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A living fossil *Tetrapus* fig wasp (Hymenoptera: Agaoninae) developing in extant Neotropical fig species (Moraceae: *Ficus,* section *Pharmacosycea*)

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Abstract: It has been assumed that *Tetrapus* female wasps (Agaonidae s.s.), the pollinators of the figs of the New World, section *Pharmacosycea*, are mainly characterized by the presence of one mandibular appendange only, and that it is the most ancestral clade of extant Agaonidae s.s., and the males are tetrapodous. The main objective of this work was to study five *Tetrapus* morphotypes, whose females have two mandibular appendages and the males are hexapodous, their fig host association and phylogenetic position to the family Agaonidae. The question of which group of fig pollinating wasps and associated figs are the sister to the rest of the pollinating agaonids, and figs respectively remain open. I report a group of New World extant *Tetrapus* morphotypes (Agaonidae: Agaoninae) provisionally assigned to *Hexapus* subg. nov. in preparation. Currently, *Tetrapus* appears as the sister taxon to all other fig pollinating taxa. Howeveer, morphologically, ecologically, geographically and historically *Hexapus* seems to be the ancestral clade of the extant Agaoninae. *Hexapus* morphotypes develop in fig species of subsection *Petenenses* (section *Pharmacosycea)*. In the known extant *Tetrapus*, the females have one mandibular appendage and the males have reduced one or two short-lobe atrophied non-functional midlegs (tetrapodous). *Hexapus* females have two free mandibular appendages, and the males have five segmented functional mid-legs (hexapodous). Molecularly *Hexapus* seems to be the ancestral clade of extant Agaoninae; e.g., a *Tetrapus* sp. of *Ficus crassivenosa* was placed by other author as the ancestral clade of 101 wasp species, representing 19 worldwide Agaoninae genera, including four *Tetrapus* species. In *Tetrapus* sp. of *Ficus crassivenosa*, the female has two mandibular appendages and the male is hexapodous. The females of *T. apopnus* and *T. delclosi,* preserved in Early to mid-Miocene amber from the Dominican Republic, also have two mandibular appendages and the general morphology of extant *Hexapus*. I suggest that *Hexapus* stands up as a living fossil and the sister clade of *Tetrapus* s.s. The presence of extant *Hexapus;* as well as extant *Tetrapus* and their fig host species, especially in South America supports a Southern Gondwanaland origin for both of them, but not a trans-Pacific migrating connection with tropical America for section *Pharmacosycea* (the host of *Tetrapus* and *Hexapus*), a long oceanic dispersal, high levels of stem extinction of *Tetrapus* or Atlantic land connections, as proposed by other authors. However, lastly it has been assumed that figs and their pollinators arose simultaneously in Eurasia during early Tertiary and spread southwards from it. Most of the morphological and molecular studies of the fig biology of Agaoninae and *Ficus*, did not include *Hexapus* morphotypes and their fig hosts, and assumed that *Tetrapus* is the most ancestral clade of the extant fig pollinating wasps. Rev. Biol. Trop. 64 (4): 1721-1735. Epub 2016 December 01.

Key words: Agaoninae, *Tetrapus*, *Hexapus*, *Ficus*, section *Pharmacosycea*, subsection *Petenenses*, living fossil wasps.

Ficus is a Pantropical group that comprises about 735 species worldwide (Berg & Corner, 2005). It is found in all regions where frost does not occur (Corner, 1958), especially in areas of the Southern Hemisphere, including very small isolated oceanic islands (McKey, 1989; Yokoyama, 2003; Azuma, Harrison, Nakamura, & Su, 2010; Ramírez, Gómez, Salazar, & Aguilar, 2011). New World *Ficus* has two endemic sections: *Pharmacosycea* (Miq.)

Benth. & Hook. f. subgenus *Pharmacosycea* (Miq.) Miq., pollinated by *Tetrapus* Mayr; and section *Americana* Miq. subgenus *Urostigma* (Gasp.) Miq., pollinated by *Pegoscapus* Cameron. Section *Pharmacosycea* is a relatively small group (Berg, 1989), comprising at least 25 species (Berg, 2009), of which 19 are found in Ecuador. *Ficus* is characterized by its unique flask-like urceolate inflorescence (fig or syconium) with internal staminate and pistillate flowers and a small circular or split-shaped terminal orifice, the ostiolum, barred by stiff bracts, through which the symbiotic pollinating wasps must pass to reach the syconial cavity, oviposit in some of the female florets that become galls, where their larvae develop, and pollinate some of them that become seeds. Dispersal of *Ficus* is a double problem, since the pollinators must accompany the host plant (Corner, 1958). Furthermore, *Ficus* pollen grains are very small (7-22 μm), ellipsoid or oblate, with smooth exine (Berg & Corner, 2005) and probably lack the vacuolated stage (Ramírez, 2007); consequently, they must suffer fast dehydration and death during long distant transportation or emigration journeys (Ramírez, 1989; 2007). Furthermore, fig seeds are small, 0.5-5 mm long (Corner, 1958), short-lived, and the seedlings initially grow very slowly (Ramírez pers. obs.). New World section *Pharmacosycea* has free standing leptocaulous terrestrial trees, although *Ficus crassiuscula* Standl. (a host of an *Hexapus* morphotype) is usually hemi-epiphytic (Berg, 2009). According to Berg and Corner (2005), events of long-distance in *Ficus* dispersal will probably rarely result in reproduction and establishment by absence of pollinators, and population of trees to allow establishment of the pollinators as well. However, Cruaud et al. (2011) proposed fruit pigeons, in part, for long distance trans-oceanic dispersal for fig fruits. Ecologically, *Ficus* provides one of the most important continuous food resources year around, e.g., Harrison (2005) noted that over 1 200 vertebrate species feed on figs. Furthermore, the eclosing new generation of female wasps is eagerly consumed by ants, flying swallows and Anisoptera dragon flies; while the wingless males are consumed by ants and staphylinid beetles. Furthermore, the dead foundresses are internally consumed by *Parasitodiplogaster* nematodes (Ramírez & Salazar, 2015). It has been suggested that the mutual relationship between *Ficus* and the pollinating Agaoninae was probably established before the break of Gondwana (Weiblen, 2002); while Berg and Corner (2005) noted that the general pattern of distribution and diversification suggest that the genus *Ficus* originated in Eastern Gondwana. Interestingly, Corner (1967) noted that subgenus *Pharmacosycea* seems to connect with an even earlier stock of *Ficus*.

Subgenus *Pharmacosycea* and its pollinators: Subgenus *Pharmacosycea* is monoecious with two sections (Berg & Corner, 2005): *Pharmacosycea* (Miq.) Benth & Hook. f. and *Oreosycea* Corner (Berg & Corner, 2005); both sections are pollinated by different Agaoninae genera: *Pharmacosycea*, by *Tetrapus* (Mayr, 1885), which is a passive pollinator; *Oreosycea*, by *Dolichoris* (Wiebes, 1979), which is an active pollinator, with the exception of one species (Wiebes, 1979). New World Section *Pharmacosycea* has ca. 25 species; while *Oreosycea,* ca. 45, ranging from Africa and Madagascar to Fiji and New Caledonia (Berg & Corner, 2005). Section *Pharmacosycea* has two subsections: *Bergianae* and *Petenenses* (Berg, 2006). *Tetrapus,* as well as most other pollinating Agaoninae, are usually tiny (no more than 3 mm in length), usually dark colored, diurnal, poor flyers, and do not feed as adults (Ramírez, Monge, & Chavarría, 2009, and references therein).

The pollinators of section *Pharmacosycea*: Currently, *Tetrapus* has been assumed to be the exclusive passive pollinator of section *Pharmacosycea* (Corner, 1960; Ramírez, 1970; Bouček, 1993; Ramírez, 1994; Wiebes, 1995). Ramírez (1978; 1991) considered that *Tetrapus* seemed to be composed of two well defined groups: in one group (*Tetrapus*), the females have one mandibular appendage and the males are tetrapodous; in the other, the females have two mandibular appendages and the males are hexapodous. Bouček (1993) referred about three *Tetrapus* with the female mandible split from the apex; while Wiebes (1995), mentioned two saw-like appendages.

Geographical origin and dispersal of subgenus *Pharmacosycea* and *Tetrapus*: Corner (1967) suggested that subgenus *Pharmacosycea* originated in the region that connected the Solomon Islands, New Hebrides and New Caledonia, it traveled via Caledonia to Asia, but very early a detachment went to America. He also noted that subgenus *Pharmacosycea* clearly has a trans-Pacific history that was not of small islands, but of high forest and that North America could not have been the source supply of *Pharmacosycea* to South America. Rønsted et al. (2005) noted that, if their age estimates are correct, this could imply that long distance oceanic dispersal is an important process in the present distribution of *Ficus* as also noted by Datwiler and Weiblen (2004); and that, the South American section *Pharmacosycea* would have separated from the rest of *Ficus* only 60 Myr ago, which postdates the separation of South America from Africa (about 90-100 Myr ago) during the break-up of Gondwana. Lopez-Vaamononde et al. (2009) noted that if their estimates and ancestral areas are correct reconstructions, it would imply that the current presence of *Tetrapus* and its associated *Pharmacosycea* figs in South America could be the result of long distance oceanic dispersal from Asia to the Neotropics; and that alternatively, high levels of stem lineage extinction could explain the observed young age for extant species of *Tetrapus and section Pharmacosycea*. Cruaud et al. (2012) estimated 74.9 Ma for the stem *Pharmacosycea/Tetrapus,* and that, biogeographic analyses indicated that the present-day distribution of fig and pollinator lineages, is consistent with a Eurasian origin and subsequent dispersal, rather than with Gondwanan vicariance. Furthermore, Cruaud et al. (2012) concluded that neither their study nor previous ones have strong basis for inferring which group was sister to all other agaonids.

Time evolution of the *Ficus*-Agaoninae association with special reference to section *Pharmacosycea* and *Tetrapus*: The age, geographical origin and major periods of diversion of *Ficus* remain controversial (Lopez-Vaamonde et al., 2009). For the geographical origin of the association of *Ficus*-Agaonidae, it has been suggested that the pollinating wasps evolved during the Cretaceous period (Corner, 1958) in the Southern Hemisphere (Gondwanaland) (Murray, 1985). According to Machado, Jousselin, Kjellberg, Compton and Herre (2001), *Ficus* and its pollinating wasps seem to be of late Cretaceous origin. Ramírez (1978, Fig. 21; 1991, Fig. 18), in his morphological cladistic analyses about the evolution of the mandibular appendage, and the pollinating syndromes of the Agaoninae, placed *Tetrapus* as the most basal clade to all other extant Agaoninae groups, and section *Pharmacosycea,* as the most ancestral group of *Ficus*. Those cladistic positions were corroborated by molecular phylogenetic analyses by Herre et al. (1996), Weiblen (2000), Rønsted et al. (2005), Rønsted, Weiblen, Clement, Zerega and Savolainen (2008), and Munro et al. (2011). Machado et al. (2001) noted that the passively pollinating *Tetrapus* appears to be the most ancient pollinator genus associated with the monoecious Neotropical subgenus *Pharmacosycea*; and that, both morphological and molecular data, support New World section *Pharmacosycea* as the earliest derivation among existing figs. Furthermore, Machado, Herre, McCafferty and Bermingham (1996) noted that the ancestors of Neotropical *Pharmacosycea* and *Tetrapus* probably arose at the origin of their mutualism ca. 90 Myr ago in Gondwana. Rønsted et al. (2005) supported that section *Pharmacosycea* is the oldest group of *Ficus*. Lopez-Vaamonde et al. (2009) also confirmed that *Tetrapus* was consistently placed as a sister group of the rest of the extant pollinating fig wasps. Furthemore, Rønsted et al. (2005) obtained confidence intervals of 98-105 Myr for the age of the root node of *Ficus,* and 66-101 Myr for the age of the root node of the wasps. While Lopez-Vaamonde et al. (2009) estimated that the crown group of Agaonidae could have originated any time between 54 and 216 Myr ago. However, Zerega, Clement, Datwyler and Weiblen (2005) suggested that the figs may have radiated more recently after the break-up of Gondwana, and gave a range of 40-51 Myr for the crown group age of *Ficus*. Furthermore, Zerega et al. (2005) results do not support the hypothesis of simultaneous diversification between figs and fig wasps and suggested that figs may have radiated more recently, during the Tertiary (*cf*. Cruaud et al. (2012) for codiversification of figs and fig-pollinating wasps). However, the *Tetrapus* species molecularly analyzed by those authors, as well as others, correspond to extant *Tetrapus* (Herre et al., 1996; Machado et al., 1996; 2001; Jousselin, Rasplus, & Kjellberg, 2003; Datwyler & Weiblen, 2004; Rønsted et al., 2005; 2008; Jian, Zhu, & Zhen, 2006; Lopez-Vaamonde et al., 2009; Azuma et al., 2010; Erasmus, van Noort, Jousselin, & Greeff, 2010; Munro et al., 2011; Cruad et al., 2009, 2011, 2012). The objectives of this work were to study the association of five *Tetrapus* morphotypes whose females have two independent mandibular appendages and hexapodous males; explain their origin and morphological relationships to other pollinating wasps of subgenus *Pharmacosycea* and other pollinating genera, their taxonomic position, and its origin.

MATERIALS AND METHODS

Syconia of *F. crassiuscula* Standl. were collected in Santa Cruz, Turrialba, Costa Rica (09°58´02” N - 83°44´15” W, at 1 430 m of elevation), from *F. macbridei* Standl. in Tapantí National Park, Costa Rica (09°46´13.2” N - 83°47´59” W, at 1 239 m in elevation) and of *F. crassivenosa* W. Burger, from near Quebrada Cataratitas, San Ramón, Alajuela, Costa Rica (10°14’3.26” N - 84°31’29.39” W, at 755 m in elevation). Naturally colonized syconia of *Ficus crassiuscula* and of *F. macbridei* were collected one day before they reached the male phase. Each syconium was individually enclosed in a tightly capped jar. Once the wasps emerged from the syconia, they were put to sleep with acetone and preserved in ethanol 70 % for further identification and dissection. *Hexapus* females and males were also collected by the author from the syconia of a cauliflorous riparian *Pharmacosycea* sp. in Jiménez, Biscucuy, Portuguesa State, Venezuela in 1966. The pollinators of *F. petenensis* Lundell was collected from Veracruz, Mexico, and described as *Hexapus ramirezi* by Xochitl M. Cuevas-Figueroa (2009, unpublished). A female of *H. ramirezi* was obtained from a preserved syconium of *F. petenensis* Lundell (=*F. apollinaris* Dugand); voucher number 2502 deposited at INBio Herbarium, Santo Domingo de Heredia, Costa Rica. The illustrations of two fossil female *Hexapus* are used with the permission of Enrique Peñalver (2006, Fig. 2a y Fig. 2b). The dissections of the wasps were made with the help of a dissecting microscope while the wasps were submerged in 70 % ethanol in a Syracuse container, the body structures were dislodged by mean of minute Nadel hook and mounted in Hoyer’s fluid to be illustrated.

The species of *Ficus* studied were identified by the author using the descriptions of Burger (1977) and Berg (2009). The scientific names of the figs were consulted in Tropicos® of the Missouri Botanical Garden and in Berg (2009). The term Agaoninae corresponds to the pollinating wasps, sensu Bouček (1988) or to Agaonidae sensu Rasplus, Kerdelhué, Le Clainche and Mondor (1998). The term *Tetrapus* refers to *Tetrapus* Mayr (1885), and *Hexapus* to a *Tetrapus* group, who’s female have two independent mandibular appendages and hexapodous males, noted as *Hexapus* subg. nov. in preparation. Since the five morphotypes of *Hexapus* studied were collected from five defined *Ficus* spp. and from different geographical areas, they are assumed to be distinct morphotypes until they are definitely described. The mandibles of *Nigeriella excavata* were also studied.

RESULTS

Females of the five *Hexapus* morphotypes studied had two free mandibular appendages (Fig. 1) and males with functional mid legs (hexapodous).They were the passive pollinators of five New World fig species of section *Pharmacosycea*, subsection *Petenenses*. The females are further characterized by an elongate head with a gular bridge without a sclerotized median carina or a felted line, mandibles with one gland only, absence of labrum, bilobed clypeus with a median setose carina, pedicel without axial spines, third segment with three completely fused anelli and a short unsegmented acuminated projection, labiomaxillary complex with distinct stipes and galea, bacilliform process pointing backward, segmented labium, forewing closed costal cell, spurious veins and moderate pilosity, and a twelfth flagellomere.

The males have two mandibular glands and antennae basically 4-segmented with an anellus, while the female has one gland and do not break off the antennae and wings when they penetrate through the ostiolum (Ramírez, 1970).

Tetrapus females have one mandibular appendage only (Fig. 2), while *Nigeriella excavata,* the pollinator of the African *Ficus tettensis (*Section *Malvanthera*) has one mandibular appendage, with two elongate lateral sclerotized median membranous areas (Fig. 3). Two fossil *Tetrapus* (*T. apopnus* and *T. delclosi*), described by Peñalver, Engel and Grimaldi (2006) preserved in early Miocene amber from the Dominican Republic, also have

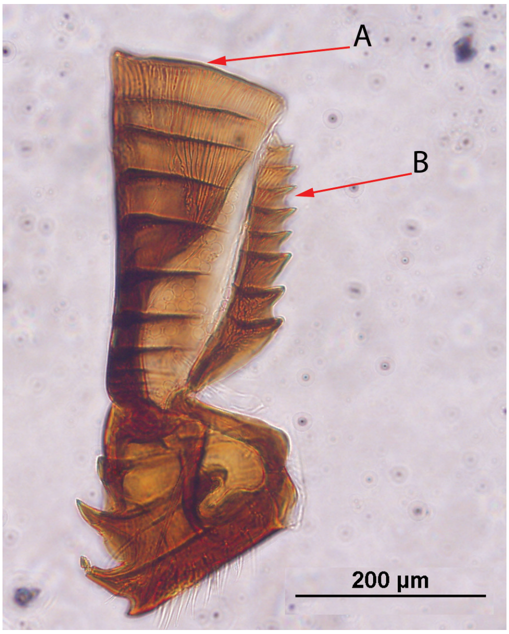


Fig. 1. Female mandible of *Hexapus* morphotype, pollinator of *Ficus macbridei*. A. External appendage. B. Internal appendage.

Fig. 2. Female mandible of *Tetrapus* morphotype, pollinator of *Ficus glabrata*. a. Mandibular appendage.

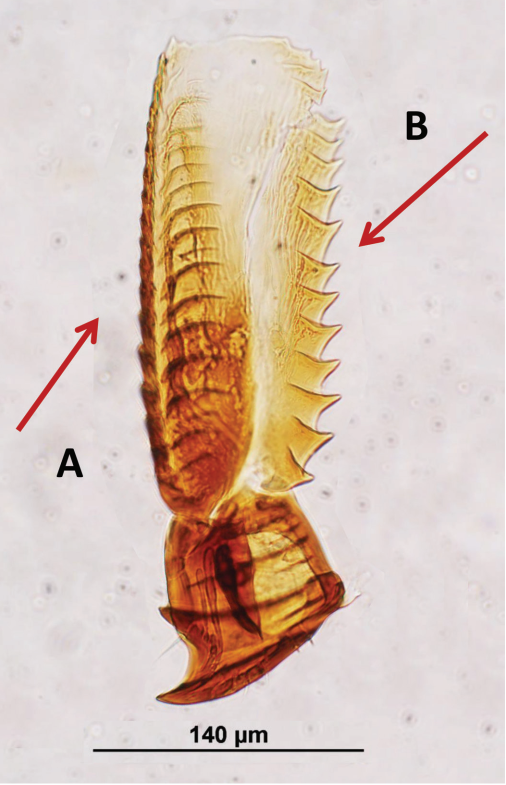
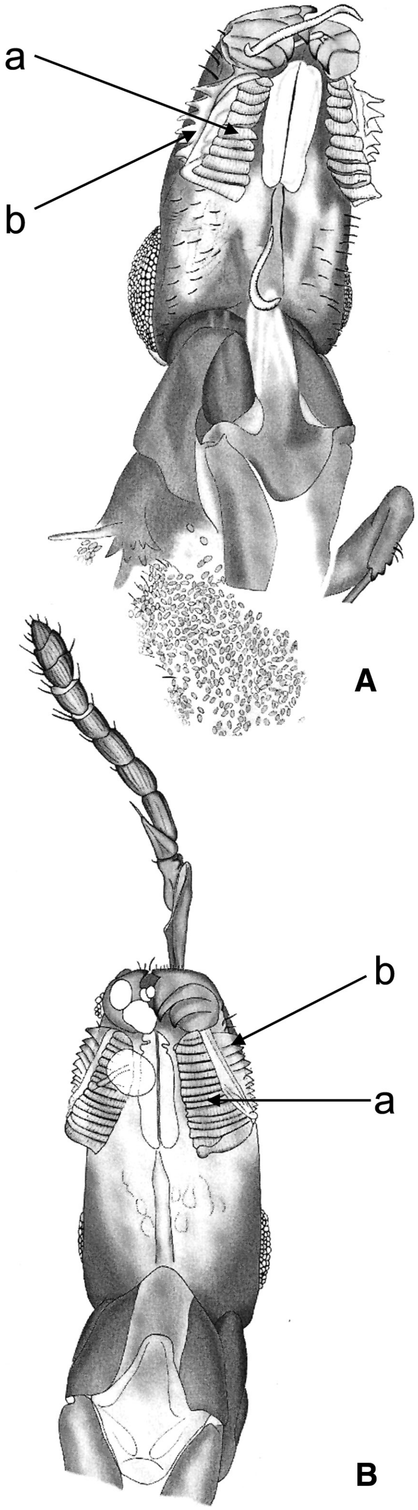


Fig. 3. Mandible of *Nigeriella excavata*, pollinator of *Ficus tettensis* (section Malvanthera). A. External sclerotized section. B. Internal sclerotized section.



two mandibular appendages (Fig. 4), and the general morphology of extant morphotypes of *Hexapus*. The ostiollum of *Ficus crassiuscula* (Fig. 5); as well as, all other fig species pollinated by *Hexapus*, is constituted by a group of few external imbricated bracts (Fig. 5a), followed by inflexed bract, forming a tubular entrance (Fig 5b). There was a relationship between the presence of two mandibular appendages of extant female morphotype of *Hexapus* and the ostiolar conformation of the five hosting fig species studied. *Hexapus* is mainly associated with subsection *Petenenses* fig species

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Fig. 4. Two fossil *Tetrapus* females preserved in Early Mioene (Burdigaliam) amber from Dominican Republic, after Peñalver et al. (2006). A. *T. delclosi*. B. *T. apopnus*. A. Internal appendage. b. External appendage.

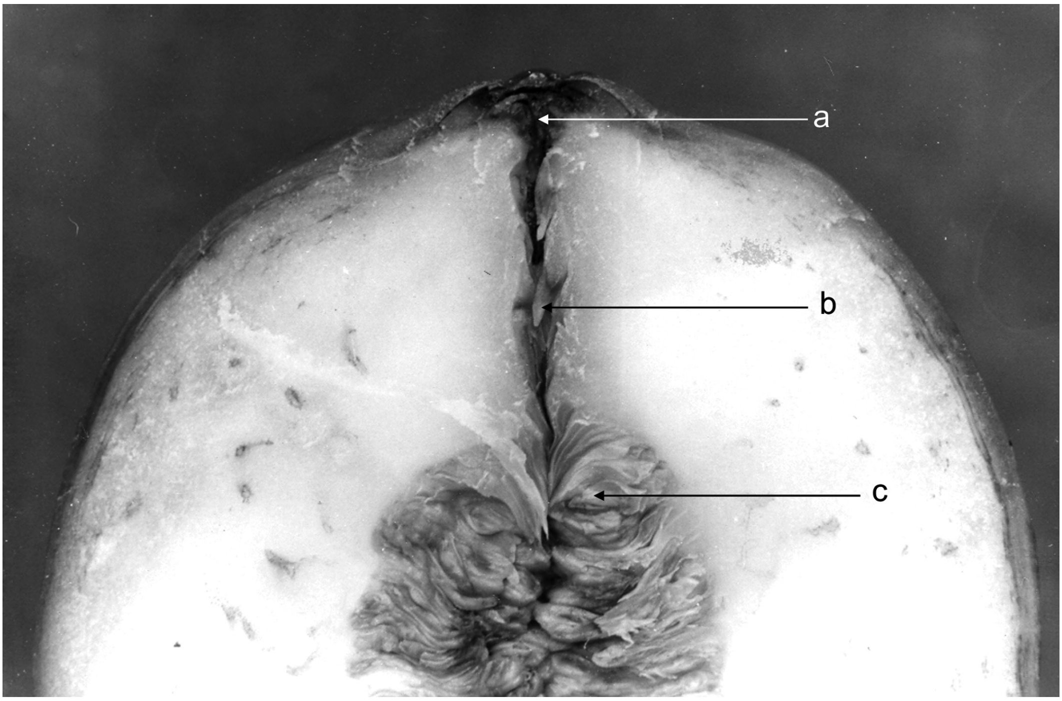

Fig. 5. Ostiolum of *Ficus crassiuscula.* a Helicoidal entrance. b. Linear entrance. c. Gall flower.

that predominate in submontane humid forests up to 2 900 m in altitude, or more; as described by Berg (2009). Due to the conformation of the ostiolum and to the distribution of the fig species in submontane, described by Berg (2009), and montane humid forest from 900 to 2 900 m in altitude or more, the author suggest that at least 10 fig species host *Hexapus* morphotypes, described by Berg (2009); among them: *Ficus bombuscaroana* C.C. Berg; *F. carchiana* C.C. Berg; *F. dulciaria* Dugand; *F. ecuadorensis* C.C. Berg; *F. gigantosyce* Dugand; *F. lacunata* T.A. Kvitvik; *F. loxensis* C.C. Berg; *F. mutisii* Dugand; *F. piresiana* Vásq. Avila & C.C. Berg; *F. quijosana* C.C. Berg. Furthermore, *Ficus crassiuscula* Standl., *F. crassivenosa* Burger, *F. macbridei* Standl., *F. petenensis* Lundell (=*F. apollinaris* Lundell); (Xochil Cuevas-Figueroa ined.), *F. pulchella* Schott (Vásquez-Avila pers. com.), and a Venezuelan *Pharmacosycea* cauliflorous species are known to host *Hexapus* female morphotypes, and differ from *Tetrapus* females by having the scape with a sharp lateral subtriangular flange (Fig. 6), instead of a bulging flange (Fig. 7), and because they have a close costal cell (Fig. 8), instead of an open costal cell

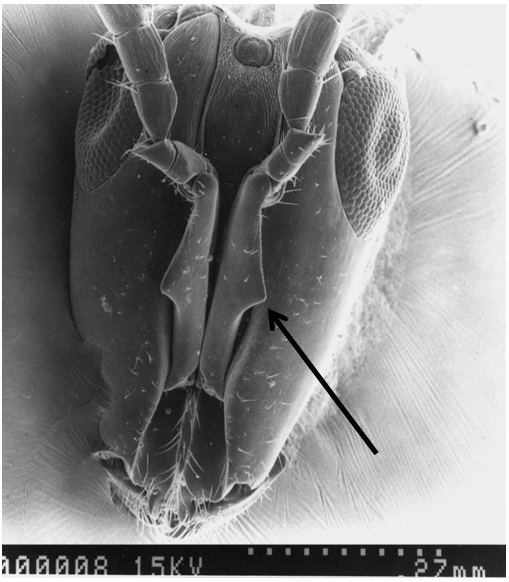

Fig. 6. Head frontal view of *Hexapus* antennal subtriangular flange (arrow).

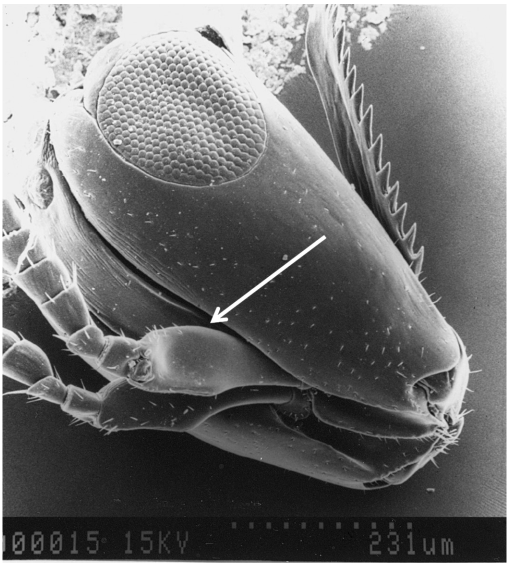

Fig. 7. Head frontal view of *Tetrapus* morphotype, antennal bulging flange (arrow).

(Fig. 9) and radiating veins, as illustrated by Peñalver et al. (2006, Fig. 4) for *T. apopnus*. By the contrary, *Tetrapus* females have open costal cell (Fig. 9). Furthermore, *Hexapus* females have elongate maxilla (Fig. 10), while *Tetrapus* have a short heart-shaped maxilla (Fig. 11). Pollen grains of section *Pharmacosycea* are cylindrical with obtuse ends (Fig. 12). All the *Hexapus* morphotypes were associated with fig species of subsection *Petenenses* and *Bergianae* of New World section *Pharmacosycea*, subgenus *Pharmacosycea.*

DISCUSSION

Hexapus subg. nov. ined., in preparation, belongs to *Tetrapus* Mayr which has five extant nominal species (Wiebes, 1995). The five *Hexapus* morphotypes studied are the specific

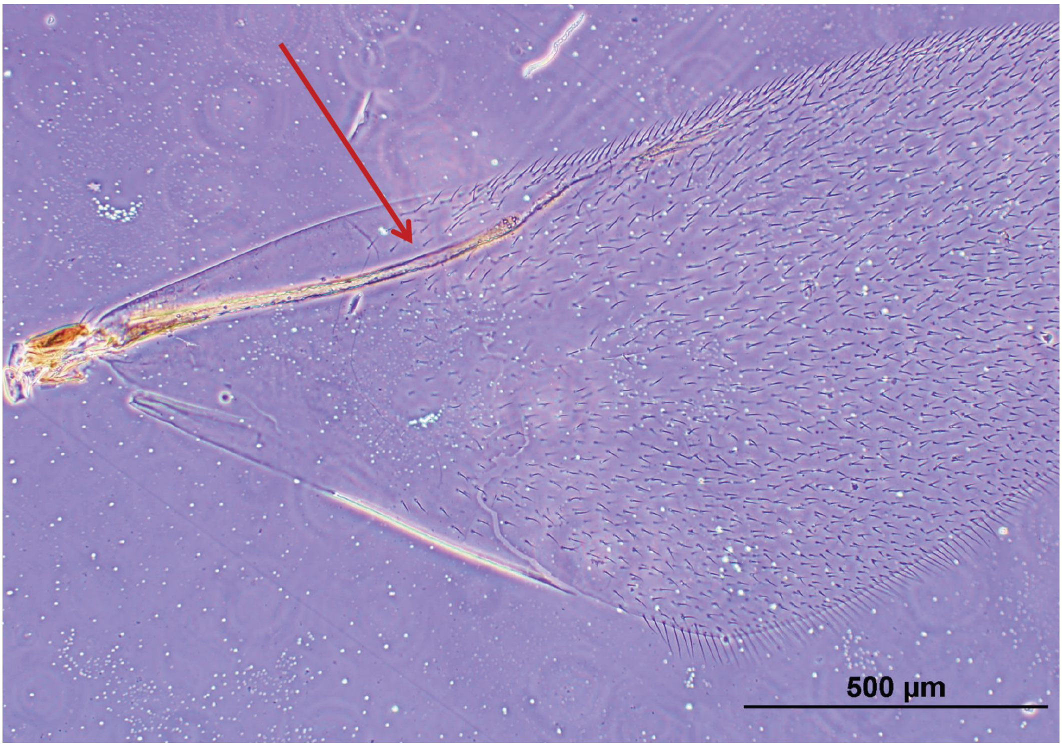

Fig. 8. Forewing basal section of *Hexapus* morphotype with close costal cell (arrow).

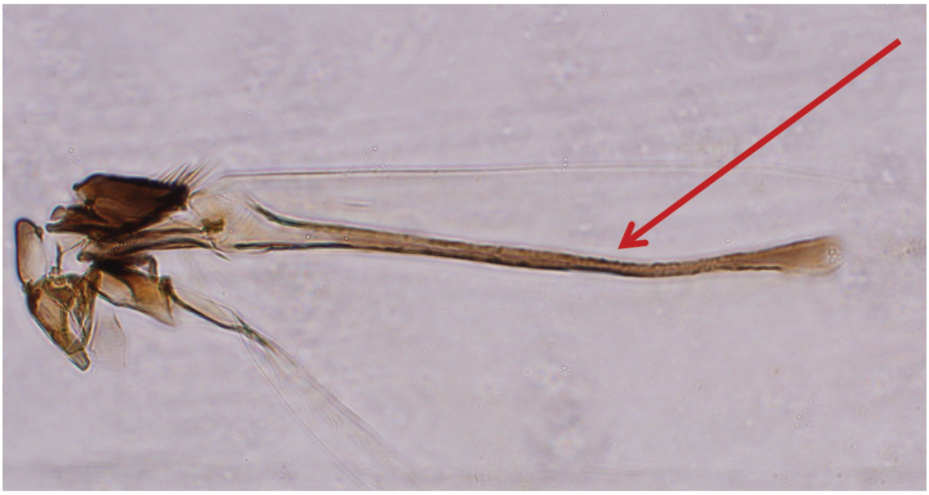

Fig. 9. Forewing basal section of *Tetrapus morphotype* with close open costal cell.

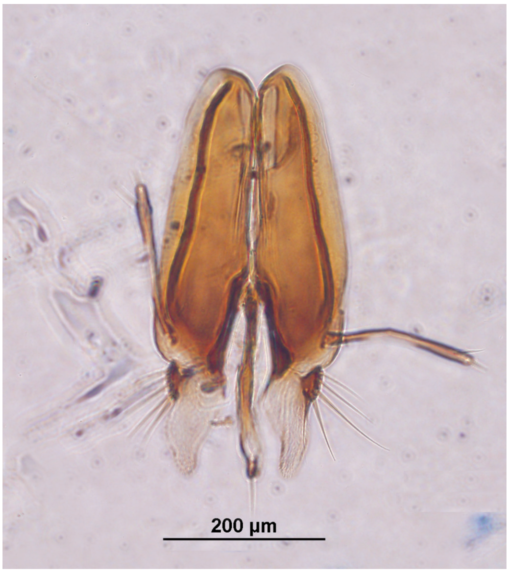

Fig. 10. Labiomaxillary complex of *Hexapus* morphotype.

Fig. 11. Labiomaxillary complex of *Tetrapus* morphotype.

pollinators of five species of section *Pharmacosycea*: subsection *Petenenses*. Two fossil female *Tetrapus* (*T. apopnus* and *T. delclosi*), described by Peñalver et al. (2006), like those of the extant *Hexapus,* also have two mandibular appendages, forewing closed costal cell, and most of the general morphology (Peñalver et al., 2006). The ostiola of fig species associated with extant *Hexapus* morphotypes, have an entrance morphology not found in other fig groups, and are usually protracted at the bottom of a cavity in the apex of the syconium (Berg, 2009); they have several layers of superficial interlocking horizontal appressed bracts; (spiral entrance), followed by a series of inflexed

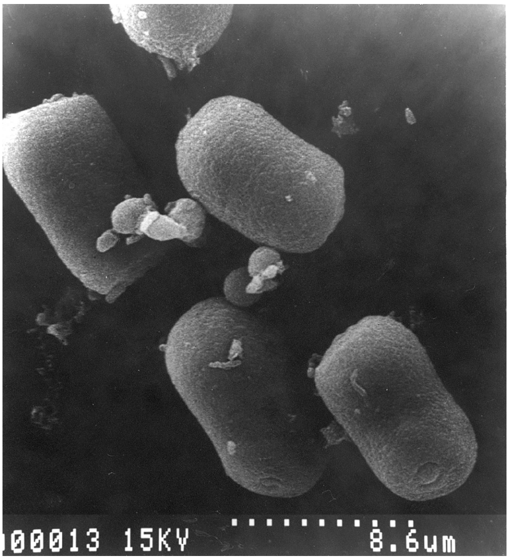

Fig. 12. Pollen grains of *Ficus glabrata*.

bracts pointing inwards into the syconial cavity; (linear entrance). On the contrary, fig species associated with *Tetrapus* usually have the ostiolum without distinct superficial interlocking bracts, but a linear tubular entrance only, and the female *Tetrapus* has one mandibular appendage. All other Agaoninae pollinators have one mandibular appendage. In *Hexapus* the internal appendage is wide, sub-rectangular, larger than the external one, and provided with lamellae, as in other female agaonines that enter syconia with a spiral ostiole; while the external appendage is narrower, with short lamellae ending in lateral teeth as in *Tetrapus*. The fossil females of *Tetrapus apopnus* and *T. delclosi* differ from extant *Hexapus* because they have an elongate sharp scale-like antennal process in the first flagellomere with an apical long spine, as depicted by Peñalver et al. (2006), which seems to be a Blastophaginae character sensu Wiebes (1982). Both *Tetrapus* and *Hexapus* are exclusively cleptoparasited by New World *Critogaster* Mayr (Sycoryctinae) (Segar, Lopez-Vaamonde, Rasplus, & Cook, 2012). *Hexapus,* as well as *Tetrapus*, are the exclusive pollinators of the New World section *Pharmacosycea*. Fossilized and extant *Hexapus* females are uniquely characterized among most extant pollinating Agaoninae groups (taxa) because they have two independent mandibular appendages as depicted by Peñalver et al. (2006) for *Tetrapus delclosi* and *T. apopnus* in Early to mid-Miocene., *ca*. 20-17 Ma (Iturralde-Vinent & MacPhee, 1996).

Extant *Hexapus* morphotypes seem to represent the most ancestral pollinating agaonines of *Ficus*, because among other pleisiomorphic characters: 1- the females do not possess a gular bridge composed of carinated sclerite, as assumed by Rasplus et al. (1988) for Agaoninae; 2- the antenna has a terminal bottom (terminal nipple), as found in Pteromalidae (Grahan, 1969), which is considered as a separate 12th flageromere, homologous to the apical segment of Rotoitidae, and also reported for some Eucharidae (Heraty et al., 2013 and references there in). Furthermore, the female *Hexapus* is uniquely characterized among other Agaoninae by the possession of two independent mandibular appendages. Due to the fact that *Tetrapus* and *Hexapus* morphotypes are passive pollinators (Ramírez, 1978), as well as to their ecology, the author considers that both groups are more ancestral than the fossilized '*Ponera*' *minuta*, described by Compton et al. (2010), and *Pegoscapus peritus* described by Peñalver et al. (2006), because both species had a general Blastophaginae morphology, sensu Wiebes (1982), and possessed pollen pockets which are associated with active pollination (Ramírez, 1978; Kjellberg et al., 2001; Cook, Bean, Power, & Dixon, 2004). Compton et al. (2010) noted that the pollen grains found in the pollen pockets of '*Ponera' minuta* were psilate and oblate as those found in other modern *Ficus* species; and that, '*P'. minuta* was an active pollinator and the host tree had dioecious breeding system. By the contrary, section *Pharmacosycea* pollen grains are cylindrical with obtuse ends, regulate and with a median constriction; as also illustrated by Ramírez and Malavasi (1997), for pollen grains carried by *Pleistodontes plebejus* (Wiebes), a passive pollinator of *Ficus hesperidiiformis* King, of section *Malvanthera* (Cook et al., 2004). According to Wang, Chen, Li, Zhang and Yang (2014) cylindrical and spherical pollen with obtuse end is only found in passively pollinated figs.

The extinct and extant *Hexapus* seem to be the sister clade of extant *Tetrapus*, the most ancestral clade of other extant Agaoninae and to represent a living fossil. It is hypothesized that the association, section *Pharmacosycea,* subsection *Petenenses* evolved in the Southern Hemisphere as assumed by Murray (1985) for Agaoninae, before the Gondwanan break (Weiblen, 2002) and that the Agaoninae ancestor could had been a *Hexapus*-like wasp; other way, the author hypothesizes that there had been an unknown ancestral Agaoninae-*Ficus* association, that originated in Eastern Gondwana, in agreement with Berg and Corner (2005); and that subgenus *Pharmacosycea* connects with an even earlier stock of *Ficus,* and suggested by Corner (1967). On the contrary, Cruad et al. (2012) stated that their ancestral recognition, suggests that figs, and their pollinators, arose simultaneously in Eurasia and the major lineages of figs, and pollinators, split during the Tertiary; and it appears that, posteriorly, they spread Southward from Eurasia. Furthermore, they noted that their, biogeography analyses indicate that *Pharmacosycea* and *Tetrapus* diverged before South America split from Antarctica (74.9-62.1 My); and that both lineages might have reached America across North Atlantic bridges (*cf.* Corner, 1967; Datwyler & Weiblen, 2004).

It is hypothesized that the ancestral Agaoninae that gave origin to *Hexapus* or their ancestors, also had two mandibular appendages, as seem to be still found as remnants, in some extant Old World pollinating agaonines e.g., *Eupristina* (*E.*) *grassi* Grandi (1916), and Saunders (1883) for *E. masoni* (Saunders); furthermore, *E. (P.) koninsbergi* Grandi, also seems to has two mandibular appendages, as illustrated by Chen and Chou (1997). *Nigeriella excavata* Compton (an African species) have a wide elongate mandibular appendage, with two lateral long sclerotized areas, each section provided with lamellae and denticles separated by a medial membranous area; the two sclerotized mandibular areas of *N. excavata,* may be remnants of two mandibular appendages that became fused. Some *Pleistodontes* females, e.g. *P. nitens* and *P. schizodontus* of Australia, as described and illustrated by Lopez-Vaamonde, Dixon, Cook and Rasplus (2002, Fig. 21 and Fig. 44), also have a mandibular appendage that recalls the presence of two fused appendages. All other described Agaoninae females have one mandibular appendage.

It is suggested that the ancestral fig ostiolum had a superficial layer with superficial interlocking bracts followed by an internal linear passage as found in subsection *Petenenses* (*cf.* Jousselin et al., 2003). The mandibular appendages of *Hexapus* and *Tetrapus* females, coevolved in response to the different ostiolar morphology of the two *Pharmacosycea* subsections (*Petenenses* and *Bergianae*). In *Hexapus* the lamellate internal appendage is used to displace the female through the superficial spiral ostiolar passage; while the external toothed appendage, is used to displace it through the internal linear passage. On the contrary, *Tetrapus* has one mandibular appendage associated with the ostiolar linear passage of subsection *Bergianae.* It is suggested that section *Pharmacosycea* and its pollinators may have evolved in submontane or montane humid tropical forest. By the way, most fig species associated with *Hexapus* are found from Southern Mexico to Ecuador, especially in humid submontane or montane forest up to 3 200 m or more. Berg (2009) noted that Ecuador gives the impression to be a “melting pot” of section *Pharmacosycea* species; e.g., out the 25 species, 19 are found in Ecuador; furthermore, out of them, 10 have an ostiolar morphology that suggests their association with *Hexapus* morphotypes (pers. obs.). Based on the morphological, ecological characters and geographical distribution of extant *Hexapus* and its fig hosts; the author thinks that this group could be the earliest divergent lineage of extant fig pollinating groups. The probable early association of *Hexapus*-section *Pharmacosycea* in Gondwana, or even an earlier stock of *Ficus* that connected with subgenus *Pharmacosycea,* as suggested by Corner (1967), calls into question the ideas of a trans-Pacific fig route connection (Corner, 1967), North Atlantic land bridges (Cruaud et al., 2012) or long distance trans-oceanic dispersal, proposed for the association *Tetrapus- Pharmacosycea* in the New World (cf. Corner, 1967; Rønsted et al., 2005; Lopez-Vaamonde et al., 2009; Azuma et al., 2010; Cruaud et al., 2011). Furthermore, it questions the radiation of *Ficus* during the Tertiary after the break-up of Gondwana proposed by Zerega et al. (2005) and Cruaud et al. (2011). Since the Agaoninae females are short-lived and do not feed as adults, long distance dispersal, or emigration, is intimately subject to ecological factors, e.g., the fig wasps must accompany the fig host in their migration (Corner, 1958; Ramírez et al., 2011). Furthermore, the seeds of *Ficus* are small 0.5-5mm long (Corner, 1958), and short lived and the seedlings grow slowly. According to Zerega et al. (2005), long distance dispersal over water seems unlikely for Moraceae, owing to the large short-living seeds. Furthermore, long distant transoceanic dispersal for particular pollinating wasps and other sycophylous invertebrates must occur inside the syconia of living fig trees (Ramírez et al., 2011). It is suggested that for migration the suit of a living fig tree or trees and associated invertebrates have to travel together on a floating riparian, or on a coastal raft. Consequently, the author suggest that dispersion of *Ficus* seeds and its suits of sycophilous wasps and other associated organisms by wind, flying, or aquatic vertebrates displacing over long transoceanic distances, do not seem to have played a historical role in the intercontinental dispersion of the main groups of sycophillous wasps and other invertebrates (*cf.* Cruaud et al., 2011) for Sycophaginae dispersal. Due to the presence of two mandibular appendages and a membranous un-carinated gular bridge, as pleisiomorphies Agaoninae characters, the author suggests that *Hexapus* is the most ancestral clade of all extant fig pollinator genera*;* as well as, the sister clade of extant *Tetrapus.* This idea is reinforced by the molecular analysis of Cruaud et al. (2009), who placed *Tetrapus* n. sp. (=*Hexapus* sp., pers. obs.) as the most basal clade of 101 Agaoninae species representing 20 world-wide pollinating genera. The author suggests that *Hexapus* may represent the supposed South America extinct stem agaonine lineage proposed by Lopez-Vaamonde et al. (2009). However, Cruad et al. (2012), concluded that neither their molecular study, nor previous ones, have a strong basis for inferring which agaonid group is sister to all other agaonids.

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RESUMEN

Un fósil viviente de avispas de los higos (Hymenoptera, Agaoninae) que se desarrollan en especies de higos (Moraceae) existentes en el Neotrópico. Se ha asumido que las hembras de las avispas *Tetrapus* (Agaonidae s.s.), las polinizadoras de los higos de la sección *Pharmacosycea* del Nuevo Mundo, se caracterizan principalmente por la presencia de un solo apéndice mandibular, que es el clado más ancestral de los Agaonidae s.s. existentes y los machos son tetrápodos. El principal objetivo de este trabajo fue estudiar algunos morfotipos de avispas *Tetrapus*, cuyas hembras poseen dos apéndices mandibulares y los machos tres pares de patas funcionales (hexápodos); su asociación con sus higos hospederos y la posición filogenética en la familia Agaonidae. Cuestiono cuál grupo de avispas polinizadoras de los higos, constituye el grupo hermano de otras avispas polinizadoras de los higos (Agaonidae). Informo sobre un grupo de avispas (*Tetrapus:* Agaoninae) existentes*,* provisionalmente asignado a *Hexapus* subg. nov. en preparación. Morfológica, ecológica, geográfica e históricamente, *Hexapus* parece ser el clado ancestral de los polinizadores de higos Agaoninae existentes. Los morfotipos de *Hexapus* se desarrollan en especies de higos de la subsección *Petenenses* (sección *Pharmacosycea*). Las hembras de *Tetrapus* existentes tienen únicamente un apéndice mandibular y los machos tienen uno o dos lóbulos cortos, en lugar de las patas medias y por lo tanto no son funcionales (tetrápodos). Las hembras de *Hexapus* tienen dos apéndices mandibulares libres y los machos tienen patas medias funcionales con cinco segmentos (hexápodos). Molecularmente, *Hexapus* parece ser el clado ancestral de los Agaoninae existentes; por ejemplo, una especie de *Tetrapus* de *Ficus crassivenosa* fue colocado por otros autores como el clado ancestral de 101 especies de avispas que representan 19 géneros de Agaoninae mundiales, incluidas cuatro especies de *Tetrapus*. En *Tetrapus* sp. de *Ficus crassivenosa*, la hembra tiene dos apéndices mandibulares y el macho es hexápodo. Las hembras de *T. apopnus* y *T. delclosi,* descritas del Mioceno Temprano (Burdigaliense), conservadas en ámbar de la República Dominicana, también tienen dos apéndices mandibulares y la morfología general de *Hexapus* existente. El autor sugiere que *Hexapus* representa un fósil viviente y es un clado hermano de *Tetrapus* s.s. La presencia de *Hexapus* y *Tetrapus* actuales, y sus higos hospederos, especialmente en Suramérica, sugieren que tienen un origen en la Gondwana meridional del Cretácico. La presencia de *Hexapus* y *Tetrapus* en el Nuevo Mundo, no parece apoyar una conexión migratoria a través del Pacífico con América tropical, para los higos de la sección *Pharmacosycea* (los hospederos de *Tetrapus* y *Hexapus*), una amplia dispersión transoceánica, altos niveles de extinción básica de *Tetrapus* y conexiones terrestres a través del Atlántico, propuesto por otros autores. Sin embargo, últimamente se ha aceptado que los higos y sus polinizadores se originaron simultáneamente en Eurasia, durante el Terciario Primario y se diseminaron hacia el sur. La mayoría de estudios morfológicos y moleculares de biólogos de los Agaoninae y *Ficus*, no incluyeron morfotipos de *Hexapus,* o sus especies hospederas de *Ficus* y aceptadaron que *Tetrapus* es el clado más ancestral de las avispas polinizadoras de los higos existentes.

Palabras clave: Chalcidoidea, Agaoninae, *Tetrapus*, *Hexapus*, sección *Pharmacosycea*, fósil viviente.

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