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A wasp species in a rolling gall at the margin of the leaflets of *Protium icicariba*: an inducer, a parasitoid or an inquiline?§

Uma espécie de vespa em galhas de bordos enrolados nos folíolos de *Protium icicariba*: um indutor, um parasitoide ou um inquilino?

André FS Merencio[1,5], Germana B Dias[2,5], Jayanne PP Souza[1,5] e Ary G Silva[3,4,5]*

§ Undergraduate monography of the first author; 1. Bachelor in Biologcal Science; 2. Post-Doctoral Fellowship FAPES; 3. Full Professor; 4. Programa de Pós-graduação em Ecologia de Ecossistemas; 5. Universidade Vila Velha – UVV. Rua Comissário José Dantas de Melo, 21, Boa Vista, Vila Velha, ES, Brasil. CEP 29102-770.
*Correpondent author: arygomes@uvv.br

Abstract Biological interactions involve several organisms in nature, and they are one of the key points in understanding biological communities. Galls are one of those interactions that may be characterized by changes in plant tissues induced by another organism. When insects cause them, galls are called entomogenous. Until now, three gall morphotypes have been described in the leaflets of *Protium icicariba* (DC) Marchand (Burseraceae). Two different researches had consensually assigned the wart-like morphotype to a Psyllidae insect species. A marginal rolled gall morphotype was reported to the leaflets of *P. icicariba* as being induced by a cecidomyiid insect species (Bregonci *et al*. 2010). However, Ramalho and Silva (2014) had found a cecidomyiid insect species in a new turbine-like gall morphotype. This paper describes a kind of falciform rolling gall that is originated by abaxial rolling of the leaflet margin. The galling insect had induced changes in the leaflet tissues organization. In the mesophyll, the cells of palisade and spongy parenchymas were transformed into polyhedral sclereids, making gall walls more resistant to compression or perforation, and protecting the galling insect until its ripping. All the developmental stages found inside the gall belonged to a wasp of the family Eulophidae, subfamily Tetrastichinae (Hymenoptera: Chalcidoidea).

Keywords: cecidogenesis, plant-insect interaction, sandbank, Tetrastichinae, Eulophidae, Chalcidoidea, Hymenoptera.

Resumo As interações biológicas envolvem diversos organismos na natureza, e são um dos pontos chaves na compreensão de comunidades biológicas. As galhas representam um tipo dessas interações que podem ser caracterizadas por alterações nos tecidos das plantas induzidas por um outro organismo. Quando os insectos são os indutores, elas são chamados de galhas entomógenas. Até agora, três morfotipos de galhas foram descritos para os folíolos de *Protium icicariba* (DC) Marchand (Burseraceae). Duas pesquisas diferentes atribuíram consensualmente o morfotipo verruga a uma espécie de inseto de Psyllidae. Um morfotipo de galha caracteriazado pelo enrolametno marginal do folíolo foi relatado para os folíolos de *P. icicariba* como sendo induzido por uma espécie de inseto cecidomyiídeo (Bregonci *et al*. 2010). No entanto, Ramalho e Silva (2014) encontraram uma espécie de inseto cecidomyiídeo num novo morfotipo de galha em forma de turbina. Este artigo descreve um tipo de galha enrolada e falciforme que é constituído pelo enrolamento abaxial da margem de folíolo. O inseto galhador induziu alterações na organização tecidos do folíolo. No mesofilo, as células dos parênquimas paliçádico e lacunoso foram transformadas em esclereídes poliédricos, tornando as paredes da galha mais resistentes à compressão ou perfuração, protengendo assim os insetos galhadores até sua eclosão. Todos os estágios de desenvolvimento encontrados no interior da galha perteciam a uma espécie da família Eulophidade, subfamília Tetrastichinae (Hymenoptera: Chalcidoidea).

Palavras-chaves: cecidogênese, interação inseto-planta, restinga, Tetrastichinae, Eulophidae, Chalcidoidea, Hymenoptera.

Introduction

There are several kinds of interactions in nature involving organisms in a broad range of reciprocal benefits and damages. The galling process is one of these relationships between plants and insects that has been drawing considerable attention of ecologists and evolutionary biologists because it contains very different characteristics from other interactions (Herrera and Pellmyr 2002).

Galls derive from changes in plant organs, usually hyperplasia or hypertrophy of certain cells or tissues. They that can be caused by several organisms, including viruses, fungi, nematodes, and insects Fernandes and Negreiros 2006). They are of distinct shapes and sizes, can be found in many plant species and different plant organs, such as branches, flowers, and leaves. The morphology of galls vary in hardness, thickness, color, air chambers and surface texture characteristics and variation of these influences predation and parasitism of galls (Inbar *et al*. 2004).

When insects are the inducers of galling process, with an intimate effect on gall morphology, the result is an entomogenous gall (Stone and Schönrogge 2003). Galling insects had found in plants a habitat, protection against environmental weather, refuge from predators and natural enemies, and overcome the difficulty in obtaining food. Therefore, they are considered specialized herbivores (Araujo and Santos 2009).

Among the Neotropical galling insects, the family Cecidomyiidae (Diptera) is the largest taxon (Fernandes and Negreiros 2006), with near 500 described species classified into 170 genera. However, the species richness of cecidomyiid is highly underestimated. A recent study estimated the global wealth galling in about 120,000 species (Espírito Santo-and Fernandes 2007), making knowledge of this essential group for ecological research. Another important to galling insect taxon is the family Psyllidae (Hemiptera)in which a group of species belonging to the suborder Sternorrhyncha, feeds the phloem sap. They have reduced size, measuring from 1 to 10 mm long in adults (Burckhradt and Queiroz 2012). Psyllids are insects that require a very particular site for their larval development. Due to this fact, they are widely used to study coevolution or cospeciation with plants and herbivores. About 4000 species of Psyllidae are described in all biogeographic regions of the world with about 1000 species distributed in Brazil (Burckhradt and Queiroz 2012).

On the genus *Protium* L. (Burseraceae), galls are found in leaflets and fruits, and some of the newly described species of galling insects receive the epithet of the plant species (Maia 2013). There is an expectative of four gall morphotypes (Maia 2013) for the leaflets of *Protium icicariba* (DC) Marchand, considering the current descriptions (Bregonci *et al*. 2010, Maia 2013, Ramalho and Silva 2014). A consensus was settled about a so-called epidermic or wart-like one that is induced by a psyllid insect species (Bregonci *et al.* 2010, Maia 2013, Ramalho and Silva 2014). However, the turbine-like morphotype described by Ramalho and Silva (2014) seemed to be a still not described one, and was induced by an apparent still not described cecidomyiid insect species. However, two gall morphotypes assumed to *P. icicariba* occurring in southeastern Brasil, the conic and the spheric ones (Bregonci *et al*. 2010, Maia 2013), were not found in the vegetation on sandbanks at Setiba, municipality of Guarapari, Espírito Santo State (Ramalho and Silva 2014)

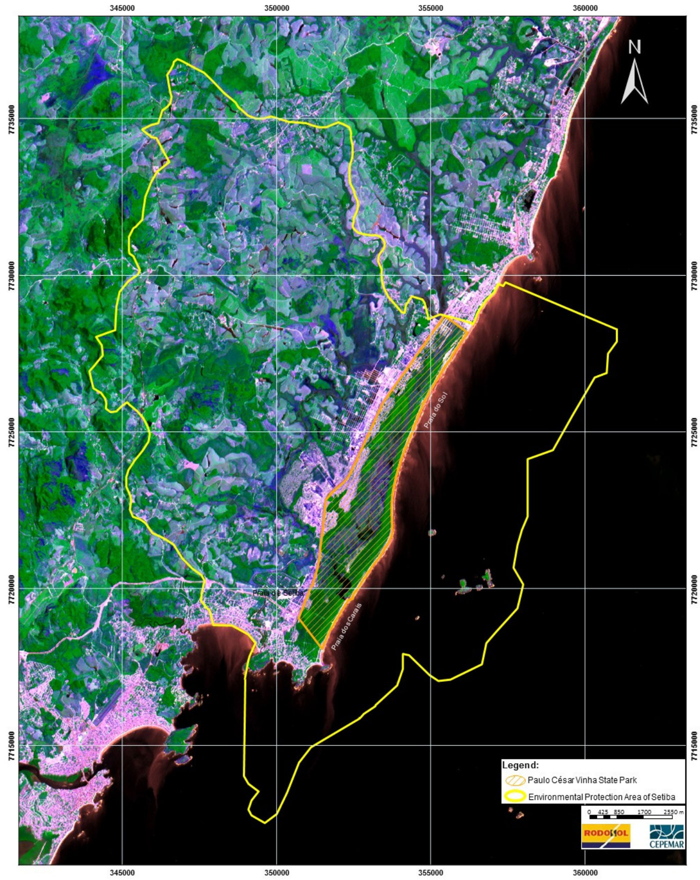

Figure 1 Localization of the Environmental Protection Area of Setiba - EPA-Setiba, and of the Paulo César Vinha State Park - PEPCV, at the municipality of Guarapari, Espírito Santo State, Brasil. Source: CEPEMAR 2007.

A marginally rolling gall morphotype was reported to the leaflets of *P. icicariba* as being induced by a cecidomyiid insect species (Bregonci *et al*. 2010, Maia 2013). It was also found at the Environmental Protection Area of Setiba – EPA-Setiba. Thus, the aim of this study was to investigate ontogenesis of the gall inducer insect and the changes in plant tissue during the galling process.

Methods

The studied area

The botanical material was collected in specimens growing in the Environmental Protection Area of Setiba - EPA-Setiba, at UTM Coordinates 24K 342,000 up to 362,000; and UTM 7.715,200 up to 7.735,250 (Figure 1). Local populations of *P. icicariba* occurs at a not-flooded open shrubland vegetation, with plant height up to 10 m. The presence of exposed white sand among the bushes of vegetation of different sizes and shapes, interspersed with areas where vegetation is sparse, was as remarkable feature in the studied area (Figure 2). It characterized this vegetation physiognomy as a typical restinga-like not-flooded open shrubland vegetation (Pereira 2003).

Figure 2 Non-flooded open shrubland vegtationf the Environmental Protection Area of Setiba - APA-Setiba, at the municipality of Guarapari, Espírito Santo State, Brasil.

Selection, collection and analysis of gall

The taxonomic identification of plant species in natural populations was carried out by Dr. Ary Gomes da Silva. A total of 12 collections of leaflets with galls were made from 2013, January, up to 2014, March 2014. Samples were collected with the aid of hermetic-sealed glass vessels, cleaned and blasted with hot air at 200°C, to eliminate any trace of chemical residues. Soon after sampling, they were packed in a thermal bag to be cooled until they reached the Laboratory of Functional Ecology, at University Vila Velha – UVV-ES. The galls were analyzed were open using a lamina to make manual longitudinal cuts, so as to avoid affecting the insect.

Morphological and anatomical analysis

Galls were morphologically characterized concerning their shape, color, site of occurrence in the host plant, number of internal chambers, and indumentum. In the case of leaflet galls, the side of their occurrence was signaled if in the adaxial or abaxial side of leaflet. Structural assessment was made at the Laboratory of Functional Ecology - UVV.

Morphological analyzes were performed on fresh leaflets with and without galls and the typology adopted for description of gall morphotype was the one proposed by Fernandes *et al.* (2012). Sample of leaflets with young galls were separated in glass pots protected with an infra millimetric mesh sheath, to monitor the emergence of adult insects. Part of the galls were dissected to obtain insect immature stages. The larval specimens were identified with the aid of specialized literature (Maia e Fernandes 2004, Narahara *et al.* 2004, Bregonci *et al.* 2010, Rodrigues e Maia 2010, Maia 2013) and photographed in stereoscopic microscope.

Anatomical analysis used leaflets without and with the two morphotypes of studied galls in different stages of development, that were fixed by immersion in a FAA 50 (Jensen 1962), since their collection in field. After near 40 minutes, the immersed leaflets were taken under a vacuum chamber for two hours to enhance the fixing solution penetration in plant tissues (Jensen 1962). Just after, leaflets were washed in distilled water and immersed in a solution of ethanol 70% for conservation.

Transversal sections were handmade for leaflet lamina nearby the morphotypes, while transversal and longitudinal sections were made for the galls in different maturation stages (Johansen 1940).

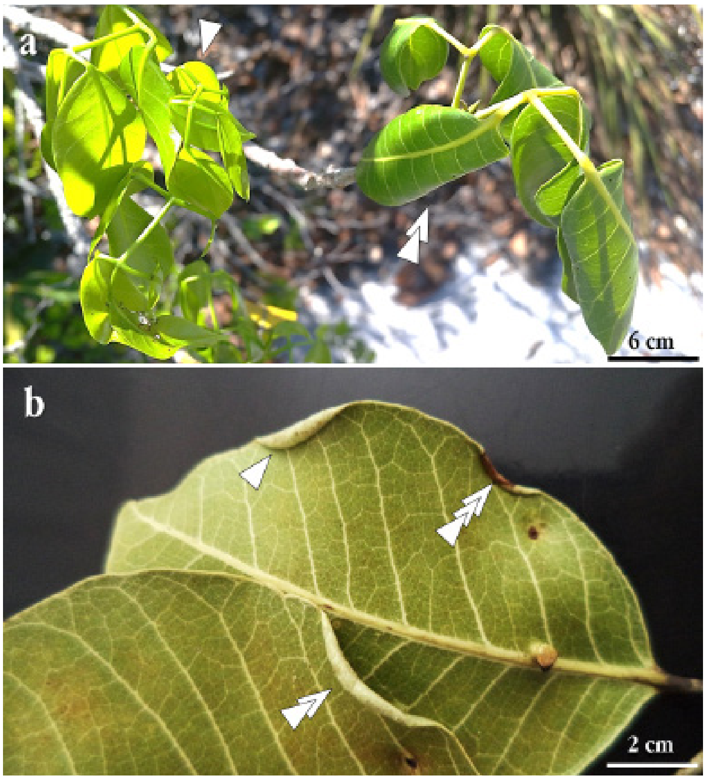

Figure 3 Leaflets of *Protium icicariba* (DC) Marchand. **a**: ungalled leaflets: young lligh -yellowish green leaflets (arrow), of low sclerification level in the mesophyll, and mature dark green leaflets (double arrow), with more sclerified mesophyll; **b**: falciform tubular rolling gall in abaxial view at intermediate (arrow), mature (double arrow) and damaged (triple arrow) stages.

Gall handmade sections were diaphanized in a water solution of sodium hypochlorite 2% (1:1), washed with distilled water, and neutralized during five minutes with a water solution of acetic acid 5%. After neutralization, sections were washed in distilled water until complete disappearance of ascetic acid scent, and then they were stained with the double coloration with Astra Blue and Safranine (Bukatsch 1972), at the proportion of 4:1. Stained sections were mounted in bidistilled glycerin and photographed in light microscopy at the Laboratory of Functional Ecology at UVV.

Results

This gall starts in anatomically mature leaflets (Figure 3a), with mesophyll differentiated into palisade and spongy parenchymas (Figure 4a), and is a result of marginal rolling of leaflet lamina towards its abaxial side, assuming a falciform tubular shape (Figure 3b). It is chlorophyllated, glabrous, and occurs only on the abaxial surface of the leaf (Figure 3a). There was a single larval chamber, bearing a single inducer insect, which may be or not be accompanied by another non-inducer arthropods, such as mites (Figure 3b). All developmental feature of insect metamorphosis happen inside the gall chamber, and even an exuviae of the final stage before adult hatching was found near a tip of an extremity of the gall chamber (Figure 3c).

In ungalled yellow-greenish leaflets the lamina had shown a

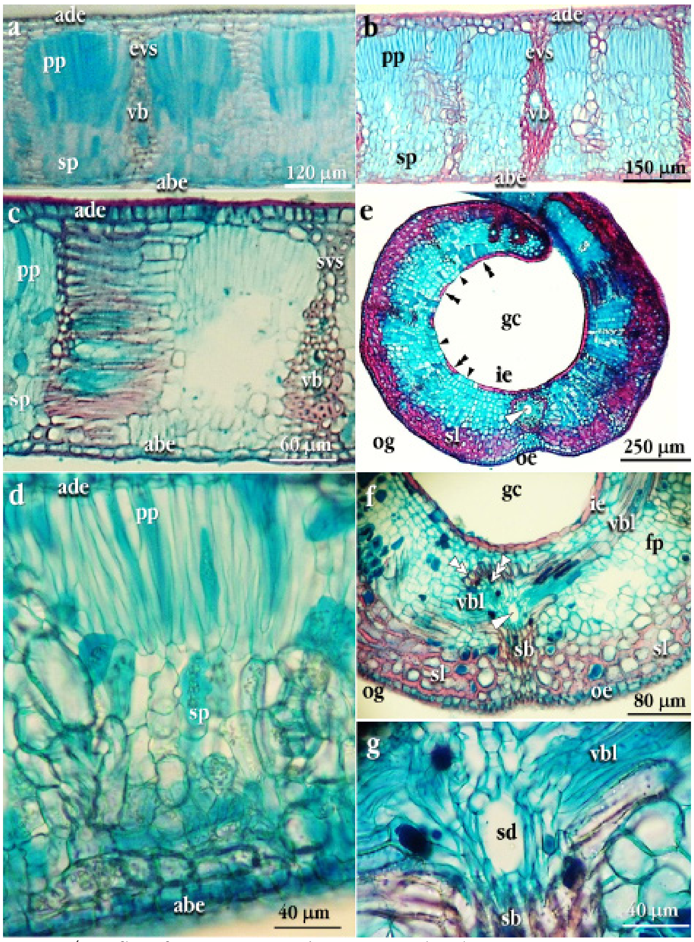

Figure 4 Leaflet of *Protium icicariba* (DC) Marchand in cross section. **a**: young yellow-greenish ungalled leaflet with lower level of lignification, exhibiting a typical dorsiventral mesophyll with adaxial epidermis (ade), palisade parenchyma (pp), spongy parenchyma (sp), but not the extended lignified vascular bundhle sheat; **b**: mature dark greenish ungalled mesophyll with a more intense level of lignification; **c**: non-galled leaflet of a alow sclerification level, exhibiting all the features of a typical dorsiventral mesophyll, and vascular bundles (vb) with their extended vascular bundle sheath (vbs); **d**: region of leaflet not involved in gall structure, showing the preserved dorsiventral mesophyll with adaxial epidermis (ade) palisade (pp) and spongy parenchymas (sp); **e**: rolling-leaflet gall. From outside (og) towards the inner gall chamber (gc), there is a strongly cutinized outer epidermis (oe) in contact with a band of four layers of sclerenchyma (sl). A secretory duct (white arrow) appears near the inner epidermis (ie) which exhibits sites with thinner (black arrow) alternated with thicker cuticle (double black arrow); **f**: detailing the gall's wall, a sclerenchyma bundle (sb) protects a secretory duct (arrow), near a vascular bundle (vbl) with xylem (double arrow) facing the gall chamber and phloem (triple arrow) facing the outer epidermis. A fundamental parenchyma (fp) fills the space between sclerenchymatous band (sl) and the vascular bundle (vbl) that surrounds the inner epidermis (ie); **g**: detail of secretory duct (sd), showing sclerenchyma sheath and the vascular bundle in longitudinal view (vbl).

typical dorsiventral mesophyll, with the palisade parenchyma on the adaxial side and spongy on the abaxial (Figure 4a). The vascular bundle sheath and its extension are not yet so intensively lignified (Figure 4a) as they are in the greenish leaflet (Figure 4b). In both of the previous leaflet stage, the vascular bundle with extended sclerenchymatous bundle sheath is regularly interspersed with palisade and spongy parenchymas in the mesophyll (Figure 4a-b).

In galled leaflets, the lamina of ungalled regions had shown the same dorsiventral pattern of ungalled dark-greenish leaflets (Figure 4 c-d). However, mesophyll of the wall of the falciform rolling gall (Figure 4e) are remarkably different. From the outer towards the inner epidermis, palisade and spongy parenchymas disappear. They

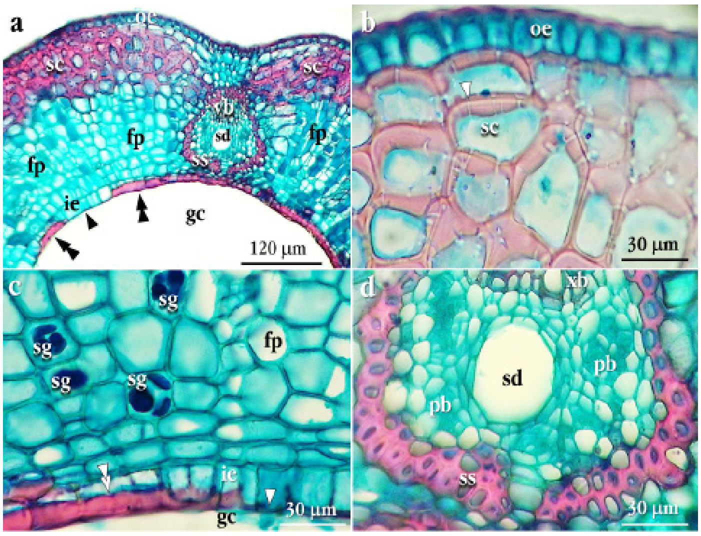

Figure 5 Mesophyll of the wall of the sickle tubular gall in the leaflets of *Protium icicariba* (DC) Marchand in cross section. **a**: the outer epidermis (oe) and the band of sclerenchymatous cells (sc) form the external harder zone, while the fundamental parenchyma (fp) and the inner epidermis (ie) form the internal zone that limits the gall chamber (gc). A secretory duct (sd) is associated with the vascular bundle (vb) and both are surrounded by a sclerenchymatic sheath (ss); **b**: external zone limited by the outer epidermis (oe) and hardened by the slerenchymatic cells (sc) with pimple pits (arrow); **c**: internal zone that limits the gall chamber (gc), comprising the fundamental parenchyma (fp) with starch grains (sg) in rare cells, and the inner unisseriate epidermis (ie) sometimes with a thinner (arrow) and other with thicker cuticle (double arrow); **d**: detailing the vascular bundle, with xylem (xb) and phloem (pb) bundles associated with a secretory duct (sd).

are replaced by a band of two up to four layers of sclerenchymatous cells, closer to the outer epidermis and hardening of the external side of gall's wall. These cell layers are followed by another band of two up to four layers of fundamental parenchyma (Figure 4f). In the gall wall, secretory ducts associated with vascular bundles (Figure 4g) were larger than the ones observed in the standard mesophyll, and they were surrounded by sclerenchymatous sheath (Figure 4g). The inner epidermis of gall's wall show sites of thicker layers of cuticle, alternated with thinner ones (Figure 4e). Vascular bundles surround the gall's wall, bellow the sclerenchymatous band, and across the fundamental parenchyma, near the inner epidermis (Figure 4f).

The mesophyll in the gall's wall may be understood as having two distinct zones. The external one that comprises the outer epidermis and a band of sclerenchymatous cells, and the inner one, mainly filled with a fundamental parenchyma and limited by the inner epidermis (Figure 5a). The external zone (Figure 5b) hardens the gall's wall. The internal one limits the gall chamber (Figure 5c), allowing the constitution of a large symplastic structure due to de existence of simple pits among the sclerenchymatous cells. Despite the fact that some starch grains may be rarely found in sparse cells of the fundamental parenchyma (Figure 5c), there was no tissue with nutritional reserve characteristics to be used during larvae development. In the interface of those two zones, there is a vascular bundles associated with a secretory duct completely surrounded by a sclerenchymatous sheath (Figure 5d).

We found a total of 307 cases of galls that contained at least remnants of galling insect, among 526 galls that were examined. Five developmental stages were found. In most of them, the insects were found near the extremities of the tubular gall, near

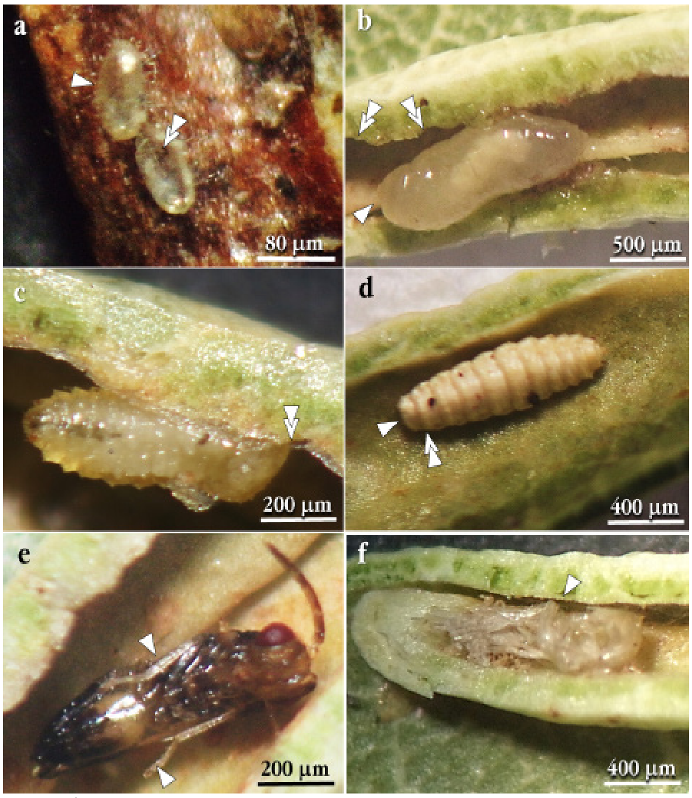

Figure 6 Developmental stages of the wasp-like Hymenotera species found inside a rolling gall in the leaflets of *Protium icicariba* (DC) Marchand. **a**: eggs in a stage previous (arrow) and after (double arrow) hatching. Larval phase (**b-d**) characterized by a still not differentiated body **b**: first instar, with translucent greenish body, still not clearly segmented. The whitish and still not mature gut is seen through transparence, and in front of the larvae head (arrow), signals of larval feeding on plant tissues of the gall wall (double arrow); **c**: second instar, still exhibiting a translucent greenish well -segmented body, exhibiting a mature reddish gut and head in a position of feeding on gall wall; **d**: third instar, showing a greenish and totally opaque body with already differentiated eyes in larvae head (arrow), spine bands (double arrow) that remark body segmentation; **e**: recently hatched adult inside the gall chamber in ventral view, showing its legs (arrow); f: exsuvia of final pupal stage in the gall extremity.

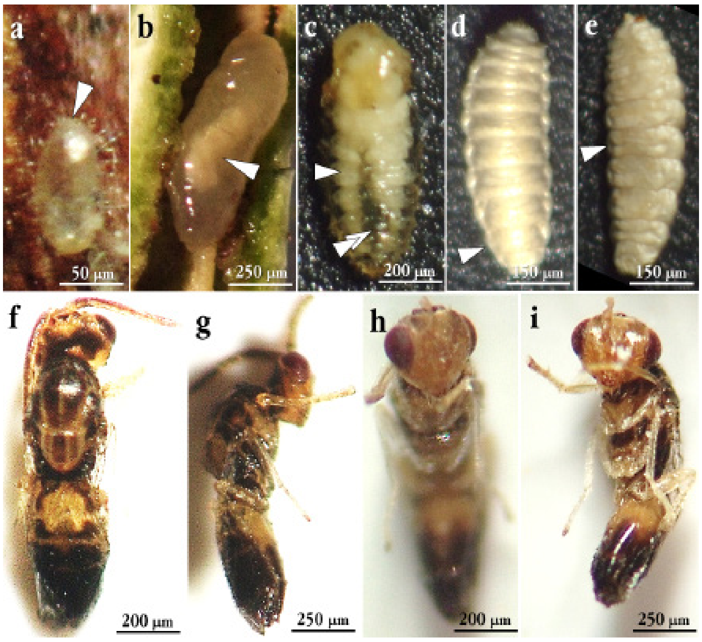

Figure 7 Details of the developmental stages of the wasp-like Hymenotera species which induces of a sickle tubular gall in the leaflets of *Protium icicariba* (DC) Marchand. a: eggs (arrow) in a stage previous to hatching. Larval phase (**b-e**) characterized by a still not differentiated body **b**: first instar, with translucent greenish body, still not clearly segmented, exhibiting through transparency a not yet mature whitish gut (arrow); **c**: second instar, still exhibiting a translucent greenish well -segmented body, exhibiting a better segmented boy with conspicuous spine bands (arrow) and a mature reddish gut (double arrow); third instar, **d**: dorsal view and **e**: ventral view, showing a greenish and totally opaque body with more clearly spine bands (arrow) that remark body segmentation. Adult (**f-i**). **f**: dorsal view; **g** lateral view; **h**: head; **i**: ventral view.

to the apertures towards the leaflet lamina. We found eggs of the galling insect (Figures 6a and 7a) near to the leaflet edge, which were laid down on the abaxial surface. We had also found four developmental stages of the wasp, comprising three larval instars and the adult phases (Figures 6b-e and 7b-i). An exuviae of final pupal stage previous to adult ripening was found in the gall extremity (Figure 6f), despite we could not find the pupa.

Among the larval stages, the first one was oval, with a transparent body, allowing to see the whitish digestory tract that was not completely mature in this developmental stage (Figure 6b and 7b). The second phase already exhibited some segmentation in the body, usually very well defined 12 segments, and the gut was entirely formed and colored in reddish-brown, which was seen by transparence through the greenish larvae body (Figures 6c and 7c). The third larval stage had already had completely formed segments, without differentiation of legs. Cutaneous transparency did not exist anymore, and the larva now has an entirely greenish color, and it is no longer possible to visualize the digestory tract (Figure 6d and 7d-e).

The adult insect (Figures 6e and 7f-i) was a chalcidoid wasp, belonging to the family Eulophidade, subfamily Tetrastichinae, that is colored in shades of brown and has red eyes. The mesonotum and metanotum have a dark brown color with three parallel light brown lines. Its abdomen has brown coloring in the last segments and segments closest to the thorax are colored in light brown (Figures 6e and 7f-i).

Discussion

The rolling of leaflet edge of *P. icicariba*, originating a gall described in this study, although not mentioned by Ramalho and Silva (2014), had already been reported by Bregonci *et al.* (2010), and was reinforced by Maia (2013). The major difference of our findings from those previous descriptions (Bregonci *et al.* 2010, Maia 2013) was the kind of inducer insect. While we found a wasp in different stages of development (Figures 6 and 7) inside the gall, Bregonci *et al.* (2010) and Maia (2013) had reported a cecidomyiid species, *Lopesia simplex* Maia, 2002 (Cecidomyiidae - Diptera). This was described as a new galling species in another study for *P. icicariba* (Maia *et al.* 2002) and was comparatively discriminated from other galling cecidomyiid species (Rodrigues e Maia 2010).

Wasps were not still described as galling inducers for leaflet in the genus *Protium* L. Studying plants of the vegetation of sandbanks, Maia (2001, 2013) had reported the occurrence of eight gall morphotypes associated with *Protium*: two types in

Protium brasiliensis, two in *Protium heptaphyllum*, and four in *P. icicariba*. However, in none of them a wasp species was cited as being involved in the galligenous process. Even when hymenopterous galls were reviewed for South America, nor even Burseraceae, the family to which species of the genus *Protium* L. belong, was listed as host plants (Maia 2012).

Species of Hymenoptera are better known as parasitoids individuals, but few of them are described as galling inducers (Cornell 1983), belonging to families such as Tenthredinidae (sawflies), Cynipidae (gall wasps), Agaonidae (fig wasps), Braconidae, Eurytomidae, Eulophidae, but others families may also be found (Felt 1940, Maia 2012). Particularly in South America, galling species of Hymenoptera comprise eight families, such as Agaonidae, Cynipidae, Eulophidae, Eurytomidae, Figitidae, Pteromalidae, Scelionidae, and Tanaostigmatidae (Maia 2012). Among them, Cynipidae induces galls in a parasitic-like relationship with the host plant (Ronquist and Liljeblad 2001), while Agaonidae galls flowers of plants of genus *Ficus*, which species they pollinate (Cook and Rasplus 2003).

The involvement of hymenopteran species in gall-inducing process does not characterize a rare event. In fact, species of the family Cynipidae (Hymenoptera) represent the second largest adaptative radiation of galling insects, after the gall midges from the family Cecidomyiidae (Diptera), and they are one of the most complex and well-organized induced galls (Cornell 1983). The most commonly reported galls induced by cynipid wasps occur in roses and oaks, but they also are hosted by herbs, particularly those ones belonging to the plant families Asteraceae, Lamiaceae, Rosaceae, and Papaveraceae (Ronquist and Liljeblad 2001).

The hymenopteran species found here was a wasp belonging to the superfamily Chalcidoidea (Mason 1993) and presents characteristics of the family Eulophidae, subfamily Tetrastichinae (Gibson 1993). Despite of the biology is still unknown for the majority of the species, the eulophids use a wide variety of habitats. Most species are parasitoids, feeding on other insects or spiders during their larval stages. Another large group of eulophids attack insects or their larvae concealed in plant tissue, such as leaf-miners, gall-formers, and leaf-rollers. A small group of eulophids are phytophagous, mostly in plant-galls induced by other organisms, but in some cases galls are induced by the eulophids themselves (Hanson and Gauld 1995 and 2006), mainly among Tetrastichinae (Gibson 1993). In fact, larval phytophagy occurs in several distant related taxa in Chalcidoidea and this habit must have evolved separately several times (Askew 1984).

No other arthropod species could be found as a previous gall inducer, even seeking on the earlier stages of gall induction. However, considering the previous report of a cecidomyiid for this gall morphotype in *P. icicariba* (Bregonci *et al*. 2010, Maia 2013), it is possible to propose three hypothesis to explain the role of the wasp species in this gall morphotype: 1. a parasitoid on the cecidomyiid species reported as gall inducer; 2. an inquiline of cecidomyiid gall chamber after eclosion of the dipteran adult; and 3. the gall inducer itself.

If hypothesis of parasitism and of inquilinism on a cecidomyiid gall inducer are considered, it is equally important to take in count that the first, the second and the third larval instars are phytophagous (Figure 6b-d), feeding on the internal zone that delimits the gall chamber (Figures 6f and 7a), where it was possible to find points of loss in cuticular thickness (Figure 6a and 6c). Despite of a pattern closer to an exophytic rolling gall (Figures 3a-b, 4 and 5) in the leaflets of *P. icicariba*, the usually reported galls induced by cynipid gall-forming wasp are typically endophytic (Cornell 1983). However, the plant tissue zonation found in the gall of *P. icicariba* (Figure 5c-d and Figure 6a-c) represent a general and common feature for wasp-galls (Askew 1984).

Despite of the different anatomical structure pattern induced by the endophytic cecidomyiid's and the exophytic psyllid's gall reported by Ramalho and Silva (2014), these three gall morphotypes showed remarkable changes in the mesophyll structural pattern that were developed after the differentiation of mature tissues, which can be seen when the mesophyll structures is sequentially studied in galled leaflets (Figures 4 and 5). No matter if a parasitoid or an inquiline, the activity of the development of this wasp inside the gall keep on induction of mesophyll changes, mainly the sclerification of the outer gall wall, up to the adult was eclosion. Sometimes it was assumed that some auxin-like substance in the larval salivary secretion could be involved in galling process, however, no effective evidence was arisen to support this assumption (Askew 1984).

If the wasp species be assumed as the galling inducer, what is possible in Tetrastichinae (Gibson 1993), the main challenge is to understand how the induction was possible, since this gall starts after the complete differentiation of mesophyll tissues, even for the juvenile yellow-greenish leaflets (Figures 3a and 4a), as well as for the dark-greenish mature ones (Figures 3a and 4b). The loss of differentiation of palisade and spongy parenchymas into bands of slerenchyma and fundamental parenchyma may be a derived character of galling inducer that involves a deep modification in leaflet mesophyll of *P. icicariba*. In evolutionary terms, these facts may be understood as a reciprocal adaptation involving the host plant and the galling inducer allows to understand the galls as extended phenotypes of galling insects, due to their influence and control over plant tissues during gall ontogeny (Dawkins 1982, Stone e Schönrogge 2003, Raman *et al*. 2005, Schoonhoven *et al*. 2005).

However, if the wasp species were the gall inducer, it is necessary to clarify how the induction starts, since so remarkable tissular changes must involve meristematic plant cells (Askew 1984, Weis and Abrahamson 1985, Price 2005, Raman 2011). Even though in the developmental stage that the leaflets are when the gall starts, the apparent absence of meristematic cells may be surpassed by totipotency of parenchyma, even a so differentiated one like palisade parenchyma (Vasil and Vasil 1972), tissues that have been used for callus induction in vegetative reproduction through cell culture (Vasil and Vasil, 1972,Leakey 1985). This fact reinforces the gall-inducer control on host plant tissues, as should be expected for the understanding of the gall structured as an extended phenotype of the galling insect species (Dawkins 1982, Askew 1984, Raman *et al*. 2005).

The tissue zonation of mesophyll that delimits the gall chamber (Figure 4e-f) into an external band of more rigid and sclerified cell layers (Figure 5c). This bands becomes harder as the larvae develops into adult, assuming a protective role (Askew 1984). In the inner zone that is closer to the gall chamber, the reorganization of vascular bundles, mainly composed by phloem (Figure 4f) and the presence of a cellulosic-wall fundamental parenchyma compose a system that may be characterized as a symplastic way of transference of water and hydrosoluble substances available in fundamental parenchyma (Evert 2005), allowing larval feeding on its tissues. In an anatomical study of epidermis in *Pistacia terebinthus* galled leaves, induced by three aphid different species had shown that the multiseriate epidermis observed in the three galls represented a structural barrier to outer water input, creating a dry and hydrophobic environment that was crucial to galling insect survival (Álvarez *et al.* 2009).

In this paper we could not conclude whether this wasp species is a parasitoid, a gall-inducer or a gall-inquiline, and this is not the only galling case that must be better studies (Raman 2007). An eventual role as a parasitoid or as an inquiline species demands an intensification in seeking for the cecidomyiid previously reported as inducer for this rolling gall in the margins of leaflets of P. icicariba. If it is the gall-inducing species, the ontogenesis of gall wall must be clarified.

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