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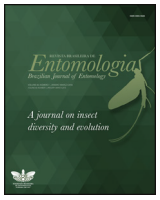

New species of *Hexacladia* Ashmead (Hymenoptera: Encyrtidae), a parasitoid of *Tibraca limbativentris* Stål (Hemiptera: Pentatomidae)

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ABSTRACT

The rice stink bug, *Tibraca limbativentris* Stål (Hemiptera: Pentatomidae), is a significant pest of rice cultivation in Brazil and attacks plants during both vegetative and reproductive phases. Natural enemy surveys conducted in plantations located in Arari, Miranda do Norte, Matões do Norte, Santa Rita, and Viana (state of Maranhão, Brazil) led to the discovery of a new species of parasitoid, which is described in this study. *Hexacladia lemosae* Costa, Noyes & Machado **sp. nov.** (Hymenoptera: Encyrtidae) is the first species of its genus associated with *T. limbativentris* and develops as a gregarious endoparasitoid in adult stink bugs, occasionally in nymphs. The parasitoid emerges from the host while host is still alive.

Introduction

Tibraca limbativentris Stål (Hemiptera: Pentatomidae), also known as the rice stink bug, is a significant pest of rice cultivation in Brazil. This insect attacks rice plants during both their vegetative and reproductive phases, causing "dead heart" and "white panicle" (Martins et al., 2009). Severe infestations can lead to crop losses of up to 90% (Souza et al., 2008). Current control measures rely heavily on the intensive use of chemical insecticides, making alternative control methods such as biological control increasingly necessary (Rampelotti et al., 2008).

Parasitoids of the genus *Hexacladia* Ashmead (Hymenoptera: Encyrtidae) are gregarious endoparasitoids that emerge from their hosts when the latter are still alive (Burks, 1972; Rasplus et al., 1990; Noyes, 2010). These endoparasitoids are known to attack nymphs or adults of phytophagous insects from several Hemiptera families, including Coreidae, Pentatomidae, Pyrrhocoridae and Scutelleridae (Noyes, 2010); Perkins (1907) also reared a *Hexacladia* species from a Cicadellidae host, *Cuerna costalis* (Fabricius), in Arizona, U.S.A. In Brazil, the genus *Hexacladia* has been reported to contain four species: *H. smithii* Ashmead (Ashmead, 1891; Costa Lima, 1930), *H. blanchardi* De Santis (De Santis and Fidalgo, 1994), *H. impiros* Noyes (Silva et al., 2017), and *H. hilaris* Burks (Marsaro Júnior et al., 2020).

None of the *Hexacladia* species occurring in Brazil have this far been associated with *T. limbativentris*. Some studies have reported egg parasitoids as natural enemies of *T. limbativentris* in Rio Grande do Sul (Idalgo et al., 2013), Santa Catarina (Riffel et al., 2010), and Maranhão (Maciel et al., 2007), while other authors have reported parasitism in adult *T. limbativentris* by *Cylindromyia brasiliana* (Townsend) and *Phasia* (*Paraphorantha*) sp. (Diptera: Tachinidae) (De Farias et al., 2012).

Therefore, this study aims to describe a new species of *Hexacladia* found in Brazil parasitizing adults of *T. limbativentris*.

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Material and methods

Adults of *T. limbativentris* were collected from rice fields belonging to family farmers in the municipalities of Arari (03° 27' 38" S and 44° 46' 56" W), Miranda do Norte (03° 34' 09" S and 44° 34' 45" W), Matões do Norte (03°37'51" S and 44°33'11" W), Santa Rita (03° 08' 37" S and 44° 19' 32" W), and Viana (03°13'14" S and 45°00'13" W) in the state of Maranhão, Brasil. The sampling was performed at morning (between 7 and 10 am), from March to October in the crop years of 2012 and 2017. Zig-zag walking was used to collect the rice stinkbugs, and each rice plantation was approximately one hectare in size and bordered by secondary vegetation. Rice was intercropped with cassava (*Manihot esculenta*) and corn (*Zea mays* L.), and babassu palm trees (*Attalea speciosa* Mart.) among food crops.

The adult rice stinkbugs collected were placed inside plastic containers with pressure lids and 500 mL capacity. These containers were then sent to the Entomology Laboratory of the Universidade Estadual do Maranhão - Campus Paulo VI, São Luís - MA. In the laboratory, specimens were sexed and kept in groups of 30 (15 females and 15 males) in plastic containers with pressure lids and 750 mL capacity. Three rice stalks, each 10 cm long, were placed inside the containers for adult rice stinkbugs to feed on. Each stem was wrapped with a cotton swab moistened with distilled water and changed every two days. The containers were kept in BOD type climatized chambers, with a temperature of 25 ± 2 °C, RH 70-80%, photophase of 12 hours, and inspected daily until the emergence of *T. limbativentris* adults.

A total of 342 females and 73 males of *H. lemosae* **sp. nov.** were reared and collected. The specimens were initially preserved in 70% ethanol and then dried in a critical point drier (Gordh and Hall, 1979) and double-mounted on cards or points (Noyes, 1982). Some of them were stored in absolute ethanol for molecular analysis, while others were slide mounted, which were made according to Noyes (1982). These specimens were examined and photographed in a Leica M165C stereomicroscope equipped with a Leica DFC 420 digital camera and a dome for diffuse light (Kerr et al., 2008). Stacked pictures were combined with Leica Application Suite v3.8 to obtain the final images with extended focus. SEM-micrographs were taken of uncoated paratype specimens in low vacuum with a Quanta 250 scanning electron microscope.

Morphological terms were based on Noyes (2010), and the following abbreviations were used: AOL = minimum distance between posterior ocellus and anterior ocellus; EL = length of eye; EW = width of eye; Fn = funicle segment *n*; FV = minimum width of frontovertex; FWL = length of fore wing (excluding marginal fringe); FWW = maximum width of fore wing (excluding marginal fringe); HH = length of head in frontal view (excluding mouth parts); HW = head width (measured in frontal view); HWL = length of hind wing (excluding marginal fringe); HWW = maximum width of hind wing (excluding marginal fringe); MS = malar space; MT = length of mid tibia; OCL = minimum distance between posterior ocellus and occipital margin; ODP = largest diameter of posterior ocellus; OEL = minimum distance between eye and occipital margin; OL = length of ovipositor; OOL = minimum distance between eye margin and adjacent posterior ocellus; POL = minimum distance between posterior ocelli; SL = length of scape; SW = maximum width of scape; TAO = minimum distance between torulus and anterior ocellus; TMM = minimum distance between torulus and mouth margin. Since Torréns et al. (2017) observed the ratio of length of fore wing in relation to length of mesosoma was useful for species separation, we used this ratio too, FWL/MM, where MM is the length of mesosoma, measured along the median line of mesosoma, from anterior margin of pronotum to posterior margin of the propodeum (Hansson 2009). Species identification followed Noyes (2010) and Torréns et al. (2017).

To complement the new species description, the barcode of the new species was generated based on its mitochondrial gene Cytochrome Oxidase I (COI). About 460 bp fragment of the mitochondrial cytochrome oxidase I (COI) gene was amplified using primers pair COI-F (5'-GATTTTTTGGKCAYCCMGAAG-3') and COI-R (5'- CRAATACRGCTCCTATWGATAAWAC-3') (Gusmão et al., 2010). DNA was extracted from one specimen using the GenElute Mammalian Genomic DNA Miniprep Kit (Sigma-Aldrich™), following the manufacturer's protocol. The product was amplified via Polymerase Chain Reaction (PCR) using the following schedule: 94°C for 2 minutes, 40 cycles of 94°C for 30 seconds, 54°C for 30 seconds, 72°C for 40 seconds, and 72°C for 4 minutes (Gusmão et al., 2010). The PCR product was purified using polyethylene glycol precipitation (Schmitz and Riesner, 2006) and sequenced using the Big Dye 3.1 reagent (Life Technologies™) and 3500 XL automatic sequencer (Life Technologies™). The sequences were aligned using ClustalW software.

Collection acronyms used in the study comprise:

NHMUK The Natural History Museum, London, UK;

MZUSP Museu de Zoologia da Universidade de São Paulo, São Paulo, Brasil.

Results

***Hexacladia lemosae* Costa, Noyes & Machado sp. nov.**

ZooBank LSID: urn:lsid:zoobank.org:act:20169A5D-0AEA-42C0-B417-0FBE1CBE2B5E

GenBank sequence OP586619

(Figs. 1A-1F/2A-2E)

Diagnosis. Female: length about 2.36–2.80 mm. Head and mesosoma varying from largely yellowish orange to brown (Figs. 1A, 1B), with mesoscutum and scutellum slightly paler. Fore wing (Fig. 1C) with an incomplete, slender fascia from parastigma and a broader, median fascia which hardly reaches posterior wing margin and is connected to a subapical fuscous area, the margins of which are more or less equidistant from wing margin. Hind tarsus honey-yellow. Gaster varying from yellowish orange, with brownish areas dorsally, to entirely brown; gonostylus same colour as the surrounding area of gaster, or paler in some darker specimens. Funicle with dorsal setae on F1 and F2 not flattened, gradually tapering and virtually straight (Fig. 1D); linear sensilla present on F2–F6; F6 not wider than clava. Mesoscutum with scattered, uniform setae; sculpture on mesoscutum clearly shallower than on scutellum. Fore wing (Figs. 1A, 1B) reaches near cerci level on specimens whose gaster is not dehydrated; marginal fringe present and complete; costal cell both dorsally and ventrally with 19–23 setae; cubital vein track with 1–8 setae, subcubital vein track with 1–5 setae; stigmal vein of fore wing 0.6× as long as marginal vein; postmarginal vein 1.2× stigmal vein; setae below parastigma relatively stout and bristle-like; posterior margin strongly sinuous opposite submarginal vein. Hypopygium (Fig. 1E) apically with lateral projections long and distinct so that median invagination is narrower than length of each projection; median incision in hypopygium relatively broad, ∩-shaped and not gradually tapering, but with an abrupt, angular protuberance about halfway along inner margin of each lateral process. Ovipositor (Fig. 1F) about as long as mid tibia; outer plate with a line of 4 setae along inner margin near apex; gonostylus not exserted.

Female (holotype): length 2.61mm (CPD-Critical Point Drier).

Head brown, paler on lower face. Radicle yellowish brown, scape yellowish brown, dorsal margin dark brown; pedicel brown, apex and venter slightly paler; funicle dark brown, clava dark brown. Mesosoma varying from largely yellowish orange to brown with mesoscutum and scutellum slightly paler, and mesopleuron slightly darker (Figs. 1A, 1B). Fore wing (Fig. 1C) with an incomplete, slender fascia from parastigma and a broader, median fascia which hardly reaches

posterior wing margin and is connected to a subapical fuscous area, the margins of which are more or less equidistant from wing margin. Hind tarsus honey-yellow. Gaster varying from yellowish orange, with brownish areas dorsally, to entirely brown (Figs. 1A, 1B); gonostylus same color as the surrounding area of gaster, or paler in some darker specimens.

Head with fine, shallow, imbricate-reticulate sculpture, very shallow and smooth, polygonally reticulate in ocellar area. Anterior ocellus more or less connected to antennal toruli by a very shallow groove that does not reach toruli; ocellar angle about 105°. Eye separated from occipital margin by about 0.4× its own length, clothed with sparse, inconspicuous, short, pale setae, each about as long as a facet. Frontovertex and face with numerous conspicuous, semi-erect setae, each about as long as diameter of anterior ocellus. Antenna with dorsal setae on F1 and F2 not flattened (Fig. 1D), gradually tapering and virtually straight; funicle with linear sensilla on F2–F6. Relative measurements: HW 97, HH 73, FV 56, ODP 5, POL 19, OOL 31, OCL 2, AOL 11, OEL 18, EL 41, EW 30, MS 19, TMM 32, TAO 20; SL 62, SW 9.

Mesosoma with fine, imbricate-reticulate sculpture on mesoscutum generally of similar mesh size to that on frontovertex, anterior part of mesoscutum with smaller mesh than eye facet, laterally and posteriorly mesh slightly larger than eye facet. Anterior part of axilla with coarse, imbricate-reticulate sculpture that is deeper than that on mesoscutum, posterior part smooth and shiny. Scutellum with coarse, imbricate-reticulate sculpture that is clearly deeper than that on mesoscutum; scutellum evenly and moderately convex in profile, most strongly convex in apical one-third or so. Setae on disc of mesoscutum and scutellum erect and about as long as those on frontovertex; apical setae on scutellum about 1.5× as long as those on mesoscutum. Mid basitarsus with a line of about 10 ventral pegs. Fore wing venation and setation as on Fig. 1C; costal cell dorsally with about 7 setae medially and 1 or 2 near at apex and ventrally with about 13 or 14 setae in distal half, otherwise naked; stigmal vein about 0.5–0.6× as long as marginal vein; postmarginal vein 1.3× as long as than stigmal vein; basal cell naked apart from the group of bristles below the parastigma, with a group of 4 setae below this and 4 setae tracking the cubital vein, and 5 setae ventrally tracking the subcubital vein; linea calva broad and usually open posteriorly, occasionally interrupted in the middle; basal cell naked but for a group of dense bristle-like setae below parastigma and about 1–5 finer setae ventrally tracking subcubital vein; marginal and postmarginal veins combined with 25 bristle-like setae dorsally; marginal fringe present and complete; posterior margin strongly sinuous opposite submarginal vein; fore wing 1.1× as long as length of mesosoma. Propodeum with about 9–12 setae scattered outside spiracle, each about as long as diameter of spiracle. Relative measurements: FWL 119, FWW 43, HWL 105, HWW 31, MM 108.

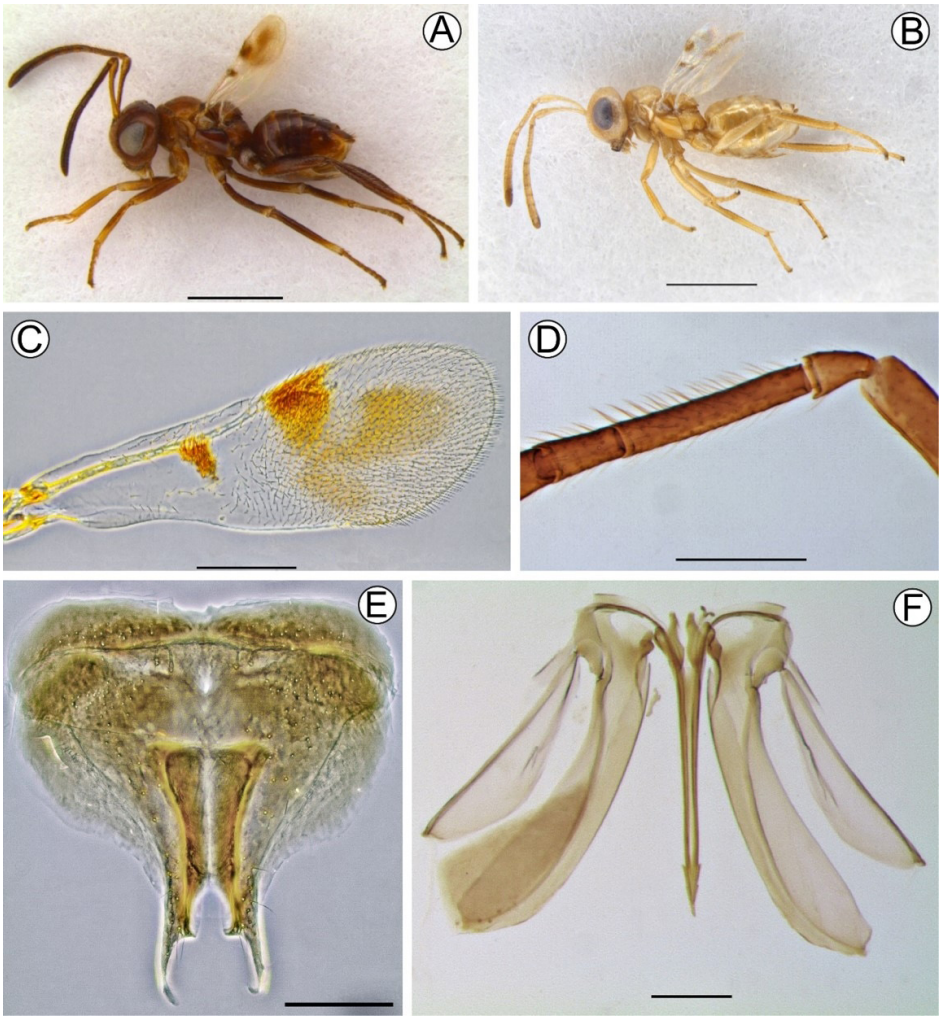

Figure 1 *Hexacladia lemosae* Costa, Noyes & Machado **sp. nov.** Female. A, habitus, lateral, holotype, brown form; B, habitus, lateral, yellowish form; C, fore wing; D, first funicle segment; E, hypopygium; F, ovipositor. Scale bar: Figs. 1A, 1B: 1 mm; Figs. 1C, 1D, 1E, 1F: 0.2 mm.

Hypopygium (Fig. 1E) reaching apex of gaster, apex with a deep, median invagination and a pair of slender, lateral curved posterior processes, each about as long as mid tibial spur; median incision in hypopygium relatively broad, ∩-shaped and not gradually tapering, but with an abrupt, angular protuberance about halfway along inner margin of each lateral process. Ovipositor (Fig. 1F) about as long as mid tibia; outer plate with a line of four setae along inner margin near apex; gonostylus not exserted. Relative measurements: OL 86 [MT 77].

Variation. Female with head, mesosoma, gaster, antennae and legs varying from largely yellowish orange to brown (Figs. 1A, 1B), with mesoscutum and scutellum slightly paler and mesopleura slightly darker; in air-dried yellowish specimens the gaster becomes dark brown. Fore wing with costal cell both dorsally and ventrally with 17–21 setae in the middle of costal cell and 0–2 at apex, with 1–5 setae tracking the cubital vein and 0–5 setae tracking the subcubital vein.

Male: length 2.03–2.53 (n = 10). Similar to female (Fig. 2A) except for following: fore wing 1.5–1.7× as long as length of mesosoma; costal cell with 28–36 setae, setae adjacent to the group of bristles below the parastigma are much more numerous and dense (Fig. 2B), 0–1 seta tracking the subcubital vein ventrally, stigmal vein with three or four placoid sensilla. Antenna with funicle segments longer than broad and all with an elongate dorsal branch; F1 the shortest funicle segment, F5 the longest; clava longer than any of the funicle segments; branch of F1 4.0–4.4× as long as F1 and longer than F1 and F2 together; branch of F6 0.5–0.8× length of F6. Ratio length:width of the antennal segments: scape 46:8, pedicel 7:7, F1 10:5, F2 15:4, F3 16:4, F4 21:4, F5 23:4, F6 22:4, Clava 37:7. Relative measurements: FWL 146, FWW 59, HWL 110, HWW 36, MM 95. Genitalia as in Fig. 2C.

Hosts. Recorded as a gregarious endoparasitoid of adult *Tibraca limbativentris* Stål (Hemiptera: Pentatomidae) (Fig. 2E), occasionally of nymphs.

Distribution. Brazil, state of Maranhão.

Material Examined

Type material. HOLOTYPE ♀: BRASIL, MA, Miranda do Norte, 03°34'09"S, 44°34'45"W, Ex adulto *Tibraca limbativentris* em *Oryza sativa*, 02.vi.2012 (K.K.G. Machado) (MZUSP). Paratypes: 10 ♀: same data as holotype (MZUSP); 1 ♀, same data as holotype but collected on 03.v.2014; 3 ♂, Matões do Norte, 03°37'51"S, 44°33'11"W, Ex *Tibraca limbativentris* em *Oryza sativa*, 10.viii.2012 (K.K.G. Machado) (MZUSP); 2 ♀, same data but collected on 10.viii.2013 (MZUSP); 2 ♀, same data but collected on 30.viii.2014 (MZUSP); 1 ♀, 2 ♂, same data but collected on 31.v.2015 (MZUSP); 8 ♀, 4 ♂, Santa Rita, [03º08'37"S, 44º19'32"W], Ex *Tibraca limbativentris* em *Oryza sativa*, 13.v.2012 (K.K.G. Machado) (NHMUK); 1 ♀, Viana, [03°13'14"S, 45°00'13"W], Ex *Tibraca limbativentris* em *Oryza sativa*, 11.iv.2017 (K.K.G. Machado) (MZUSP).

Etymology. The species was named in honor of Professor Raimunda Nonata Santos de Lemos, former advisor of the first author, for her long career dedicated to alternative pest control in agriculture.

Discussion

Based on the shape of the projections on the apex of the hypopygium, the basic infuscation of the fore wing and the non-flattened setae on the dorsal part of F1, females of *Hexacladia lemosae* **sp. nov.** are similar to those of *H. felas* Noyes and *H. hilaris* Burks. However, *H. lemosae* differs from both species by having linear sensilla on F2–F6, fore wing not reaching apex of gaster and having a strongly sinuous posterior margin in the proximal half, and stigmal vein about 0.5–0.6× as long as marginal vein. In *H. felas* and *H. hilaris*, linear sensilla absent on F2, fore wing reaching apex of gaster and with posterior margin only slightly sinuous, and stigmal vein as long as marginal vein.

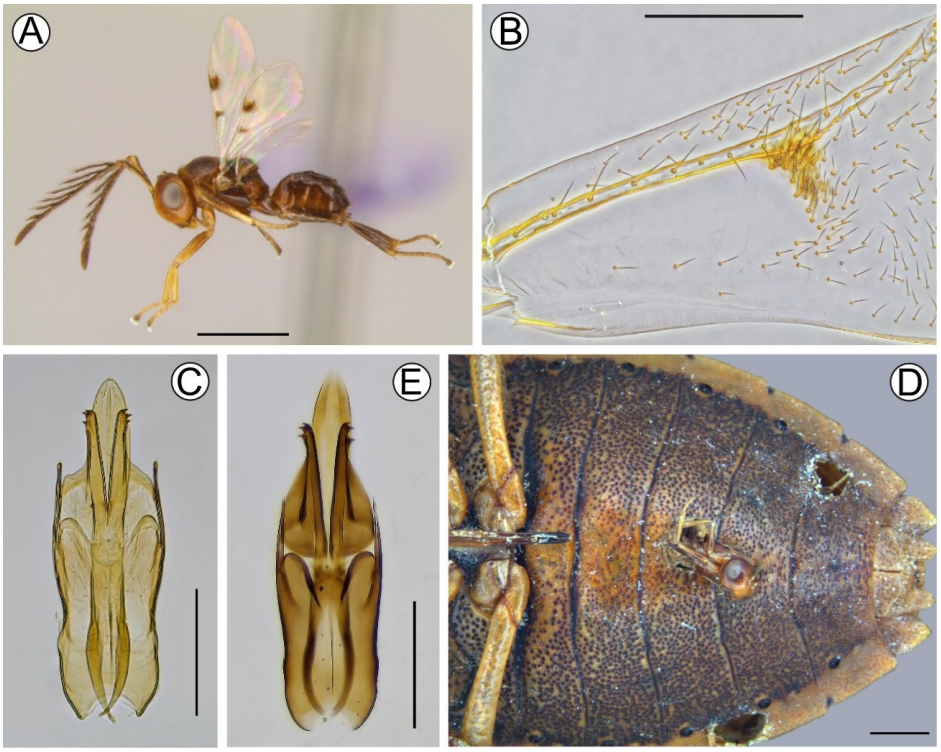

Figure 2 *Hexacladia* species. *Hexacladia lemosae* Costa, Noyes & Machado **sp. nov.** Male. A, habitus, lateral; B, basal area of fore wing; C, genitalia; D, Adult parasitoid emerging from host. *Hexacladia hilaris*. Male. E, genitalia. Scale bar: Figs. 2A, 2D: 1 mm; Figs. 2B, 2C, 2E: 0.2 mm.

The difference in the venation between *H. lemosae* and the two species may be as a result of wing shortening in *lemosae*. In the males, the genitalia of *H. lemosae* are similar to those of *H. hilaris*, including the proportion aedeagus to mid tibia (0.7×). However, the aedeagus is less pointed in *H. lemosae* than in *H. hilaris* (compare Figs. 2C and 2E).

The hypopygium of *H. lemosae* resembles that of *H. townsendi* (Crawford) (Noyes, 2010: fig. 96), although the lateral projections are slightly slender in *H. lemosae*. These two species can be distinguished from each other by the setae on dorsal part of F1 and F2, the linear sensilla on F2–F6 and the ratio of stigmal and marginal veins. In *H. lemosae*, funicle with dorsal setae on F1 and F2 not flattened, gradually tapering and virtually straight, linear sensilla present on F2–F6, stigmal vein about 0.5–0.6× as long as marginal vein. In *H. townsendi*, antenna with dorsal setae on F1 and F2 flattened in apical half and abruptly bent subapically, linear sensilla present only on F3–F6 and stigmal vein about as long as marginal vein (Noyes, 2010).

Hexacladia lemosae females have fore wings with marginal fringe present and complete, so they could run to *H. dichelopsis* Torrens & Fidalgo in Torréns et al. (2017) key to known South American species. However, in *H. lemosae* the head, body and appendages are largely yellowish orange to brown, in female the fore wing is only 1.1× as long as the mesosoma and hypopygium with apex having a pair of long, curved lateral processes, each about as long as mid tibial spur. In *H. dichelopsis* females, the general color of the body is dark brown to black, fore wing is 1.3× as long as the mesosoma, and the pair of lateral processes at apex of hypopygium is short. In the males, F1 1.2–1.3× as long as pedicel and branch of F1 4.0–4.4× as long as length of F1 in *H. lemosae*, while in *H. dichelopsis* F1 1.9× as long as pedicel and branch of F1 2.1× as long as F1 length.

Hexacladia blanchardi De Santis is a South American species known only by the male. *Hexacladia lemosae* males differ from *H. blanchardi* males by the proportions of the length of F6 to its branch and fore wing to mesosoma and by the setation of the basal third of the fore wing. In *H. lemosae* males, the branch of F6 is 0.5–0.8× as long as F6, and the basal third of the fore wing is glabrous, with only five or less setae tracking the cubital and subcubital veins (Fig. 2B). In *H. blanchardi*, the branch of F6 is 0.1× as long as F6, and the basal area of the fore wing is very setose (De Santis, 1964). Also, in *H. lemosae*, the fore wing is 1.5–1.7× as long as the mesosoma, while in *H. blanchardi*, this ratio is 2.5, according to Torréns et al. (2017).

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Conflicts of interest

The authors declare no conflicts of interest.

Author contribution statement

All authors contributed materially to this scientific research. RNSL and KKGM planned the work, KKGM, EAC, JMM and RNSL performed the experiments and KKGM carried out the specimen collection. VAC and JN carried out the taxonomic works, dissections, photographs, and description of the new species *Hexacladia lemosae*. RNSL, KKGM, VAC and JN wrote the initial manuscript. All authors revised and edited the final version of this work.

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