



<http://dx.doi.org/10.11646/zootaxa.3861.5.6>

<http://zoobank.org/urn:lsid:zoobank.org:pub:66573913-A017-4C35-93D3-8C6D0A71E754>

The histo structure of galls induced by aphids as a useful taxonomic character: the case of *Rectinasus* (Hemiptera, Aphididae, Eriosomatinae)

RAFAEL ÁLVAREZ^{1,7}, PILAR MOLIST², SILVIA GONZÁLEZ-SIERRA³,
JEAN JACQUES ITZHAK MARTINEZ^{4,5} & JUAN M. NIETO NAFRÍA⁶

¹Departamento de Biología Molecular-Biología Celular, Universidad de León, León, Spain

²Department of Functional Biology and Health Sciences, University of Vigo, Vigo, Spain

³Laboratorio de microscopía, Centro Nacional de Investigación sobre la Evolución Humana (CENIEH), Burgos, Spain

⁴Dept. Zootechnology, Faculty of Sciences and Technology, Tel Hai College, Israel

⁵Animal Ecology Lab, Migal, Galilee Research Institute LTD, POB 831 Kiryat Shmona, 11016, Israel

⁶Departamento de Biodiversidad y Gestión Ambiental, Universidad de León, 24071 León, Spain

⁷Corresponding autor. E-mail: ralvn@unileon.es

Abstract

Morphological differentiation of gall tissues induced on plants may play a role to characterize the real taxonomic position of the gall inducer. We verified this hypothesis with galls induced by *Rectinasus buxtoni* on *Pistacia palaestina*. There is controversy about the taxonomic localization of genus *Rectinasus*: in one classification it is situated with the genera *Forda* and *Paracletus* while in another it is linked to the genera *Geoica* and *Baizongia*. Histological examination of the walls of the galls reveals the presence of two opposed vascular bundles and an inner surface of the gall with cavities. These features place *Rectinasus* in the same group as *Geoica* and *Baizongia*, and not with *Paracletus* and *Forda*, whose galls have a different histological structure, as generally admitted.

Key words: *Rectinasus buxtoni*, gall, histology, Eriosomatinae, taxonomy

Introduction

Gall morphology and anatomy can be used in insect taxonomy, considering that galls may play the role of an extended phenotype of the inducer insect (Stone & Schönrogge 2003). This was assumed in particular with gall inducing aphids (Stern 1995). For example in galls created on *Pistacia* trees by aphids of the superfamily Aphidoidea, the structure of the modified leaflet is diverse, in accordance with different gall shapes, under the influence of the inducer. In summary: (1) the walls of the galls induced by *Paracletus* and *Forda* have a single vascular bundle (phloem – xylem) and the inside is dimpled and without cavities (Álvarez *et al.* 2009); (2) the walls of the galls induced by *Geoica* and *Baizongia* have two vascular bundles facing each other (phloem – xylem / xylem – phloem) and the interior of the galls has conspicuous cavities (Álvarez 2012).

Two classifications of superfamily Aphidoidea are currently used, which have been named the “Remaudière, Stroyan and Quednau extended” and the “Heie and Wegierek revised” classifications by Nieto Nafría and Favret (Nieto Nafría *et al.* 2011). They mainly differ from each other in the ranks given to several family-group taxa, and also in the groups of some of them. A taxon structured around *Forda* (von Heyden, 1837) with a taxonomic width of 18 genera is present in both classifications: the tribe Fordini Acloque, 1897 (Aphididae Eriosomatinae) in the first mentioned classification and the subfamily Fordinae Acloque, 1897 in the second one, which is placed in Eriosomatidae (Favret 2013).

In the “Heie and Wegierek revised” classification the subfamily Fordinae is divided into two tribes: Melaphidini Baker (A.C.), 1920 and Fordini. The latter tribe is divided into three subtribes: Fordina, Baizongiina Börner, 1944 (1914) and Geoicina Mordvilko, 1921 (Heie & Wegierek 2009). In the “Remaudière, Stroyan and Quednau extended” classification the tribe Fordini is not divided, although Remaudière used subordinated taxa,

and the tribe Fordini could be divided into two subtribes without epistemological problems, Melaphidina and Fordina (Remaudière & Remaudière 1997). The current diagnoses of Fordini, Baizongiina, Geoicina and Fordina (according to Heie and Wegierek) are derived from those established by Börner (Börner 1952), who used characteristics of genera present in Central Europe. If these diagnoses are used then: Fordina includes *Forda* von Heyden, 1837, *Paracletus* von Heyden, 1837, *Rectinasus* Theobald, 1914, *Smynthurodes* Westwood, 1849, and *Tramaforda* Manheim, 2007; Baizongiina contains *Aloephagus* Essig, 1950, *Aploneura* Passerini, 1863, *Asiphonella* Theobald, 1923, *Baizongia* Rondani, 1848, *Geopemphigus* Hille Ris Lambers, 1933, and *Slavum* Mordvilko, 1927; and finally Geoicina includes *Geoica* Hart, 1894, and *Chaetogeoica* (Remaudière & Tao 1957).

The taxonomic position of *Rectinasus* in Fordinae, which had already been exposed by Börner (Börner 1952) was considered doubtful by Remaudière and Davatchi (Remaudière & Davatchi 1956) and was rejected by Remaudière and Tao (Remaudière & Tao 1957) and Davatchi (Davatchi 1958) who included this genus together with *Geoica* and *Chaetogeoica* in Geoicina. The reasons adduced are morphological, but also bionomical: in *Geoica*, *Chaetogeoica*, and also in *Baizongia* and related genera, the fundatrix induces a definite gall, which is closed and more or less bag- or blister-shaped, whilst in *Forda* and its related genera the fundatrix induces a provisional gall, and the fundatrigeniae induce a definitive gall, which is fold-shaped with a long and partially occluded slit.

There are some molecular studies about aphids (for example Zhang & Qiao (2008), Ortiz-Rivas & Martínez-Torres (2010)) but none refers to the genus *Rectinasus*.

Microscopic study of the plant traits transformed in the galls by *R. buxtoni* on *Pistacia palaestina* may provide useful data to establish the exact taxonomic position of the genus.

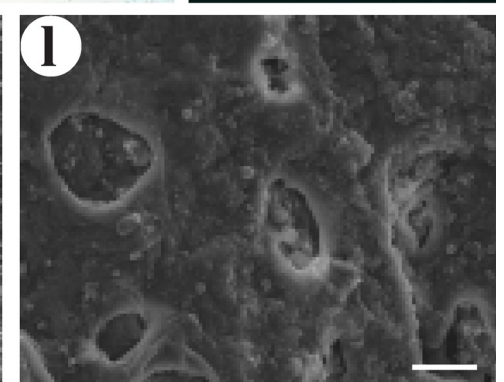
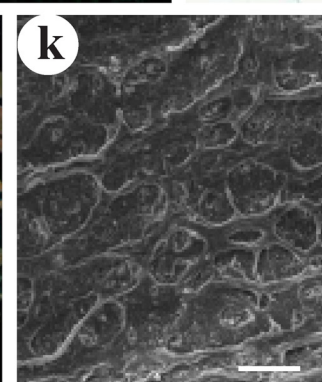
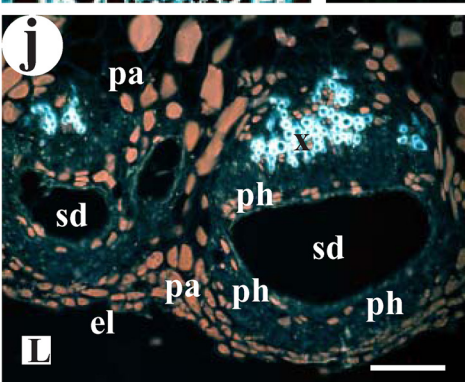
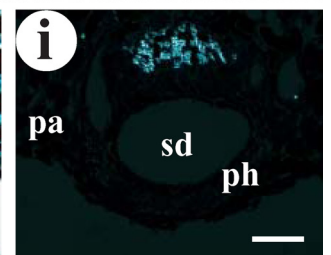
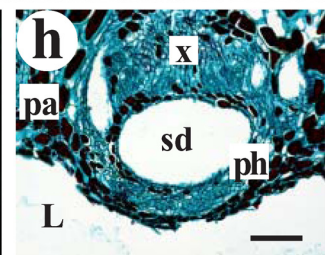
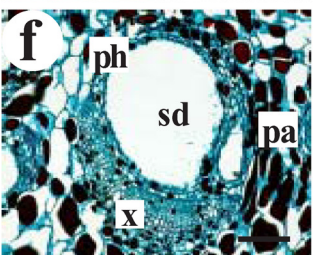
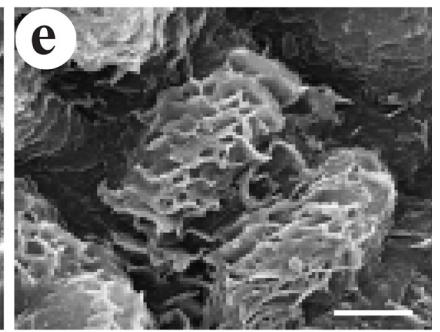
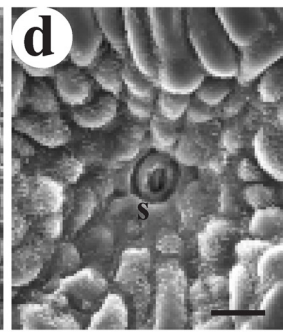
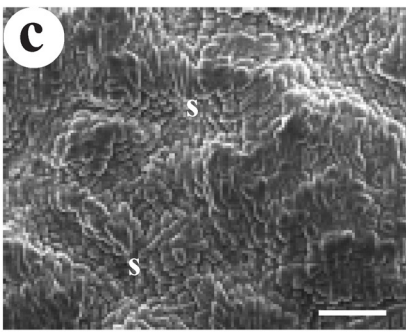
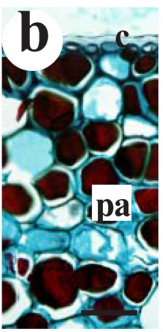
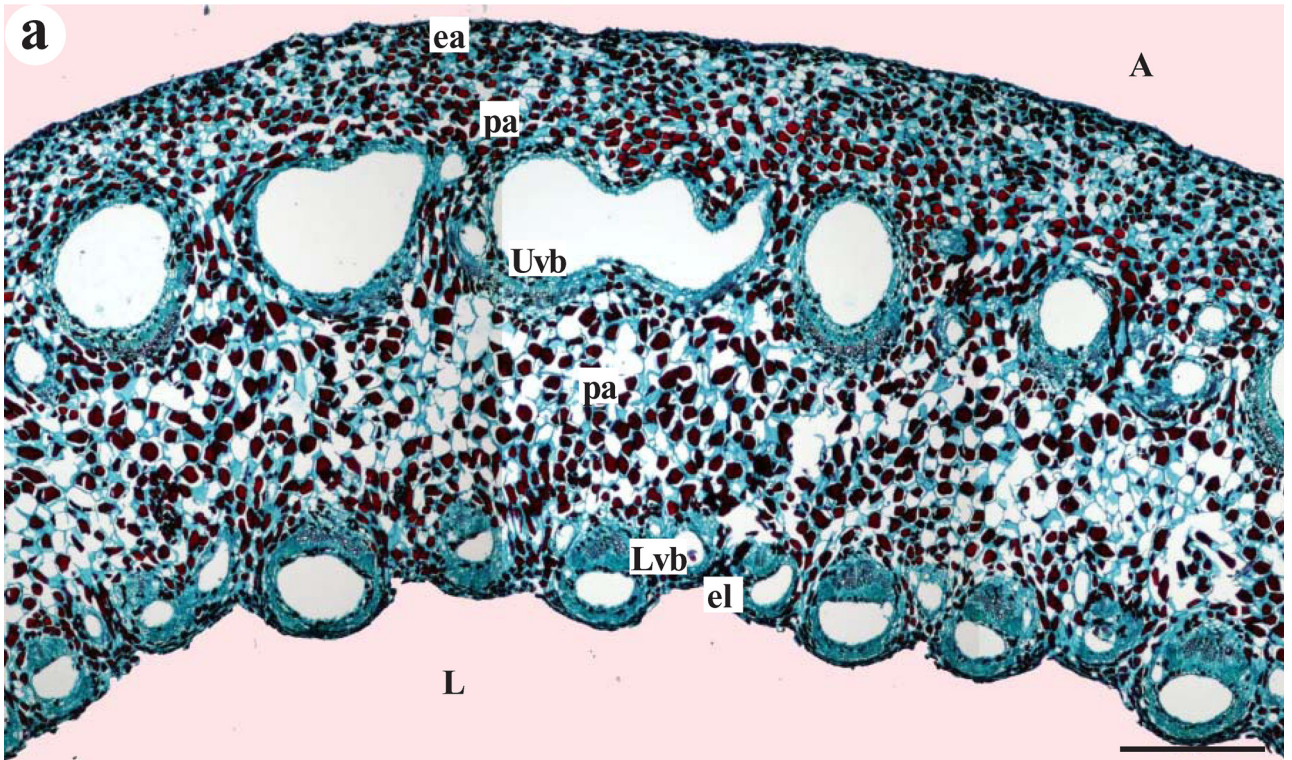
Material and methods

The study included 3 galls induced by *Rectinasus buxtoni* on *Pistacia palaestina*, collected in Baram (Israel) in August 2012. Samples were opened with a scalpel and fixed in formaldehyde, acetic acid and ethanol (FAA). Wall fragments of about 0.5 cm² were taken, embedded in Paraplast, and cut on a rotatory microtome (12µm thick sections). Some sections were stained with Safranin-Fast Green and mounted permanently on microscope slides. Other sections were mounted permanently on microscope slides without prior staining. The sections were examined by bright-field microscopy. The unstained sections were studied with a polarized light microscope and an epifluorescence microscope. Other wall fragments were dehydrated and coated with gold for observation under a scanning electron microscope (SEM).

Result

Going from the outside to the inside of the gall, the wall of the gall induced by *R. buxtoni* shows (Fig. 1a):

- Uniseriate epidermis with a thick cuticle and a few stomata (Fig. 1b–d). The epidermal cells present epicuticular waxes (Fig. 1e).
- Storage parenchyma. Many of its cells show tannin inclusions (Fig. 1b). This is observed in this parenchyma located immediately below the epidermis and in the remaining wall parenchyma.
- Upper vascular bundle (Fig. 1f–g). The phloem is oriented towards the outside and the xylem towards the inside of the gall.
- Storage parenchyma.
- Lower vascular bundle (Fig. 1h–i). The xylem is oriented towards the outside of the gall and the phloem is oriented towards the lumen of the gall. All vascular bundles are closed collateral vascular bundles (Fig. 1j). The phloem presents schizogenous ducts.
- Storage parenchyma.
- Interior surface without a defined epidermis, and consequently no cuticles were observed on the inner surface of the galls. Dimples are observed (Fig. 1k–l).



Discussion

Microscopic examination of the wall of the gall induced by *R. buxtoni* reveals the presence of two opposed vascular bundles. Specifically, the upper vascular bundle presents the xylem oriented towards the lumen of the gall, whereas the lower vascular bundle is observed in a mirror arrangement with respect to the former. In other words, the xylem faces the xylem of the upper vascular bundle, and the phloem is oriented towards the lumen of the gall.

The vascular bundles are always closed collateral vascular bundles, similar to the vascular bundles present in the leaflets of *Pistacia* and in various aphid-induced galls in these leaflets (Álvarez *et al.* 2008; Álvarez *et al.* 2009; Álvarez, 2012). The presence of schizogenous ducts associated with the phloem is a general characteristic of both the family Anacardiaceae (Simpson 2006) and the genus *Pistacia* (Álvarez *et al.* 2008). Chian turpentine, a resin, circulates through these ducts. This resin, along with abundant tannins present in the leaflets of the genus *Pistacia*, is a deterrent to the chewing of the leaves by herbivores (Costa *et al.* 2001).

The arrangement of the vascular bundles of *R. buxtoni* is similar to that observed in the walls of the galls induced by *Geoica utricularia* and *Baizongia pistaciae* (Álvarez 2012). This feature distinguishes these galls on a net basis from those induced on *Pistacia* leaflets by *Paracletus cimiciformis*, *Forda marginata* and *F. formicaria*, which present a single vascular bundle (Álvarez *et al.* 2009).

At the same time, galls induced by the first group mentioned (*Rectinasus*, *Geoica* and *Baizongia*) present another characteristic common to all 3 species, which again differentiates them from the second group (*Paracletus* and *Forda*). In the first group, the inner surface of the gall shows cavities (Álvarez 2012), while in the second group this surface has a dimpled appearance without any cavities (Álvarez *et al.* 2009). The presence of these cavities can be interpreted along the lines of Kutsukake *et al.* (2012) as an adaptation of the species that induce closed galls, and which allows them to totally or partially remove honeydew from the inside of the galls to the vascular tissues of the plant.

Macroscopic differences are also apparent. On the one hand, the galls of *Paracletus* and *Forda* are formed as a fold of the distal portion of the leaflets. On the other hand, galls of *Rectinasus*, *Geoica* and *Baizongia* are globose galls, are larger, and are associated with the midvein of the leaflets (Inbar 2006). This relationship takes shape in the case of *Geoica* and *Baizongia*, where the aphid is interested in the vascular bundle of the leaflets and also in their small supernumerary vascular bundles (Álvarez *et al.* 2008). It is these two – hypertrophied – vascular bundles that are observed in the walls of said galls (Álvarez 2011). Grouping *Rectinasus* with *Geoica* and *Baizongia*, which is evident in the histological study, agrees with one of the taxonomic groupings presented and is in disagreement with the other such as it has been indicated in the Introduction section: following the classification of “Remaudière, Stroyan and Quednau extended” *Rectinasus* is linked with *Geoica* and *Baizongia*; and following the classification of “Heie and Wegierek revised” *Rectinasus* is linked with *Forda* and *Paracletus*. Thus, the histological study presented here supports the classification of “Remaudière, Stroyan and Quednau extended”.

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FIGURE 1. Galls induced by *Rectinasus buxtoni*. Microscopic structure. a Transverse section of the wall of the gall. Two vascular bundles are observed, one closer to the outside (Uvb) and the other closer to the inside of the gall (Lvb). Specifically, going from the outside (A) to the lumen (L) of the gall, the following is observed: epidermis-air (ea), storage parenchyma (pa), upper vascular bundle (Uvb), storage parenchyma, lower vascular bundle (Lvb), and epidermis-lumen (el). b–e Epidermis-air. b Uniseriate epidermis-air with thickened cuticle (c). Below storage parenchyma (pa) is observed, many cells of which have tannin inclusions. c View of the outer surface the gall. Between the epidermal cells a few stomata (s) are observed. d Detail of the foregoing. A stoma (s) and the presence of epicuticular waxes are observed in the epidermal cells. e Detail of the epicuticular waxes. f–i Vascular bundles. The vascular bundles are seen to be facing each other: the xylem of the upper bundle f–g is facing the xylem of the lower bundle h–i. f–g Upper vascular bundle. Xylem cells in f are the only ones that polarize light in g. h–i Lower vascular bundle. Xylem cells in h are the only ones that polarize light in i. The phloem is oriented towards the lumen (L) of the gall. j Detail of a vascular bundle: Closed collateral vascular bundle. Very characteristic of the vascular bundles of *Pistacia* sp. (and of the galls) is the presence of intraphloematic schizogenous ducts (sd). k–l Interior surface of the gall. k The interior surface of the gall has conspicuous dimples. l Detail of the foregoing. a b f h Safranin-Fast Green. Bright-field microscope. c d e k l SEM. g i Polarized light microscope. j Epifluorescence microscope. Abbreviations: A outside of the gall, c cuticle, ea epidermis-air, el epidermis-lumen, L lumen of the gall, Lvb lower vascular bundle, pa storage parenchyma, ph phloem, s stoma, sd intraphloematic schizogenous duct, Uvb upper vascular bundle, x xylem. Scale bars a 500 µm; b 50 µm; c f–k 100 µm; d l 20 µm; e 5 µm.

This disagreement about the taxonomic position of *Rectinasus* invites to conduct a thorough review of the genus and perhaps, in general, of all the Fordinae and even the Eriosomatinae (according to Remaudière, Stroyan and Quednau extended). In these future studies, the molecular, populational and morphological aspects of the different forms of the species (fundatrix, fundatrigeniae, virginogeniae, sexuparae) should be considered, as well as the macroscopic and microscopic characteristics of the galls.

Acknowledgements

The authors are grateful to Adoración Candelas for critically reading the manuscript and to Ron Hartong of Teccientifica for translating the manuscript into English. The authors wish to acknowledge the positive collaboration of editor David Ouvrard.

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