/2 of gx IX length).

This character is contingent on (or associated with) presence of gph IX composing a structure with two elon-

gate rods (“hypovalva”) in males, and it is better observed in lateral view. *Indianoinocellia mayana* has no “hypovalva”, so it is coded as inapplicable “–”. *Agulla* (*Ag.*) *bicolor*, and most part of *Alena*, have males with gph IX apex surpassing the tergite IX apex, while *Al.* (*Az.*) *michoacana* and *Al.* (*Az.*) *horstaspoecki* share male gph IX with its apex not reaching the tergite IX apex.

23. Arrangement of gph IX apex (when composing the “hypovalva”), ventral view: (0) parallel; (1) divergent.

This character is contingent on (or associated with) the presence of gph IX composing a structure with two elongated rods (“hypovalva”) in males, and it is better observed in ventral view. *Indianoinocellia mayana* has no “hypovalva”, so it is coded as inapplicable “–”. The arrangement of the apex of male gph IX is highly variable between the groups of Raphidiidae, including species of *Alena*. The apomorphic state has two independent origins, in *Al.* (*Al.*) *distincta*, and in the clade composed of *Aztekorphidia* minus *Al.* (*Az.*) *alanae* **sp. nov.**, which have a reversion to gph IX apex parallel in *Al.* (*Az.*) *schremmeri*.

24. Shape of gph IX apex (when composing the “hypovalva”), ventral view: (0) rhomboid; (1) tapered.

This character is contingent on (or associated with) the presence of gph IX composing a structure with two elongated rods (“hypovalva”) in males, and it is better observed in ventral view. *Indianoinocellia mayana* has no “hypovalva” so it is coded as inapplicable “–”. Similar to character 23, it is highly variable in Raphidiidae, nevertheless most species of *Alena* have male gph IX with a tapered apex, which was recovered as autapomorphic to the genus. Two reversions to rhomboid apex occurred independently, in *Al.* (*Az.*) *schremmeri* and *Al.* (*Az.*) *michoacana* + *Al.* (*Az.*) *horstaspoecki*.

25. Gph IX (when composing the “hypovalva”), ventral view: (0) weakly or not inserted on the basal sclerites of gx IX; (1) broadly inserted on the basal sclerites of gx IX.

This character is contingent on (or associated with) the presence of gph IX composing a structure with two elongate rods (“hypovalva”) in males, and it is better observed in ventral view. *Indianoinocellia mayana* has no “hypovalva”, so it is coded as inapplicable “–”. Males with gph IX strongly inserted in the basal sclerites of gx IX is autapomorphic to *Alena*, highlighted by Haring et al. (2011).

26. Complex of gx X: (0) located at the base level or laterally to gph IX; (1) completely amalgamated with gph IX; (2) located apically to gph IX.

Males with the complex of gx X located at the base or laterally to gph IX are present in *I. mayana* and *Ag.* (*Ag.*) *bicolor*, and this is considered the plesiomorphic state in our analysis. U. Aspöck (2002) hypothesized that some species of Raphidiidae suffered amalgamation of gph IX and complex of gx X, which subsequently was confirmed with the *Alena* (*Az.*) *horstaspoecki*, *Al.* (*Az.*) *michoacana*, and *Al.* (*Az.*) *tenochtitlana* genitalia. Posteriorly, Haring et al. (2011) considered this amalgamation

as an autapomorphy of *Alena*, which is herein confirmed. The clear separation of complex of gx X, with it apically located to gph IX, have two independent origins, in *Al.* (*Az.*) *horstaspoecki* + *Al.* (*Az.*) *michoacana* and *Al.* (*Az.*) *tenochtitlana*. There are doubts about whether *Al.* (*Az.*) *infundibulata* has the complex of gx X completely separated in the apical region of gph IX, so this species was coded as missing data “?”.

27. Projections of complex of gx X (when not amalgamate with gph IX): (0) absent; (1) present.

This character is contingent on presence of complex of gx X separated from gph IX in males. *Alena* (*Az.*) *tenochtitlana* has the plesiomorphic state, while the presence of projections is synapomorphic to *Al.* (*Az.*) *horstaspoecki* + *Al.* (*Az.*) *michoacana*. All the other species of *Alena* have the complex of gx X amalgamated with gph IX, so there are coded as inapplicable “–” for these.

28. Shape of ectoproct, dorsal view: (0) subquadrangular; (1) rectangular; (2) trapezoidal, with anterior margin narrower than posterior margin.

Males of *I. mayana* and *Ag.* (*Ag.*) *bicolor* have subquadrangular ectoprocts. Most species of *Alena* present males with rectangular ectoprocts, elongate in antero-posterior axis, and considered autapomorphic to the genus. The change to trapezoidal ectoproct, with anterior margin narrower than its posterior margin, is synapomorphic to *Al.* (*Az.*) *minuta* + *Al.* (*Az.*) *australis* + *Al.* (*Az.*) *tenochtitlana*.

29. Shape of median region of ectoproct posterior margin, dorsal view: (0) approximately straight; (1) with median V-shaped incision; (2) convex.

Indianoinocellia mayana and *Ag.* (*Ag.*) *bicolor* have the male ectoproct with posterior margin approximately straight, while the male ectoproct with a median V-shaped incision on its posterior margin is autapomorphic to *Alena*. Nevertheless, the clade *Al.* (*Az.*) *minuta* + (*Al.* (*Az.*) *australis* + *Al.* (*Az.*) *tenochtitlana*) have the median posterior margin of the ectoproct convex, clearly synapomorphic to this group.

30. Shape of ectoproct, lateral view: (0) rounded or subquadrangular; (1) subtriangular; (2) C-shaped, with apex ventrally folded; (3) arm-like.

The lateral shape of the male ectoproct is also highly variable in Raphidioptera. Herein the rounded or subquadrangular ectoprocts on males are considered the plesiomorphic state, present in outgroups. Subtriangular ectoproct is synapomorphic to *Alena* species, suffering two changes: (1) C-shaped ectoproct in *Al.* (*Al.*) *distincta*, and (2) arm-like ectoproct, elongate and narrow in lateral view, in *Al.* (*Az.*) *minuta* + (*Al.* (*Az.*) *australis* + *Al.* (*Az.*) *tenochtitlana*).

31. Trichobothria on ectoproct: (0) present; (1) reduced or absent.

Most part of the Neuropterida groups have trichobothria in its male ectoprocts, nevertheless their amount,

presence/absence, and distribution are variable. *Indianoinocellia mayana* and *Ag. (Ag.) bicolor* clearly possess trichobothria. Most part of *Alena* species have male ectoprocts without trichobothria or in small number, which is autapomorphic to the genus. Two distant species of *Aztek-*

oraphidia, *Al. (Az.) horstaspoecki* and *Al. (Az.) schremmeri*, possess males with ectoprocts bearing trichobothria, which is considered two independent reversions.

Female abdomen and genitalia

As *Alena (Az.) michoacana* has no described female, it is coded as missing data “?” in the following characters of female.

32. Tergite VIII: (0) ventrally separated, not forming a ring; (1) with strong ventral extension; (2) fused ventrally, forming a ring.

Generally, the tergite VIII of females is ventrally separated in snakeflies, which is also observable on most part of *Alena*. However, *Al. (Mx.) americana* has its tergite VIII with a strong extension, which is considered its autapomorphy. Other important apomorphic state was originated inside the *Aztekorphidia* subgenus, with *Al. (Az.) australis* + *Al. (Az.) tenochtitlana* possessing females with tergite VIII ventrally fused, forming a ring.

33. Tergite VIII, lateral view: (0) base narrower than, or as large as, its median region; (1) base clearly larger than its median region.

Character highly variable in Raphidioptera. Most part of *Alena* species have females possessing the plesiomorphic state, but there are three independently origins of the female tergite VIII, having its base clearly larger than its median region, in *Al. (Mx.) americana*, *Al. (Az.) australis* + *Al. (Az.) tenochtitlana*, and *Al. (Az.) infundibulata*; state also present in *Ag. (Ag.) bicolor*.

34. Atrium bursae: (0) inconspicuous; (1) large, robust; (2) tube-like.

The atrium bursae of *I. mayana* females is inconspicuous. Large and robust atrium bursae is herein synapomorphic to *Agulla* and *Alena*, while females possessing this structure tube-like is clearly an autapomorphy of *Aztekorphidia*.

35. Receptaculum seminis: (0) not adhered to the atrium bursae; (1) adhered or fused with the atrium bursae; (2) absent or strong reduced.

Females with receptaculum seminis not adhered to the atrium bursae are present in the outgroups, and suffered two different changes in *Alena*: (1) receptaculum seminis strongly reduced or absent in *Al. (Al.) distincta* + *Al. (Mx.) americana*; (2) adhered or fused with atrium bursae in *Aztekorphidia*.

36. Glandulae receptaculi: (0) short; (1) elongate; (2) inconspicuous.

Short glandulae receptaculi in females are considered as the plesiomorphic state, present in *I. mayana*, *Ag. (Ag.) bicolor*, and *Al. (Mx.) americana*. Inconspicuous glandulae receptaculi represent an autapomorphy of *Al. (Al.) distincta*, while elongated glandulae receptaculi are autapomorphic to *Aztekorphidia* species.