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Laticifer distribution in fig inflorescence and its potential role in the fig-fig wasp mutualism

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ABSTRACT

Although in Moraceae the presence of laticifers is considered to be a synapomorphy, little is known about the distribution and morphology of this type of secretory structure in the reproductive organs of its species. *Ficus*, the largest genus of Moraceae, is characterized by an inflorescence known as syconium and by an obligate mutualistic interaction with pollinating wasps. The objectives of the present study were to evaluate the distribution and morphology of laticifers in syconia of 36 species belonging to different *Ficus* sections and to survey traits of taxonomic and adaptive value for the group. Syconia containing flowers in a receptive state were collected, fixed and processed for anatomical analysis. All species studied have branched laticifers distributed in the syconium receptacle, in the ostiolar bracts and in the pedicel of staminate flowers. Almost all species show laticifers in the pedicel of shorter-styled flowers. Laticifers also occur in the pedicel of longer-styled flowers in most *Ficus* sections, except *F. curtipes* (*Conosycea* section) and more than 75% of the studied species of the *Americanae* section. Laticifers are observed in the sepals of 25 of the 36 species studied and occasionally in the pistil. The presence of laticifers in the pedicel of shorter-style flowers and its absence in the pistil suggest that the distribution of this secretory structure in the fig flower was selected by pressures imposed by the fig-fig wasp mutualism. The laticifers in the pedicel of shorter-styled flowers may confer protection to the developing wasp larvae against natural enemies. However, the absence of laticifers in the pistil of most *Ficus* species studied was probably selected by the mutualistic relationship with the agaonid pollinating wasps since the latex could interfere with oviposition through the style, with the larval development of the pollinating fig wasps, and the emergence of pollinator offspring from their galls.

1. Introduction

Laticifer is a structure specialized in the secretion of latex, an exudate composed of several substances such as polyisoprene hydrocarbons, triterpenes, sterols, fatty and aromatic acids, carotenes, phospholipids, proteins, alkaloids, inorganic compounds, etc., dispersed in a liquid with a distinct refractive index (Fahn, 1979; Ascensão, 2007). Structurally, laticifers can be nonarticulated (constituted by a single multinucleated cell which grows with the plant development) or articulated (constituted by a row of several cells whose end walls can remain intact, become porous or disappear completely). Both types can branch out or not, producing a complex system similar to tubes that permeate different tissues of the plant body (Evert, 2006). These structures act in the plant defense against herbivores, since their toxic or repellent content, which is under strong turgor pressure, is readily released to the outside in response to any injury caused to the plant (Fahn, 1979; Agrawal and Konno, 2009; Konno, 2011). In addition, the latex coagulant property acts on the sealing of wounds, preventing the entry of pathogens (Fahn, 1979; Farrell et al., 1991).

In Moraceae, a family with 40 genera and about 1200 species (The Plant List, 2013), the presence of nonarticulated-branched laticifers (Metcalfe and Chalk, 1950; Fahn, 1979; Evert, 2006) is considered to be a synapomorphy (Judd et al., 2009). There are numerous studies on the distribution and morphology of laticifers in vegetative organs of Moraceae species (Tippo, 1938; Vreede, 1949; Milanez, 1954; Topper and Koek-Noorman, 1980; Davies et al., 1982; van Veenendaal and den Outer, 1990; Balaji et al., 1993; Kang et al., 2000; Jacomassi and Machado, 2003; Quintanar and Castrejón, 2004; Jacomassi et al., 2007;

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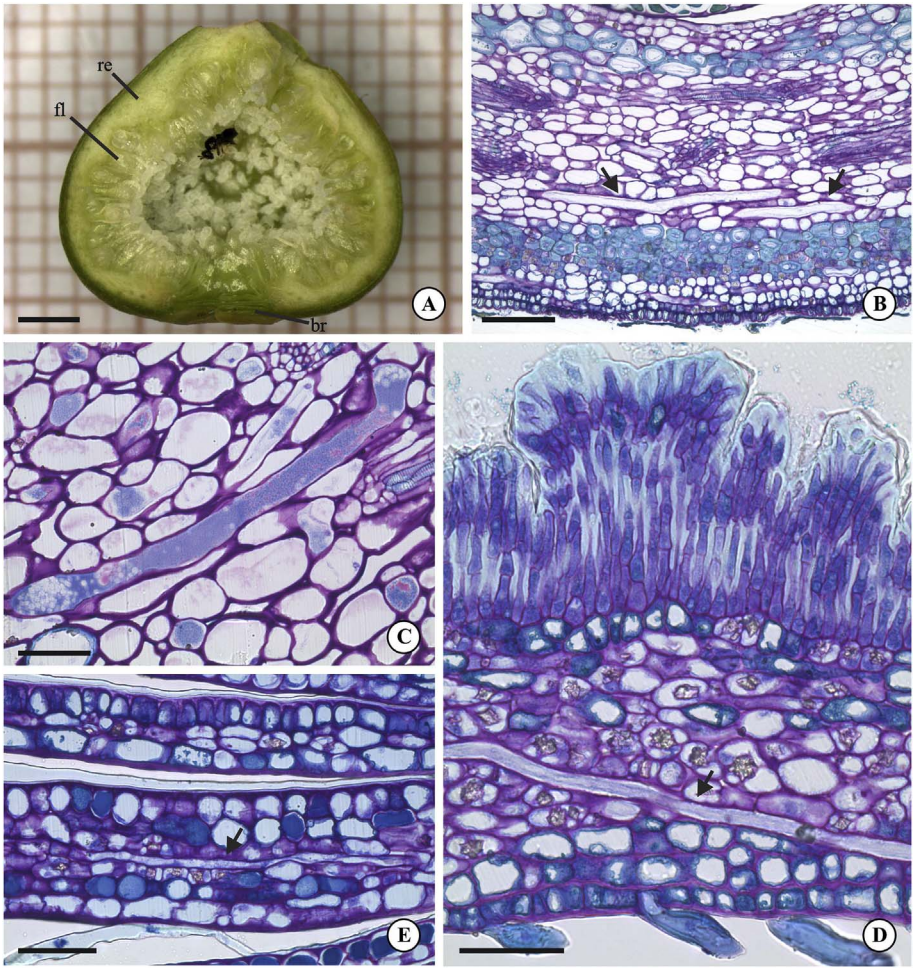

Fig. 1. Photomicrographs of longitudinal sections of *Ficus* inflorescences (*Americanae* section). A: Overview of *F. citrifolia* fresh syconium (image credits M.F.B. Costa). B–C: Laticifer in the syconium receptacle of *F. clusiifolia* (B) and *F. mariae* (C). D–E: Laticifer in the outer ostiolar bract of *F. eximia* (D) and in the inner ostiolar bract of *F. pertusa* (E). Staining: Toluidine blue (B–E). arrows = laticifer, br = ostiolar bracts, fl = flower, re = receptacle. Scale bars: 2 mm (A); 100 μm (B); 50 μm (C–E).

Palhares et al., 2007; Ramadan et al., 2008; Duarte et al., 2012; Alonso et al., 2013; Araújo et al., 2014; Bercu and Popoviciu, 2014; Kajii et al., 2014; Sharma et al., 2014); however, studies in reproductive organs are scarce. In organs related to plant reproduction, studies of laticifers have been reported for *Sorocea bonplandii* (Souza and Rosa, 2005), *Brosimum gaudichaudii* (Jacomassi et al., 2010), *Maclura tinctoria* (Oyama and Souza, 2011), and some *Ficus* species (Machado et al., 2013; Subramanian et al., 2013; Souza et al., 2015).

In *Ficus*, 11 out of the approx. 750 described species had their laticifers studied in reproductive organs. Besides being the richest genus of Moraceae, *Ficus* exhibits a wide diversity of habits and geographic distribution, as well as a unique type of inflorescence called syconium (Clement and Weiblen, 2009; Judd et al., 2009). The syconium exhibits an urn-shaped receptacle containing several diclinous flowers, and opens to the outside through an ostiole, which is lined by bracts (Datwyler and Weiblen, 2004; see Fig. 1A). It is through the ostiole that the fig wasps enter the syconium, pollinate the flowers and lay eggs in some of them, ensuring the production of seeds and wasp offspring and setting up a very specialized and one of the most currently studied mutualistic interactions (Galil and Eisikowitch, 1968a; Anstett et al., 1997; Cook and Rasplus, 2003). Female pollinating wasps are attracted to receptive syconia (B phase) by a scent produced in osmophores present on the surface of the syconium and on the ostiolar bracts (Souza et al., 2015). After entering the syconium the wasps oviposit while they pollinate the pistillate flowers. The oviposition generally occurs in flowers with ovaries located closer to the fig cavity and therefore with longer pedicels and shorter styles. In this process, the pollinating wasp introduces its ovipositor through the style and deposit an egg between the inner integument and the nucellus. The flowers with the ovary closest to the syconium receptacle, with a shorter pedicel and a longer style, form the seeds. The emergence of the wasp offspring coincides with the anthesis of the staminate flowers; thus, the female wasps leave the fig loaded with pollen and ready to restart the cycle in a new syconium (Galil and Eisikowitch, 1968a).

The role of scent in the fig fig-wasp mutualism (Grison et al., 1999; Grison-Pigé et al., 2002; Dudareva and Pichersky, 2006; Hossaert-McKey et al., 2010) and its associated glands (Souza et al., 2015; Machado et al., 2013) is better known than that of the latex and, consequently, of the laticifers, probably due to the higher concentration of studies on the pollination biology of fig trees (Galil and Eisikowitch, 1968a; Anstett et al., 1997; Cook and Rasplus, 2003) than on the commensal or parasitic microfauna of the mutualism (Cruaud et al., 2011).

Considering the ecological role of *Ficus* syconium and the scarcity of information about the protective glands in these inflorescences, we compared here the distribution and morphology of laticifers in syconia of 21 *Ficus* species belonging to the *Americanae* section and 15 other species belonging to nine additional sections, namely *Conosycea*, *Dammaropsis*, *Ficus*, *Galoglychia*, *Pharmacosycea*, *Sycidium*, *Sycocarpus*, *Sycomorus* and *Urostigma*. Traits of taxonomic and adaptive value were surveyed for the group. Evolutionary aspects were also addressed using the phylogeny published by Cruaud et al. (2012) as reference.

2. Material and methods

Syconia at the receptive phase (B phase) of 36 species of *Ficus* (Table 1) were collected and fixed in FAA_{50} (Johansen, 1940) or neutral buffered formalin (Lillie, 1965). Vouchers were deposited in the HITBC,