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New species of *Tetradiplosis* (Diptera: Cecidomyiidae) inducing galls on *Prosopis caldenia* (Fabaceae) in Argentina

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Abstract

Two new species of *Tetradiplosis* inducing galls on *Prosopis caldenia* are described from Argentina: *Tetradiplosis panghitruz* Martínez **n. sp.** and *Tetradiplosis rayen* Martínez **n. sp.** *Tetradiplosis panghitruz* induces multilocular galls on vegetative stems, whereas *T. rayen* induces unilocular galls containing multiple larvae on the rachis of the developing inflorescences. The adult male, female, pupa and larva are described and illustrated for both species. A key to the known species of the genus is provided.

Key words. *Caldén,* Gall midges, Neotropical, Taxonomy

Introduction

The *caldén* (*Prosopis caldenia* Burkart) (Fabaceae) is a deciduous tree endemic to the central semiarid plains of Argentina. Its dominance defines the *caldén* district within the *espinal* biogeographical province, as defined by Cabrera & Willink (1973). Despite its economic, cultural and ecological importance, very little is known about the insect species associated with the numerous galls which develop on this legume species.

According to Gagné (1994), species of four cecidomyiid (Diptera) genera are known to induce galls on species of *Prosopis* L.: *Hemiasphondylia* Möhn, *Rhopalomyia* Rübsaamen, *Liebeliola* Kieffer & Jörgensen and *Tetradiplosis* Kieffer & Jörgensen. Apart from *Hemiasphondylia*, all of them are known to occur in Argentina. Species of *Rhopalomyia* induce bud galls on *P. alba* Griseb., *P. alpataco* Philippi, *P. campestris* Griseb., and *P. flexuosa* De Candolle; *Liebeliola* induces stem galls on *P. strombullifera* (Lam.) Benthan; and representatives of *Tetradiplosis* are known to induce stem galls on *P. alpataco* and *P. campestris* (Gagné 1994). However, there are no records of cecidomyiid species associated with *P. caldenia*.

The genus *Tetradiplosis* Kieffer & Jörgensen 1910 was erected to include one species, *T. sexdentata* Kieffer & Jörgensen, which was reared from stem galls on *P. alpataco* in western Argentina. No other species have been formally described in the genus, but undescribed species have been mentioned from NW Argentina (Gagné 1994).

As a part of an ongoing study of the galls on *P. caldenia*, two undescribed species of Cecidomyiidae were reared from stem galls on this plant species in central Argentina. Both species are considered to be members of the genus *Tetradiplosis* based on the wing venation, male and female antennal morphology, acropod morphology and male and female postabdomen.

Material and methods

Galls containing mature larvae were collected from three sites in suburban areas of Santa Rosa, La Pampa, Argentina: Laguna Don Tomás (36º37'03"S, 64º19'29"W), Facultad de Agronomía (36º38'47"S, 64º18'16"W) and Inti Hué (36º38'28"S, 64º19'39"W). Some galls were dissected to obtain mature larvae, while others were kept in jars covered with commercial tissue paper until adult emergence.

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Larval and adult cecidomyiids were fixed in ~80% ethanol and mounted following Gagné (1989, 1994). Adults were keyed to genus following Gagné (1994).

All material is deposited in the Entomology Division of the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia", Buenos Aires, Argentina (MACN).

Results

Genus *Tetradiplosis* Kieffer & Jörgensen

Tetradiplosis Kieffer & Jörgensen 1910: 421; Gagné 1994: 169, catalog of Neotropical species; Gagné 2010: 390, catalog of World species.
Type species: *Tetradiplosis sexdentata* Kieffer & Jörgensen, by monotypy

The genus was described based on the female, pupa and larva (Kieffer and Jörgensen 1910; syntype series presumed lost, Gagné 1994), and the male was described later by Gagné (1994). It is characterized by the following characters: wing venation with vein R_5 joining C beyond wing apex, Rs weak but evident and beyond midlength of R_1, base of M slightly curved, and M_3 fold present; male flagellomeres with first and third circumfila with long loops, contrasting with the much shorter loops of the second circumfilum; tarsal claws with two teeth, the basal one smaller; female cerci elongate-ovoid, with closely set setae apicoventrally; pupa with pointed antennal horns, sometimes with a conical prothorax which projects beyond them, with or without spines on abdominal terga; larva with a robust spatula bearing 4–6 teeth anteriorly.

Comments: The new species of *Tetradiplosis* broaden the concept of the genus compared with the original description and the definition provided by Gagné (1994). Kieffer & Jörgensen stated that the type species of the genus has a four segmented palpus, whereas Gagné (1994), based on specimens of undescribed species, stated that *Tetradiplosis* has a three segmented palpus. The study of both the original description, the specimens collected and studied by Gagné in northern Argentina, and the new species described herein, allows us to conclude that *Tetradiplosis* has a variable number (3–4) of palpal segments. The larval and pupal morphology also exhibits considerable variation (see discussion below).

Tetradiplosis panghitruz Martínez, new species

(Figs 1–11)

Adult: Body length 3.2–4.1 mm (n= 20)

Head: Eyes large, widely connate (Fig. 1), eye bridge about 8–9 facets long, facets hexagonal, closely adjacent throughout. Occiput with a distinct dorsal protuberance. Frons with 3–5 setae per side. Palpus four segmented. Labella evenly convex in frontal view, each with 6–8 lateral setae. Male third flagellomere (Fig. 2) binodal, with three circumfila, first and third circumfila loops distinctly long, second circumfilum loops much shorter. Female third flagellomere (Fig. 3) cylindrical, 2.7–2.8 times longer than its median width, with two appressed circumfila

Thorax: Wing length, male 2.5–2.6 mm (n= 5); female 3.0–3.3 mm (n= 16). Wing veins M_3, Cu_1 and Cu_2 faint apically (Fig. 4). Anepimeron with 20–24 setae. Acropods with claws bent before middle length and bidentate (Fig. 5). Empodium as long as bend in claw or slightly longer. Pulvilli about half as long as empodium.

Male abdomen: Tergites 1–6 rectangular with an irregular row of setae apically and a few setae laterally; tergites 7 and 8 much shorter, irregular in shape, and mostly devoid of vestiture (similar to tergite 8 in figure 6). All tergites with a pair of anterior trichoid sensillae. Sternites 2–7 rectangular. Genitalia (Fig. 6): Cercus well developed, as long as hypoproct or slightly longer, with the outer apical edges acute. Hypoproct bilobed. Aedeagus slender and slightly longer than hypoproct and cerci. Gonocoxites rugose on outer surface and somewhat slender, wider basally and slightly constricted in the middle, setose on outer surface and on inner surface near gonostylus. Gonostylus elongate and slightly bumped basally, setulae almost reaching half the length of the gonostylus.

Female abdomen: Tergites 1–7 rectangular, with an apical row of setae and a few lateral setae, and with a pair of anterior trichoid sensillae; tergite 8 not sclerotized, devoid of vestiture except for a single pair of trichoid

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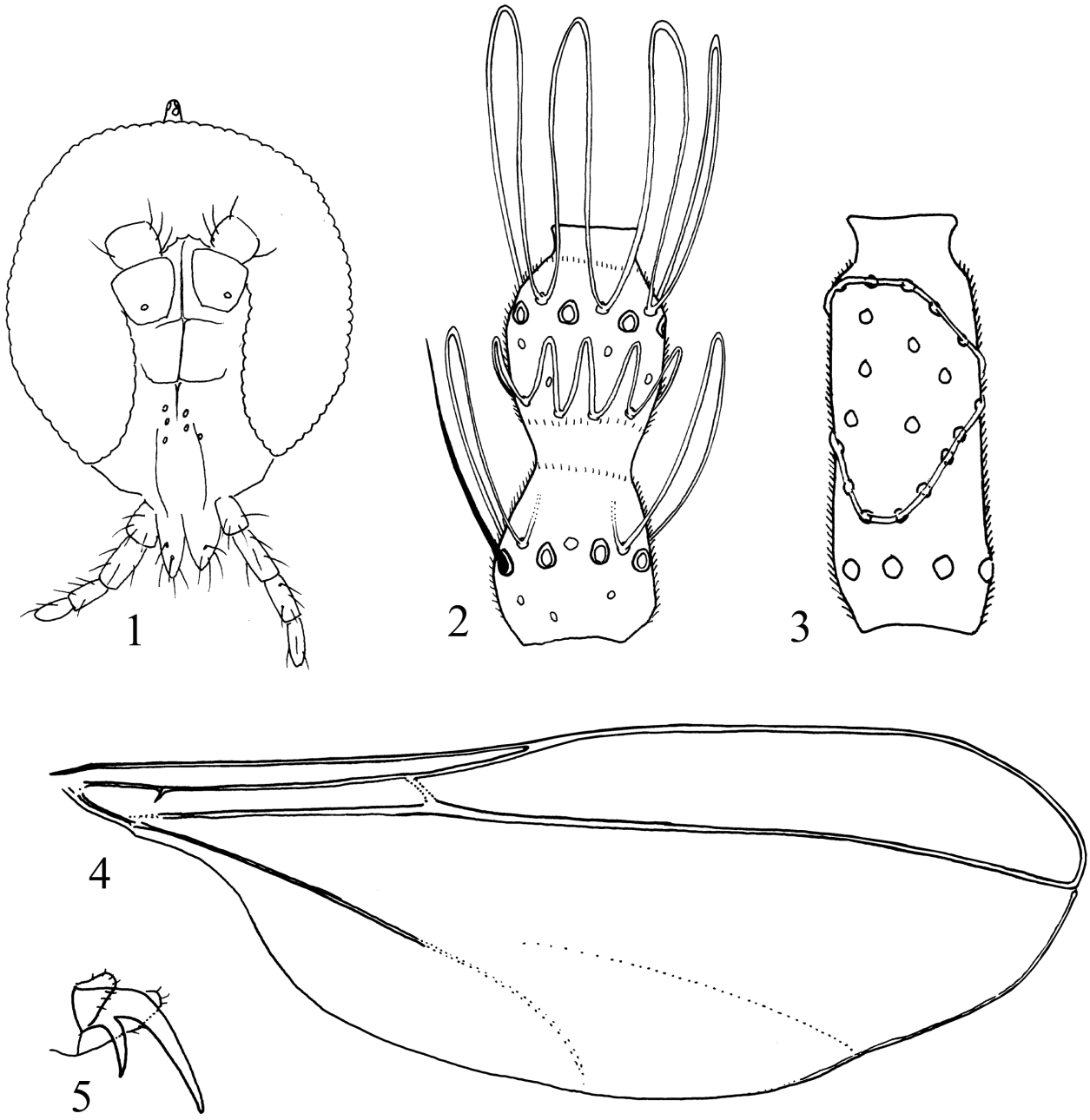
sensillae (Fig. 7). Cerci uniformly ovoid, not tapering apically, with a set of closely set setae apicoventrally (Fig. 8).

Pupa: Each antennal base with a small and acute antennal horn (Fig. 9). Pronotum largely smooth without a median tooth-like projection. Abdominal tergites bare apically.

Third instar larva: Length 4.2–4.9 mm (n= 11). Integument mostly covered with spicules. Antennae about two times longer than basal width. Spatula robust, with two well developed anterior lobes, each bearing a pair of teeth, and an additional median basal tooth between lobes (Fig. 10). In some specimens the median tooth is poorly developed or entirely missing, thus the spatula can be 5-toothed or rarely 4-toothed. Two groups of three small setiform papillae present on each side of the spatula. Dorsal, pleural and terminal papillae bearing a stout, conical seta. Terminal segment with four papillae bearing conical setae on each side in dorsal view (Fig. 11).



FIGURES 1–5. *Tetradiplosis panghitruz*. 1, head frontal; 2, male third antennal flagellomere; 3, female third flagellomere; 4, wing; 5, tarsal claw, empodium, and pulvillus of hind leg.

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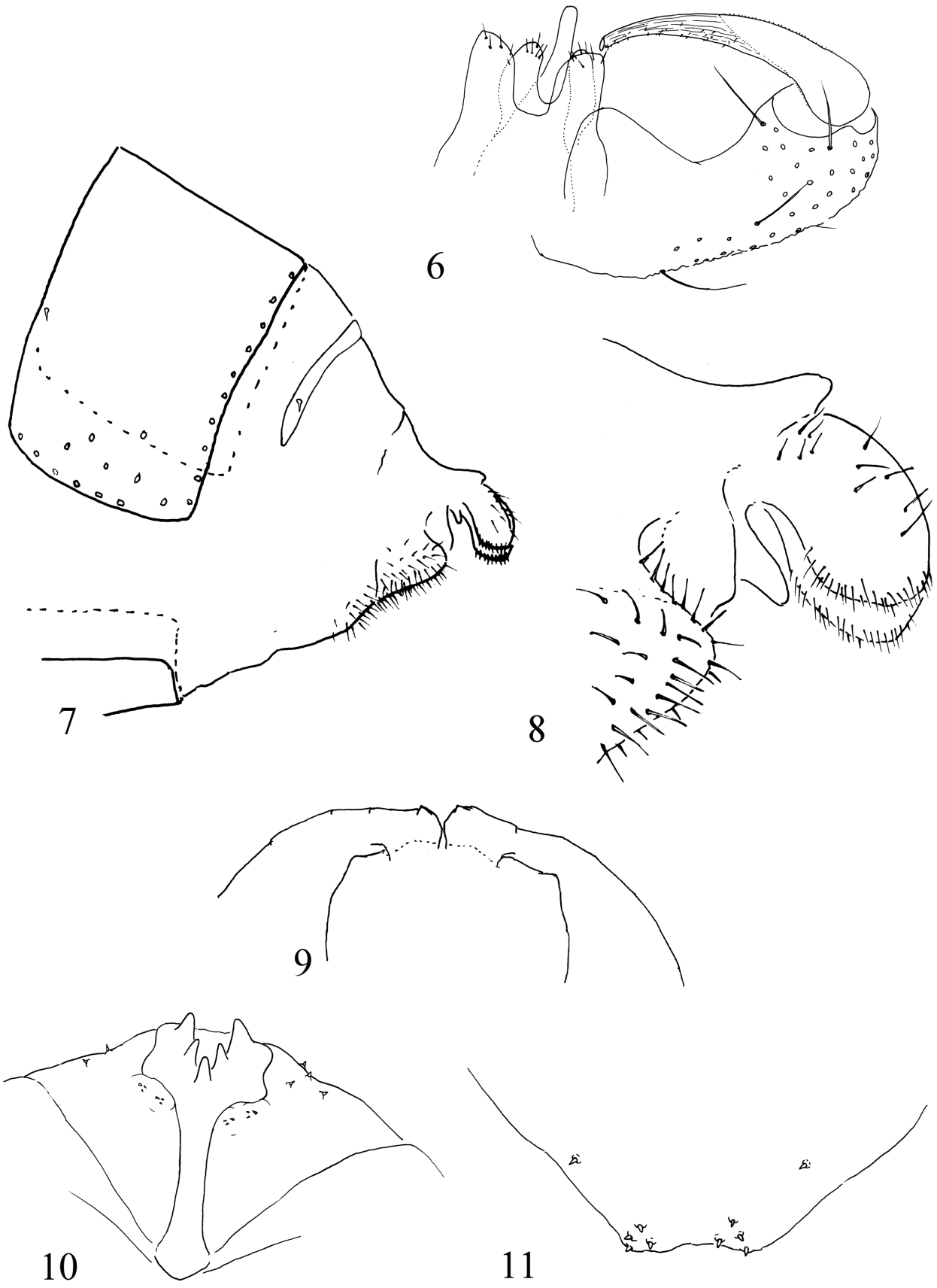

FIGURES 6–11. *Tetradiplosis panghitruz*. 6, male genitalia, dorsal 7, abdomen of female, lateral; 8, female terminalia, lateral; 9, pupal antennal horns; 10, larval spatula and associated papillae, ventral; 11, larval terminal segments, dorsal.

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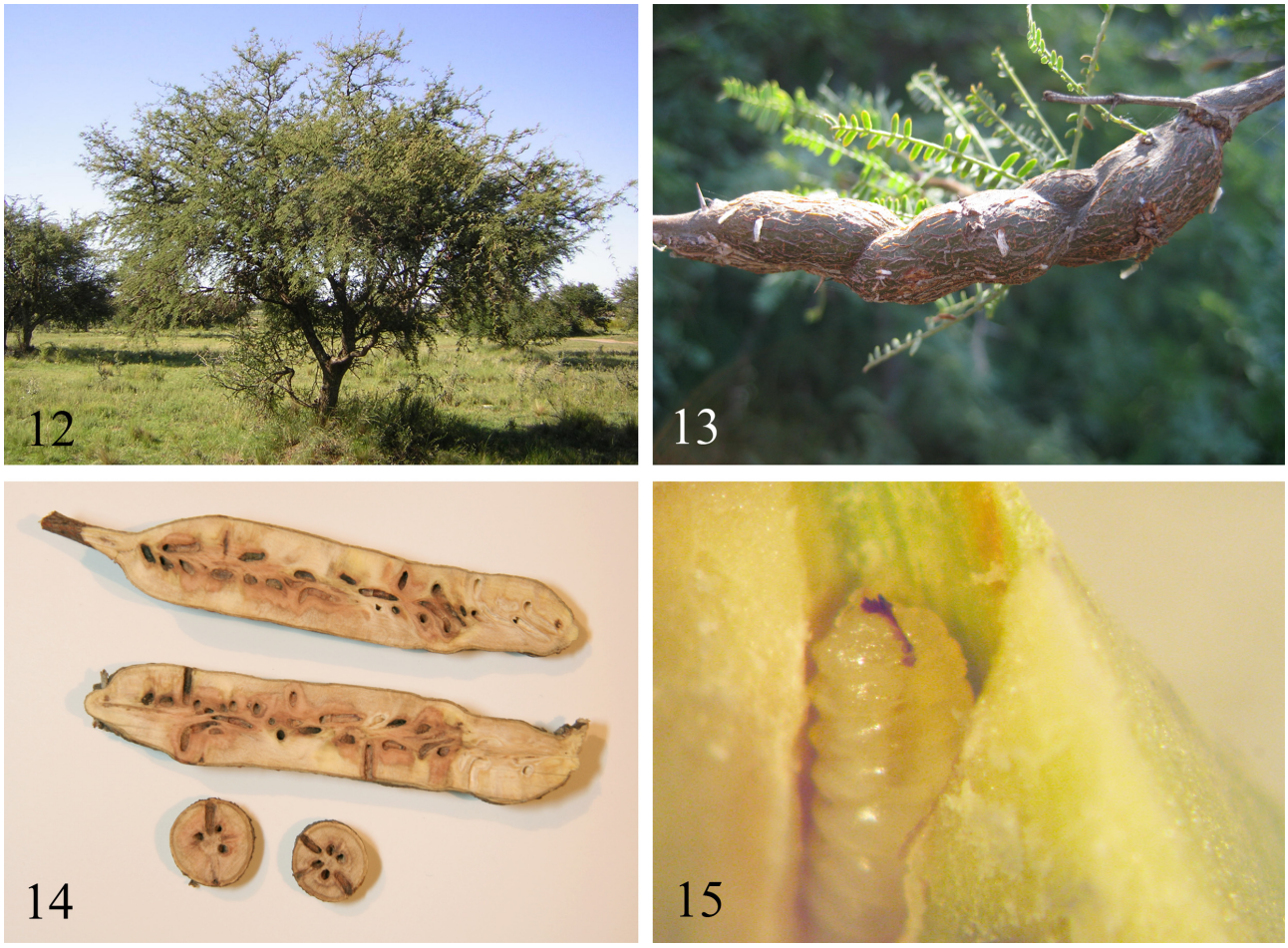
Etymology: The new species is named after Panghitruz Güor (*ca.*1820–1877), a leader of the Ranquel people, inhabitants of the *caldén* forests of central Argentina.



FIGURES 12–15. 12, *Prosopis caldenia*; 13, external morphology of galls induced by *T. panghitruz*; 14 longitudinal and transversal sections of galls induced by *T. panghitruz*; 15, mature larva of *T. panghitruz* inside the gall.

Material examined: HOLOTYPE MALE: Argentina, La Pampa, Santa Rosa, Laguna Don Tomás, 27.xii.2010 de agallas caulinares en *Prosopis caldenia*, J.J. Martínez col. (MACN) PARATYPES, four males, 16 females and three pupal exuviae, same data as holotype (MACN); two larvae, Argentina, La Pampa, Santa Rosa, Inti Hué, 18.i.2009, Martínez col. (MACN); nine larvae, Argentina, La Pampa, Santa Rosa, Facultad de Agronomía, 5.i.2010, J.J. Martínez col. (MACN).

Comments: The pupal morphology was described based on three pupal exuviae, the prothoracic spiracles were not observed due to the condition of the specimens.

Biological observations: Tetradiplosis panghitruz induces multilocular stem galls (larvae in individual chambers) on young stems of *Prosopis caldenia* (Figs 12, 13) similar to those of other *Tetradiplosis* induced galls on other *Prosopis* species (Kieffer & Jörgensen 1910; Jörgensen 1916, 1917; Gagné 1994). The abnormal tissue is induced in the xylem of one year old stems (Fig. 14). In December the first mature larvae can be observed (Fig.15) and in late December pupae are present inside the galls, and the first adults begin to emerge. Most adults emerge during January, although a few mature larvae can be found in the galls in late summer. Apparently, *T. panghitruz* has one generation per year. Our preliminary observations indicate that adults oviposit on developing stems in which immature larvae can be observed at the end of the summer, although at this point galls are barely recognizable as very subtle swellings on young stems. The first instar larva spends autumn and winter inside small longitudinal chambers, remaining largely inactive until the following spring, when galls become fully developed. According to the classification provided by Yukawa and Rohfritsch (2007), the life history strategy of *T. panghitruz* would fit in type II B, with the first instar larva as the overwintering stage. Galls induced by *T. panghitruz* support a complex community of parasitoids and inquilines of at least nine arthropod species including: an unidentified

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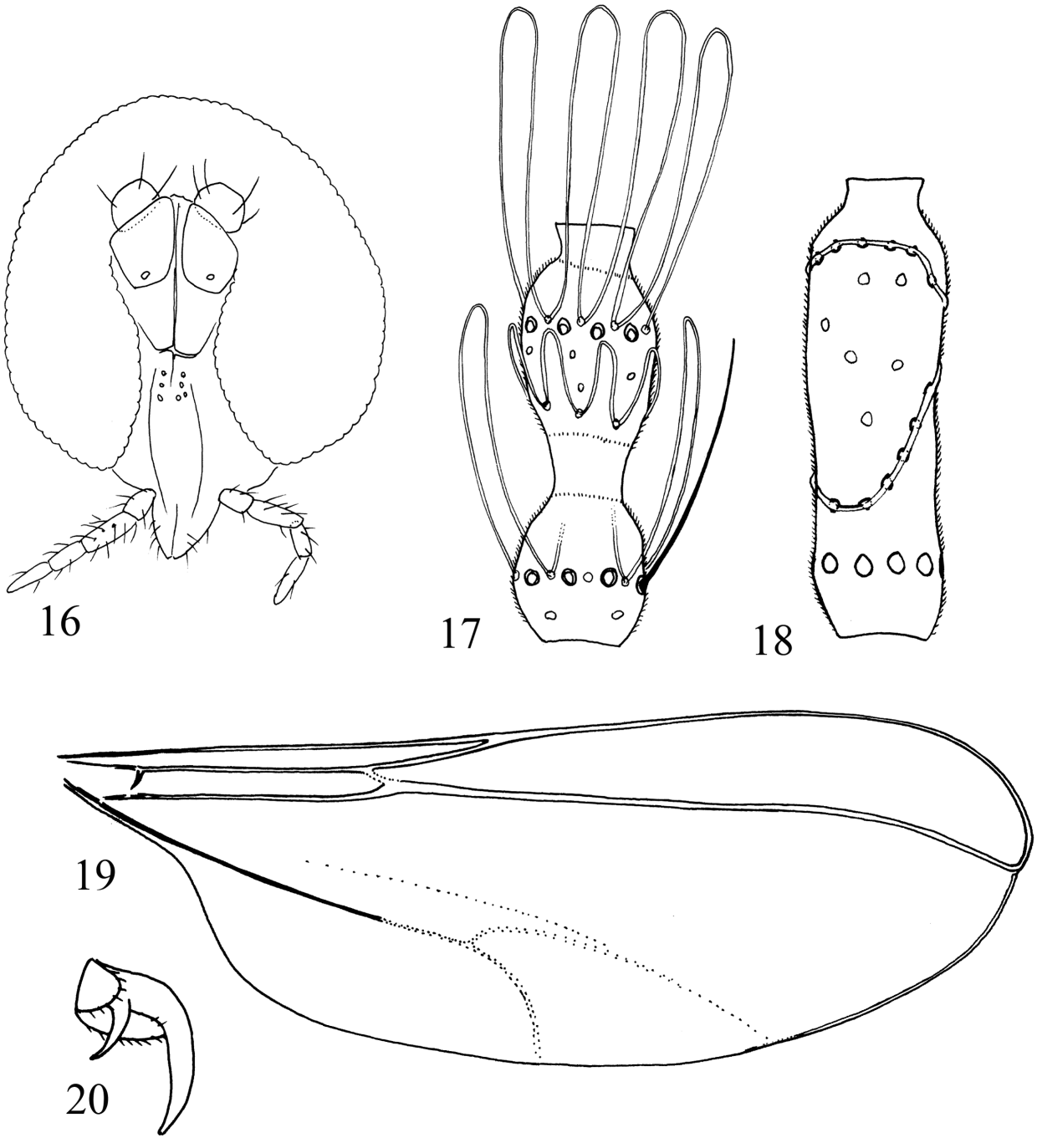
inquiline species of *Contarinia* Rondani (Diptera: Cecidomyiidae), three species of *Allorhogas* Gahan (Hymenoptera: Braconidae), one undescribed species of *Percnobracon* Kieffer & Jörgensen (Hymenoptera: Braconidae), one unidentified species of Torymidae (Hymenoptera), one species of Eupelmidae (Hymenoptera), one species of *Sycophila* Walker (Hymenoptera: Eurytomidae) and one species of *Apion* Herbst (Coleoptera: Brentidae). Additionally, abandoned galls are frequently occupied by *Myrmelachista gallícola* Mayr (Hymenoptera: Formicidae).



FIGURES 16–20. *Tetradiplosis rayen*. 16, head frontal; 17, male third antennal flagellomere; 18, female third flagellomere; 19, wing; 20, tarsal claw, empodium, and pulvillus of hind leg.

***Tetradiplosis rayen* Martínez, new species**
(Figs 16–26)

Adult**:** Body length 4.1–5.0 mm (n= 7)

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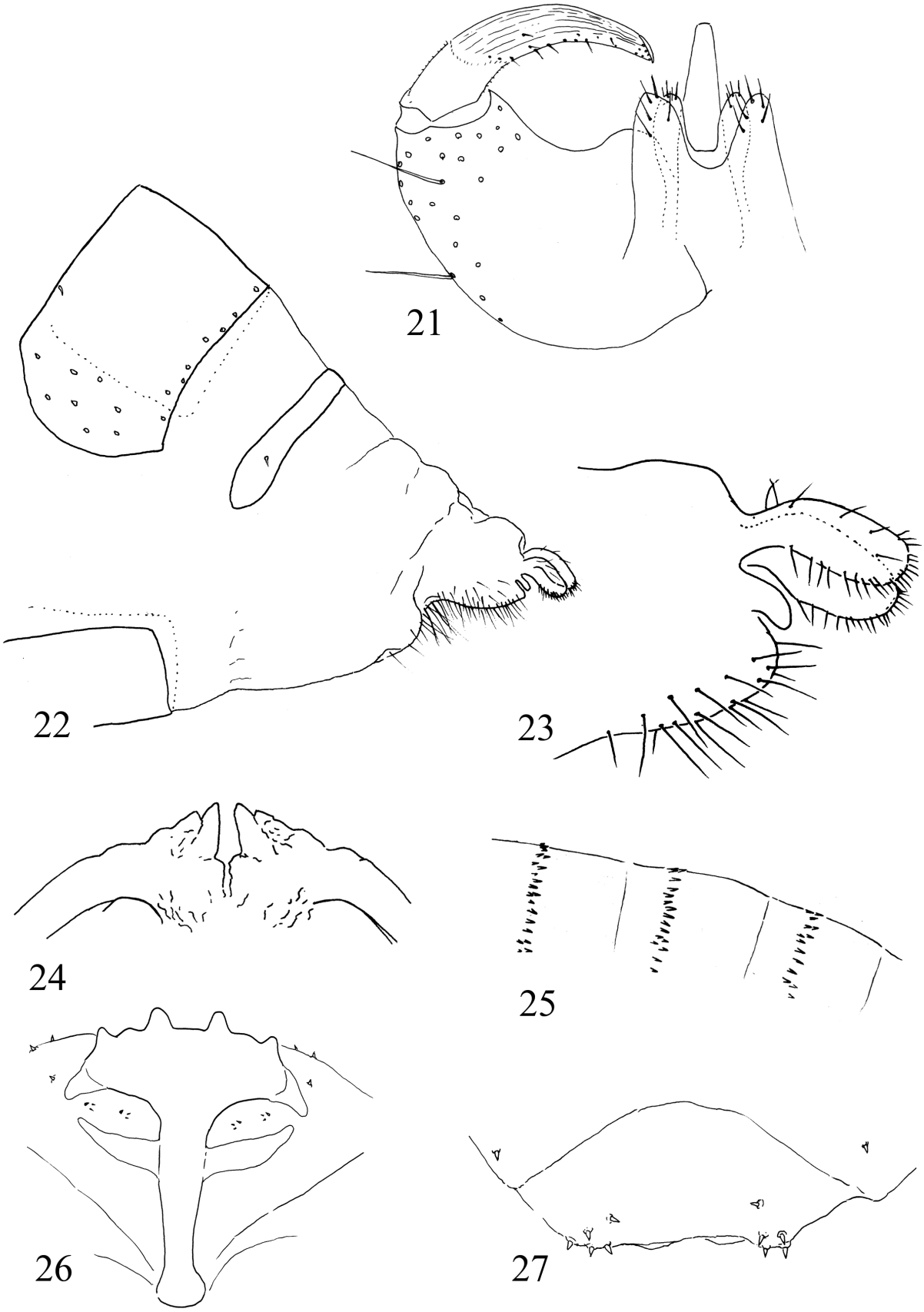

FIGURES 21–27. *Tetradiplosis rayen*. 21, male genitalia, dorsal; 22, abdomen of female, lateral; 23, female terminalia, lateral; 24, pupal antennal horns; 25, pupal abdominal tergites, lateral; 26, larval spatula and associated papillae, ventral; 27, larval terminal segments, dorsal.

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FIGURES 28–30. 28 external morphology of galls induced by *T. rayen*; 29, detail of the subapical exit hole; 30, immature larvae of *T. rayen* inside the gall.

Head: Eyes large, widely connate (Fig 16), eye bridge about 9 facets long, facets hexagonal, closely adjacent throughout. Occiput without dorsal protuberance. Frons with 3–4 setae per side. Palpus four segmented. Labella evenly convex in frontal view, each with 6–8 lateral setae. Male third flagellomere (Fig. 17) binodal, with three circumfila, first and third circumfila loops distinctly long, second circumfilum loops much shorter. Female third flagellomere (Fig. 18) 3.7–3.8 times longer than its median width, with two appressed circumfila.

Thorax: Wing length, male 3.2–3.4 mm (n= 4); female 3.5–3.7 mm (n= 3). Wing veins M_3, Cu_1 and Cu_2 fading apically (Fig 19). Anepimeron with 18–23 setae. Acropods with claws bent before middle length and bidentate (Fig. 20), sometimes basal tooth reduced. Empodium as long as bend in claw or slightly longer. Pulvilli slightly shorter than half the length of empodium.

Male abdomen: Tergites 1–6 rectangular with an irregular row of setae apically, a few setae laterally and a pair of anterior trichoid sensillae. Tergites 7 and 8 much shorter, and irregular in shape (similar to tergite 8 in figure 22). Tergite 7 with a few scattered setae and a pair of anterior trichoid sensilla. Tergite 8 mostly devoid of vestiture except for a pair of trichoid sensillae. Genitalia (Fig. 21): Cercus well developed, as long as hypoproct or slightly longer, with the outer apical edges acute. Hypoproct bilobed. Aedeagus slender and slightly longer than hypoproct and cerci. Gonocoxites wide, not constricted in the middle. Gonostylus elongate, setulose basally.

Female abdomen: Tergites 1–7 rectangular, with an apical row of setae, a few lateral setae, and a pair of anterior trichoid sensillae. Tergite 8 very slightly sclerotized, devoid of vestiture except for a pair of trichoid sensillae (Fig. 22). Cerci uniformly ovoid, not tapering apically, with a set of closely set setae apicoventrally (Fig. 23).

Pupa: Antennal base coarsely rugose. Antennal horns bidentate (Fig. 24). Pronotum coarsely rugose and without a median conical projection. Abdominal tergites with a single transverse row of spines (Fig. 25).

Third instar larva: Length 4.2–4.8 mm (n= 6). Integument entirely covered with spicules. Antennae about two times longer than basal width. Spatula robust, with an anterior transversal lobe bearing four major teeth (Fig. 25), sometimes with much smaller teeth between them. Spatula bears two lateral basal projections (Fig. 26), apically almost meeting the outer edge of the transversal lobe. Two groups of three small setiform papillae present on each side of the spatula, between the lateral projections and the transversal lobe. Dorsal, pleural and terminal papillae bearing stout, conical setae. Terminal segment in dorsal view with five papillae on each side bearing conical setae (Fig 27).

Etymology: The specific epithet derives from the Ranquel language, meaning “flower”, in reference to the location of the galls induced by this species.

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Material examined: HOLOTYPE MALE: Argentina, La Pampa, Santa Rosa, Laguna Don Tomás, 2.vii.2009, de agallas en inflorescencias de *Prosopis caldenia*, J.J. Martínez col. (MACN) PARATYPES, three males, three females, six larvae and one pupal exuvia, same data as holotype (MACN)

Comments: Pupal description is based on a single pupal exuvia, it was not possible to observe the prothoracic spiracles due to the condition of the specimen.

Biological observations: Tetradiplosis rayen induces stem galls on the rachis of the developing inflorescences of *P. caldenia* causing complete flower and fruit abortion. The resulting gall is a lignified pedicelate fruit like structure (Fig. 28) with a single central longitudinal chamber containing several cecidomyiid larvae (Fig. 30). Adults emerge in spring from the previous year's galls and probably oviposit on or near developing inflorescences. Immature larvae can be found inside the galls during spring and early summer. Mature larvae remain in the galls from late summer until the following spring, when adults emerge through a single subapical emergence hole (Fig. 29). The life history strategy of *T. rayen*, cannot be clearly included in any of the types described by Yukawa and Rohfritsch (2007). The overwintering stage inside the gall is the third instar larva is similar to their definition of the type II A, but in Yukawa and Rohfritsch's classification the development of the first instar larva seems to take more time compared with our observations on the development of *T. rayen*. The galls induced by *T. rayen* support a much simpler community than that of galls induced by *T. panghitruz*. An unidentified species of Torymidae is the only common parasitoid in this gall; additionally one braconid larva was collected while dissecting the galls, probably belonging to the genus *Allorhogas*. After the emergence of the adult cecidomyiids, some galls are occupied by *Myrmelachista gallicola* (Hymenoptera: Formicidae) (Quirán & Martínez 2008).

Key to known species of *Tetradiplosis*.

1. Occiput without a distinguishable protuberance (Fig. 16); pupa with a row of spines on abdominal tergites (Fig. 25), antennal horns bidentate (Fig. 24); larval spatula with a massive transverse anterior area bearing four teeth, and with two lateral projections (Fig. 26); gall inducing species on the rachis of *Prosopis* inflorescences (Fig. 28), larvae gregarious in a single chambered gall (Fig. 30) . . . . . *T. rayen* Martínez
- Occiput with a distinct protuberance (Fig. 1); pupa without an apical row of spines on abdominal tergites, antennal horns simple (Fig. 9); larval spatula bearing two lobes anteriorly, each one with 2–3 teeth on its inner margin (Fig. 10); gall inducing species on vegetative stems of *Prosopis* (Fig. 13); larvae in individual chambers in a multilocular gall (Fig. 14). Adults not currently distinguishable beyond this point 2
2. Spatula of the mature larva with six teeth, three on each lateral lobe; pupa with a conical projection on mesoscutum; species inducing stem galls on *Prosopis alpataco* . . . . . *T. sexdentata* Kieffer & Jörgensen
- Spatula of mature larva with five teeth (Fig. 10), rarely four, two on each lateral lobe and in most cases with a median tooth between them; pupa with mesoscutum entirely smooth; species inducing stem galls on *Prosopis caldenia* . . . . . *T. panghitruz* Martínez

Discussion

The finding of two new species broadens the current concept of the genus *Tetradiplosis*. *Tetradiplosis rayen* exhibits the most relevant morphological differences compared with the diagnosis provided by Gagné (1994). Larval and pupal morphology show considerable variation compared with the type species of the genus (i.e. morphology of the spatula, presence/absence of spines on pupal abdominal tergites, presence/absence of conical projection on pupal mesoscutum), but these differences are found among congeneric species in other cecidomyiid genera (Gagné, personal communication). Adult morphology is more conservative and congruent with the diagnosis of *Tetradiplosis*, with the only exceptions being the variable number of palpomeres and the absence of the occipital protuberance in *T. rayen*. Despite these features, the specimens studied in this work should clearly be included in *Tetradiplosis* following Gagné's (1994) diagnosis. The undescribed specimens collected by Gagné in NW Argentina (studied but not described here, as they were represented by an incomplete series of larvae or adults), and the finding of similar galls by the senior author in NE Argentina on *P. alba,* indicate that the genus probably includes several undescribed species.

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