Syconium development in Ficus petiolaris (Ficus, sect. Americanae, Moraceae) and their relationship with pollinator and parasitic wasps.

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Syconium development in *Ficus petiolaris* (*Ficus, sect. Americanae, Moraceae*) and their relationship with pollinator and parasitic wasps.


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Abstract

For the first time in a Neotropical Ficus species (F. petiolaris), the external and internal structural changes in its syconia are described together with the development of its fig wasps. Ficus petiolaris is endemic to Mexico and represents the northernmost limit of the geographical distribution of the genus in America. Considering the large variation in syconium morphology, we evaluate whether there are differences between syconium development in F. petiolaris and that described for Paleotropical species. We recorded the development of 428 syconia in two individuals, weekly for a period of five months, from initiation to maturity, as well as that of their associated insects. The time required for completion of syconia development ranged from 91 to 126 days. The external morphology of the syconia (color, ostiole size and thickness of syconium wall) enabled phase recognition. Male and female of pollinators (Pegoscapus sp.) and parasitic wasps (Idarnes sp.) were registered. High seed germination values (50-90 %) tested indirectly the positive role of pollinators. Our results are similar to those reported in Paleotropical Ficus species and should contribute to our understanding about syconium development as well as to the relationships that they maintain with their Agaonidae wasps.

Key words: Fig wasps, Phenophases, Seed germination, Seedlings, Syconium morphology.
**Introduction**

The genus *Ficus* (Moraceae) is characterized by its peculiar inflorescence-infrutescence with one apical aperture known as ostiole (the fig or syconium), which is commonly referred to as a “fruit”, while the true fruits (achenes or drupes depending on authors) as “seeds” (Verkerke 1989). The syconium is an ellipsoid to spherical receptacle, which encloses structurally and functionally unisexual flowers, as well as the fruits (Berg 1990). Within the monoecious species (Berg 1989), trees produce protogynous syconia which contain two types of female flowers that differ in style length (short-styled and long-styled flowers) and male flowers. In contrast, in gynodioecious species, plants produce either “gall-syconia”, with male and short-styled female flowers (male individuals) or “seed syconia”, generally only with long-styled female flowers (female individuals) (Berg and Wiebes 1992; Weiblen 2001). Mature syconia represent a food resource for many vertebrate frugivores, particularly birds and mammals (Shanahan et al. 2001; Ragusa-Netto 2002; Domínguez-Domínguez et al. 2006; Lomáscolo et al. 2010). Members of this genus are therefore considered key components for the maintenance of tropical forest diversity (Janzen 1979; Shanahan et al. 2001; Harrison 2005).

*Ficus* species are also notable for the completely dependent mutualistic relationship they maintain with their pollinator wasps (Hymenoptera, Chalcidoidea, Agaonidae). The wasps, in turn, require the syconium in order to grow and reproduce (Ramírez 1974; Weiblen 2002; Harrison 2005). Originally, this interaction was considered a model of strict coevolution, where each species of *Ficus* had its specific pollinator (Janzen 1979; Jousselin et al. 2003; Ronsted et al. 2005; Herre et al. 2008). However, there is now evidence of some *Ficus* species being pollinated by more than one wasp species and, equally, one species of these insects can act as a pollinator for more than one species of *Ficus* (Ramirez 1970; Michaloud et al. 1996; Cook and
Rasplus 2003; Harrison 2005; Lin et al. 2011). Moreover, there are five subfamilies of non-pollinator wasps (Epichrysomallinae, Otitesellinae, Sycoecinae, Sycophaginae and Sycoryctinae), with life cycles that are also strictly associated with the syconia and can be parasites of the pollinator wasps or of the ovaries of female flowers (Bouček 1993; Cruaud et al. 2011).

Diversity in the genus *Ficus* is estimated to be around 750 species, which are found in the Neo and Paleotropical areas of the world (Berg 1989). Despite the high number of species described in *Ficus* and the notable variation that exists in the structure, color, shape or number of flowers of their syconia, developmental phases have been characterized only in few species and typically categorized from A (initiation of syconia) to E (mature syconia) phases (Galil and Eisikowitch 1968a; Smith and Bronstein 1996). A total of 22 species of *Ficus* have been reported in Mexico, of which three are endemic (Ibarra-Manríquez et al. 2012). One of these endemic species is *F. petiolaris* Kunth, which also represents the northernmost distributional limit of the genus in America. Its monoecious tree grows exclusively on rocky substrates (Fig. 1), mainly in the tropical dry forest (Ibarra-Manríquez et al. 2012). Detailed ecological information about the reproductive aspects of this species (e. g., syconium phase duration, records about their pollinator and parasitic wasps, which animals could disperse their syconia or data about seed germination), are virtually unavailable.

The main objectives of the present study are therefore to: i) describe and illustrate the main changes, both external and internal, in the developmental phases of the *F. petiolaris* syconia and ii) determine the duration of each phase. Given that the development of the syconium of any species of *Ficus* cannot be adequately understood without also considering the development of its wasp community (Galil and Eisikowitch 1968a), another important goal of this study is characterize general aspects
of the development of the wasps associated with *F. petiolaris*. We also wish to provide evidence of the activity of bats, which are likely to be their seed disperser, as additional indicators of the phase E. Finally, we aim to estimate the seed viability, in order to indirectly evaluate the effectiveness of the pollinator wasps and further our knowledge of the seedling morphology. Considering the findings reported for paleotropical species, we expected to find characters in the syconium external structure (e.g. color and size) with which to recognize internal developmental changes that occur throughout its different development phases, particularly in the female (B) and dispersal (E) phases.

**Materials and methods**

*Selection of *F. petiolaris* trees*

Observation of the main external and internal changes in the syconia was conducted in two individuals of *F. petiolaris* located in Morelia city, Michoacán, Mexico. The first individual (A) was located in the gardens of the Comisión Forestal del Estado de Michoacán (19°41’50.15” N, 101°10’53.45” W). The individual (B) is in the Botanic Garden of the Instituto de Investigaciones en Ecosistemas y Sustentabilidad, Morelia campus, of the Universidad Nacional Autónoma de México (19°38’56.23” N, 101°13’47.21” W). It had been planted in the spring of 2011 by one of the authors (GI-M), and originally came from a seasonally dry forest in the La Huacana municipality, Michoacán. Vouchers of both monitored individuals will be deposited at MEXU and MO herbaria (Piedra-Malagón 618, 619).

*Development of syconia in *F. petiolaris***

The syconia were measured and monitored in both trees, from initial development (phase A1) to maturation (phase E). The recording frequency was weekly and covered the period from July to November 2017. Twenty branches with floral buds were selected (15 branches in A and 5 in B individuals) and marked once the development of
buds began and differentiation of the syconium structure became evident. Each syconium was assigned a key for identification and its growth and any external morphological changes recorded. Simultaneously, syconia of equivalent size and color to those of the marked syconia were collected from other non-marked branches. We measured and dissected these syconia in order to record the internal characteristics and accurately identify their developmental phase based on the proposal of Galil and Eisikowitch (1968a, b), while including the modifications of phases A and C suggested by Smith and Bronstein (1996). In total, eight attributes were recorded in the collected syconia and seven in the marked syconia (Table 1, Fig. 2). The difference in the number of characteristics recorded between both groups was due to the fact that manipulation of the marked syconia had to be minimized in order not to affect their normal development. In total, 48 syconia were marked (12 IA, 36 IB) and 380 syconia were dissected in different phases. All measurements were recorded using a digital vernier (Mitutoyo, Absolute model (CD-6” CSX)), accurate to 0.01 mm and with instrumental error margins of ± 0.02 mm (≤ 200 mm) and ± 0.03 mm (> 200 mm). To assess whether the syconia measurement values differed statistically among phases, we applied a repeated measures ANOVA, with a Tukey post hoc test, using SPSS 22 (IBM Corp 2013).

Fig wasps material

Fig wasps were collected from both individuals observed. All wasps were collected from closed syconia in phase D, placed in sealable plastic bags to allow wasps emerge naturally and then, with a fine brush, stored in alcohol 70 %.

Scanning electron microscopy

Floral syconium structures and the associated fig wasps were observed and photographed under a JEOL JSM-IT300 LV scanning electron microscope (SEM). In
To avoid charge effects, the samples were observed in low vacuum mode, at between 20 and 50 Pa depending on the sample. Micrographs obtained by SEM were pseudocolored in Adobe Photoshop CS6, using the color substitution tool in order to highlight the floral syconium structures as well as the fig wasps.

Germination of seeds in F. petiolaris

Syconia in phase E were collected from each individual (2 syconia from individual A and 4 from individual B). We extracted 20 “seeds” of each syconium and placed on filter paper in Petri dishes, in order to evaluate if seeds were viable. The dishes were then placed in a germination chamber at 25 °C and the “seeds” watered every third day. Germination (radicle emergence) was verified daily (n = 120 seeds).

Results

Development of syconia and fig wasps in F. petiolaris

Phase A1 (Differentiation of structures; duration 21–28 days). Like in all other species of the subgenus Urostigma, syconia are also initiated here in pairs in the axil of a leaf and begins their development simultaneously as two green ellipsoid buds, each protected by a pale green bract (Fig. 3). The first structure to differentiate in the interior of each syconium is the ostiole, which is composed of a series of overlapping bracts, with no clear differentiation of the syconium cavity. However, there is a slight widening in its lower part that, in future phases, gives rise to the collar of the syconium and the peduncle. As the syconium continues its development, the bracts of the buds acquiring a brown color, separate and drop. Around the 15th day, the syconium is as wide as its collar and maybe covered with yellow to red macules, depending on the level of insolation to which it has been exposed (Table 1, Fig. 3).

Phase A2 (female; 7–21 days). In this phase, important growth of the syconium occurs with respect to the previous phase (Table 1, Fig. 3). The basal bracts of the syconium
become more visible due to widening of the receptacle and may change in color from green to brown. The macules increase in size and their outline is more defined. The stigmas of the female flowers are observed with a uniform distribution in the cavity of the receptacle; the collar of the receptacle, the peduncle and the ostiole are clearly delimited. In some syconia, it was possible to observe wasps in different phases of development, mainly around the ostiole and in ovaries close to the collar of the syconium, as is the case in phase C1 or C3. All of these emerged wasps belonged to the genus *Idarnes* sp. (non-pollinators). The females presented red eyes, an ovipositor that exceeded their body length and a metallic green exoskeleton (Fig. 4).

**Phase B (pollination; 1–7 days).** Given its short duration, this phase is difficult to observe. Externally, a similar morphology to that described in phase A2 is presented in terms of coloration and size (Table 1). However, unlike in A2, the syconium has a more defined globular form and the ostiole is slightly sunken (Fig. 3). Strictly, this phase begins with the arrival of the female wasps (*Pegoscapus* sp., Fig. 4), which enter the syconium through the bracts of the ostiole. When this event has recently taken place, it is possible to observe the wings inserted between the internal bracts of the ostiole or between its most external bracts (Fig. 4). Once within the syconium, the female wasps loaded with pollen (Figs. 4) pollinate the female flowers and oviposit on some. The flowers have white stigmas (Fig. 5) and their number range from 394 to 562, with an average of 228 (SD ± 63.06) for those with long styles, and of 247.8 (SD ± 37.83) for those with short styles. The overlap of the ostiole bracts causes the wasps to remain trapped in the syconium cavity and eventually die. It is most common during this stage to find only one wasp (Fig. 3). However, in around of 30 % of the observed syconia, up to three were recorded. Finalization of this phase is recognized when the bracts of the ostiole become more constricted (Fig 5).
Phase C1 (early interfloral; 14–21 days). At this stage of development, the syconium continues increasing in size (Table 1), while the “seeds” and wasps begin to develop (Table 1). The external bracts of the ostiole are slightly flattened. New macules are observed, although of smaller diameter than those observed previously. Inside of the syconium, the cavity is well defined and no difference is detectable between the ovaries that contain the immature seeds and those that contain the wasps. However, due to their loss of receptivity, the stigmas of all flowers acquire a pale brown color (Fig. 3). In some cases, a hyaline and viscous liquid covers the syconium cavity.

Phase C2 (mid interfloral; 14–21 days). The syconium continues growing, particularly the ostiole, and the peduncle reaches its maximum size (Table 1, Fig. 3). However, all of the external changes during phase C are barely perceptible and the change in size is particularly gradual, which complicates differentiation among the C phases. The changes are more evident in the interior. The wasp larvae and “seeds” continue developing, such that the syconium cavity becomes smaller. The flowers that contain the wasps and the “seeds” differ in that the latter are smaller and flatter. Besides, both female flower types also differ in terms of the tonality of the yellow color of the ovaries. The exoskeleton of the progenitor wasp is compressed, dehydrated and can be found in different degrees of decomposition. It is also frequently possible to observe nematodes feeding on the exoskeleton. In other cases, the wasps and the syconium cavity are covered by fungal hyphae and a dark brown hyaline liquid (Fig. 3) partially or totally covers the syconium cavity.

Phase C3 (late interfloral; 14–21 days). The ostiole in this phase appears flattened, becoming a small apical protuberance (Fig. 3). In the interior of the syconium, the wasps and “seeds” have almost completed their development, such that the syconium cavity is reduced markedly. The bodies of the new generation of wasps become visible
through the ovary walls, which acquire a dark coloration, while those that contain the “seeds” are yellowish. The anthers begin to emerge among the remains of the female flowers and the interfloral bracts.

**Phase D (male; 1–7 days).** The syconia increase notably in size and change in color from green to light green and their walls become slightly softer, with pale external macules (Table 1, Fig. 3). Inside, 12 to 45 (mean 26.9, SD ± 9.07) male flowers reach anthesis, and exert their stamens between and above the gall and “seeds” flowers (Fig. 5). The male wasps of the genus *Pegoscapus* sp. are the first to emerge (Fig. 4), breaking the walls of the ovary with their mandibles, copulating with the females and facilitating their exit from this structure. Most of the males remain within the syconium but some excavate a tunnel through the bracts of the ostiole (Fig. 5), which is used by the fecundated, pollen carrying females to leave the syconium. The males non-pollinator wasps of the genus *Idarnes* sp. can be also observed (Fig. 4).

**Phase E (post-floral; 1-7 days).** In this final phase, the syconium decreases faintly in size (Table 1) and acquires a pale green to yellow brown color, with opaque macules (Fig. 3). The mature syconium is completely soft to the touch and a sweet smell can be perceived. The ostiole is almost flattened and compact (Fig. 5). Internally, the structures within the syconium cavity acquire an overall dark brown color. In the ovaries of the female flowers that had been occupied by wasps, the holes through which the wasps emerged can be observed (Fig. 5). The stamens begin to lose turgidity and the “seeds” are now mature (122–214 per syconium; mean 155, SD ± 41.43; n=4) and surrounded by a mucilaginous and hyaline tissue layer (Fig. 5).

**Seed germination in *F. petiolaris***

Seed germination in the studied species, from radicle emergence (started at ninth day) until the presence of foliaceous cotyledons (Figure 1), occurs at between 25 and 30
days, with 67.5 % of the seeds germinating by day 25. Seed germination percentage registered in individual A were 55 and 65 % in two syconia, respectively, whereas in the four compared for individual B they were 50, 60, 85 and 90 %.

Discussion

Syconia development phases in F. petiolaris

The time required of phases A1 to E in the syconia of F. petiolaris ranged from 91 to 126 days. This result agrees with that reported by Verkerke (1989), who reported very wide variation in this trait (between 40 and 181 days). Similarly, Zhang et al. (2006) reported a wide range (around 44–111 days), but it varied among the seasons of the year and was longer during the foggy-cool season. Other studies have indicated a shorter development of between 28 and 55 days (Galil and Eisikowitch 1968b; Baijnath and Naicker 1989; Smith and Bronstein 1996; Zhang et al. 2006). Smith and Bronstein (1996) mentioned that their findings may be explained by the fact that the species were found on the boreal limit of their geographic distribution, which could affect different aspects of their reproductive cycles and those of their Agaonidae pollinators.

Phases A1 and A2 of F. petiolaris also coincide with that described in previous studies (Verkerke 1986, 1988; Zhang et al. 2006) in that the most notable development was the increased size of the syconium, which almost doubled in our study (Table 1). There is also agreement in the differentiation of the ostiole and of the female flowers. However, the duration of these phases in F. petiolaris (4–7 weeks) was greater than the 1.5-4 weeks indicated by both Verkerke (1989) and Zhang et al. (2006). In contrast, the relatively brief duration (1–7 days) of phase B recorded in F. petiolaris coincides with that indicated by these authors.

Phase B of the syconia is easily recognized when remains of the wings of the foundress wasps are observed in the ostiole (Fig. 4). In this sense, the record of one, and
rarely up to three, wasp(s) per syconium in the studied species is comparable to the 1–4 foundress wasps reported by Gibernau et al. (1996) and Hernández-Sosa and Saralegui-Boza (2001), while in other species (Galil and Eisikowitch 1968a; Compton and Nefdt 1990), the range is wider (between 1 and 10 wasps). This variable influences the number of wasps of next generation and “seeds” produces that can be found in the syconium and can thus affect the population dynamics of both partners in this mutualist interaction. The development of this phase differs from that reported previously by Smith and Bronstein (1996) and Zhang et al. (2006). In the former study, it was hypothesized that the syconium stops growing during this phase, with entry of the female wasps to the interior of the syconium. However, we observed gradual and constant growth in the diameter and length of the syconium in phases subsequent to phase B (Table 1, Fig. 3). Throughout the growth of the syconium, we also observed a clear thickening of the syconium wall in phases A–D; this structure showed significant differences between phases A1 and D (Table 1).

On the other hand, based on a phenological study conducted by Morrison on the Isla de Barro Colorado, Panamá, Janzen (1979) inferred that the separation between phases B and D was four weeks. The records for *F. petiolaris* present a longer period (6 to 9 weeks), which matches exactly the range indicated for *F. racemosa* (Zhang et al. 2006). This result can be explained by the proposal of Verkerke (1989), who left open the possibility of a longer duration of phase B, depending on the arrival of the female wasps to the syconium. This situation was later corroborated in other subsequent studies (Bronstein 1988; Khadari et al. 1995), in which an extension of this phase of up to four weeks was recorded when there was no pollination of the female flowers. These flowers then rapidly lose their receptivity once pollination occurs.
For phase C, Verkerke (1989) suggested that the development of the syconia could present marked variations as also demonstrated for *F. sycomorus* (Galil and Eisikowitch 1968a, b) and *F. racemosa* (Zhang et al. 2006). While it was also detected in *F. petiolaris* that it is these phases (C1–C3) together that require more time in the overall development of the syconium, only gradual external changes were detected in its size (Table 1). In contrast, important modifications are observed inside, especially the growth, color and texture of the ovaries of the female flowers. All of these variations eventually derive in a reduction of the syconium cavity, although this does not completely disappear. This latter finding differs from that described by Smith and Bronstein (1996), who indicated that the syconium cavity closes completely in phase C2. On the other hand, there is currently no certainty regarding the function of dark brown hyaline liquid detected in the syconium cavity of *F. petiolaris* in C1 and C2, which was also described during phase C in *F. ottoniifolia* (subgen. *Urostigma*) by Verkerke (1986), in the subgenus *Sycomorus* (Berg and Wiebes 1992) and in *F. racemosa* (Zhang et al. 2006).

Phase D of the syconium of the studied species presented a loss of turgidity as the most prominent external trait of the syconium, although it also began to become slightly paler (Fig. 3). Comparatively, the syconium presented greater dimensions than those recorded in the previous phases (Table 1), enabling clear differentiation of the female flowers with apical holes, as a result of the emergence of the new generation of wasps *Pegoscapus* sp., as well as the ovaries that contained “seeds” and the male flowers (Figs. 3 and 5). All of this is in general agreement with previous descriptions (Verkerke 1989, Smith and Bronstein 1996 but see Zhang et al. 2006). In contrast to that reported in *F. aurea* with respect to the fact that the exit hole for the female *P. jimenezi* wasps is excavated by the males through the lateral walls of the syconium
(Bronstein and Hassaert-McKey 1996, Hernández-Sosa and Saralegui-Boza 2001), in F. petiolaris, this hole is always found in the ostiole, which possibly represents an easier alternative when attempting to escape the syconium, since the ostiolar bracts are probably less compact than the wall. However, validation of this hypothesis would require future anatomical studies. We found only one exit hole in the ostiole but it was possible to observe two or three such holes on rare occasions (Fig. 5). Other authors reported the same observation in F. sycomorus (Galil and Eisikowitch 1968a) and F. aurea (Hernández-Sosa and Saralegui-Boza 2001).

Phase E in F. petiolaris, in which the syconium and the “seeds” are mature, is externally distinguishable by a very soft texture, green yellow to green brown color (Fig. 3) and slightly sweet odor. This phase is very brief (1–7 days) and is similar to that found by Zhang et al. (2006) for F. racemosa. In this phase, the syconia of the studied species were rapidly removed by frugivores that, given the characteristics of the bite (Fig. 5), were probably bats. The night watchmen at the site in which individual A is located reported visits of these mammals around the crown. In addition, it should be noted that the syconia of F. petiolaris present the typical bat-syndrome traits proposed by Lomáscolo et al. (2010).

**Syconia flowers and fig-wasp community**

The number of flowers of the syconium of F. petiolaris (406–607) is within the very broad range of 50 to 7000 flowers reported for the genus (Verkerke 1989). However, a recently reported pattern is that the number of female flowers is always higher than that of the male flowers (Verkerke 1989; Ibarra-Manríquez and Wendt 1992; Compton 1993, Hernández-Sosa and Saralegui-Boza 2001), presenting a proportion of 10:1 (Berg and Wiebes 1992). In the subgenera Pharmacosycea and Sycidium, the percentage of male flowers can vary by between 10 and 30 % (Berg 1989; Ibarra-Manríquez and
Wendt 1992). In *F. petiolaris*, this percentage was lower (3–7.4 %), a finding that is similar to that reported by Compton (1993) for *F. burtt-davyi* Hutch. (1.4 to 4.8 %).

While the number of flowers in the syconia depends to a large extent on their size (Verkerke 1989, Ibarra-Manríquez and Wendt 1992), other more complex relationships can arise with variation in these proportions. For example, the numbers of wasps and “seeds” increase directly with the number of flowers recorded in a syconium (Bronstein and Hossaert-McKey 1996).

Under laboratory conditions, the female fig wasps found in *F. petiolaris* can survive outside of the syconium for three to eight days; a similar period is reported by Compton (1993) and Janzen (1979). However, it was observed that the lifespan of females of *Pegoscapus* sp. is shorter than that of *Idarnes* sp. Another notable finding was the presence of wasps of the genus *Idarnes* sp. in phase A2, with an absence of wasps of *Pegoscapus* sp. Wasps of the former genus are characterized by being smaller or of the same size of the pollinator wasps but presenting an ovipositor that exceeds their body length (Fig. 4). These characteristics enable them to oviposit on the female flowers from outside of the syconium, days before the presence of pollinators is detected (Elias et al. 2008). Cruaud et al. (2011) also suggest that these wasps can oviposit on the female flowers through the stigmas and style, depositing their eggs between the interior of the integument and the nucellus. In this sense, the oviposition of these non-pollinators is similar to that of the pollinators, but in this case, no pollination will occur and there is current uncertainty regarding the possibility of development of viable syconia in the absence of pollinators, or if they simply abort. It should be noted that, in the syconia of individual A in phase C1, females of *Idarnes* sp. were observed ovipositing through the walls of syconia (Fig. 4) that already had pollinator wasps in the interior, as inferred by the presence of their wings in the ostiole.
Seed germination in *F. petiolaris*

The seeds of *F. petiolaris* presented high germination percentage values (50–90 %). Previous studies report similar germination values of between 57 and 100 % in other *Ficus* species (Verkerke 1986, 1988; Utzurrum and Heideman 1991; Ibarra-Manríquez 1992). The seeds required around nine days for the radicle to emerge and *ca.* 25 days to exhibit green and foliaceous cotyledons (Fig. 1). This development closely comparable with the findings of Ibarra-Manríquez (1992), who germinated five species of the section *Pharmacosycea*. Based on the classification proposed by Garwood (1996), *F. petiolaris* has the phanerocotylar epigeal seedling type with foliaceous cotyledons, which were associated with small seeds in tree seedlings in a tropical rain forest (Ibarra-Manríquez et al. 2001). The high germination percentage recorded in *F. petiolaris* also can be considered an indirect measure of the positive role of female wasps *Pegoscapus* sp. as pollinators, despite the apparently isolated condition of the monitored individuals. The known natural populations of *F. petiolaris* are located at distances of *ca.* 59 (Tacámbaro, Michoacán) and 79 (Tuxpan, Michoacán) km from Morelia. These locations could serve as sources from which the wasps can migrate towards Morelia.

Future genetic studies are required in order to corroborate the validity of this hypothesis.

**Conclusions**

For the first time in a Neotropical *Ficus* species, the external and internal structural changes in its syconia are described, and these events related to the development of its fig wasp communities. Our study shows that the different developmental phases of the syconium in *F. petiolaris* conform, in general terms, to those described previously for others species in the genus. However, since this is the only species in America to be characterized in this respect, certain characteristics emerge that should be evaluated in
other species in order to determine the breadth of similarity that exists among the congeneres. For example, the frequency at which the emergence of female wasps occurs in phase D through a tunnel in the ostiole, or the role of the hyaline substance found within the syconium cavity in phases C2–C3. One important consideration is that the development of the syconia and their fig wasps is, in general, similar to that observed in natural communities throughout the geographical range of *F. petiolaris* in Mexico (Fig. 6). Nevertheless, it is recommended to examine and observe the most critical syconium developmental stages (B, D and E) more frequently (e.g. each week), and it is very important to determine how many animal species interact as “seed” dispersal vectors in the natural communities where *F. petiolaris* are found.

Another particular aspect is the record of new species of wasps of the genera *Pegoscapus* and *Idarnes*. The record of wasps of the genus *Heterandrium* is of note since there is little information available about this genus due to the fact that it is only occasionally recorded, and there is no certainty regarding the type of interactions it has with the syconium and with other groups of wasps. Our study confirms that priority studies in *Ficus* should integrate the morphology of the syconia and their interactions with the Agaonidae wasps. These types of studies will be more valuable among species that belong to different sections and subgenera, particularly during phases B and D. Finally, we expect to generate interest in conducting similar studies in other species of the genus, especially in American species, which would advance our understanding of the complex relationships maintained by species of *Ficus* with their pollinators and dispersers.

**Acknowledgements**

This study is part of the activities carried out by EMPM during her postdoctoral research (scholarship DGAPA–Universidad Nacional Autónoma de México, UNAM),
and BHR as part of an investigation conducted within the Summer of Science Program. We thank the authorities of the Comisión Forestal del Estado de Michoacán for providing facilities for this study, particularly Sr. Daniel Cuamva, who provided us with information about the activity of bats around individual A, and Dr. Orlando Hernández Cristóbal, head of the Microscopy Laboratory of the ENES Morelia (UNAM), who helped us to obtain the SEM photographs. We greatly appreciate the support of Dr. Jean-Yves Rasplus, who provided us with valuable taxonomic data about wasps found in our study (all of them are undescribed species). Finally, we thank Dr. Christian Lacroix, Dr. Julien Bachelier and two anonymous reviewers for their valuable comments that have improved the manuscript.

References


TABLE 1. Traits measurement for syconium development phases of *Ficus petiolaris*.

Except for syconium wall thickness, for each structure, we indicate width and length in mm (± DS). Means with the same superscript do not differ statistically (Repeated measures ANOVA with Tukey post hoc test < 0.05) from other means among phases.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Syconium</th>
<th>Peduncle</th>
<th>Ostiole</th>
<th>Syconium wall</th>
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<tr>
<td></td>
<td>Thickness</td>
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<td></td>
<td></td>
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<td>A1</td>
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<td>2.22 (0.23)&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>2.32 (0.27)&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
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<td>1.33 (0.24)&lt;sup&gt;c&lt;/sup&gt;</td>
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FIGURES

FIGURE 1. Habit and germination aspects of *Ficus petiolaris*. A) Rupicole habit and trunk with yellow bark. B) Rupicole young plant with cordiform leaves. C) Germination of the seed showing a white radicle. D) Seedling with radicle, hypocotyl and foliaceous cotyledons.


FIGURE 3. Internal and external characteristics of syconia development phases in *Ficus petiolaris*. The photographs on the right hand side present details of different structures of the syconia and fig wasps. A) Floral bud cut showing the beginning of differentiation of the syconium structure (ostiolar bracts and basal neck). B) Differentiation between ostiole (OS), syconium cavity (SC) and basal neck (BN). C) Development of female flowers (PF) and gall flowers (GF) of parasitic wasp *Idarnes* sp. D) Syconium cavity widening with a female foundress wasp (FW) *Pegoscapus* sp., ovipositing on female flowers (PF). E) Initiation of the constriction of the ostiole internal bracts with no differences detectable in the cavity between the ovaries of female and gall flowers (GF and PF, respectively); the body of the foundress wasp (FW) is visible. F) The wasp larvae and “seeds” continue developing, such that the syconium cavity constricts even further; the flowers that contain the wasps (GF) and “seeds” (SF) differ mainly in their color (yellow blackish and brown yellowish, respectively) and the
body of the foundress wasp (FW) is still visible. G) In the interior of the syconium, the wasps (GF) and “seeds” (SF) have almost completed their development, such that the syconium cavity is reduced almost totally. H) Syconium increases in proportion to its dimensions, male flowers (SF) are visible among the gall flowers (GF) and “seeds” (SD). The fig wasps emerge. I) Syconium mature size increase and ostiole is almost flattened and compact; internally, the cavity of the fig presents a dark brown color. The holes in the ovaries of the gall flowers (GF) through which wasps emerged and “seeds” (SD) can be observed.

**FIGURE 4.** Fig wasps associated with *Ficus petiolaris*. A) Female *Idarnes* sp. B) Females *Idarnes* sp. ovipositing externally through the walls of syconia. C) Female *Pegoscapus* sp. attempting to enter the syconium cavity through the ostiole. D) Female *Pegoscapus* sp. E) Remains of pollinator wasp wings (WW) in the ostiole bracts (OB). F) Female wasp corbicle of *Pegoscapus* sp. showing the pollen load. G) Male *Pegoscapus* sp. H) Male *Heterandrium* sp. I) Male *Idarnes* sp.

**FIGURE 5.** Details of syconium morphology of *Ficus petiolaris*. A) Ostiole in early phases of syconium development. B) Female flowers. C) Stamen of the male flower (SF) and gall flower (OG). D) Broken ovary gall. E) Three exit holes excavated by males through the bracts of the ostiole. F) Detail of exit hole. G) Ostiole flattened, characteristic in phases D-E of syconium development. H) “Seeds” (SD) and ovary galls (OG). I) Syconia, probably bitten by bats.

**FIGURE 6.** Syconium development phases in different populations across natural distribution of *F. petiolaris*. Locations of the populations in Mexico (locality, state) are
showed with a number in superscript. A) Floral bud ¹; B) Syconia ¹; C) Syconium cavity ²; D) Syconia ²; E) and F) Female wasps of *Pegoscapus* sp., ¹,³; G) Syconia with remains of pollinator wasp wings in the ostiole bracts and parasitic wasps *Idarnes* sp. ¹; H) Syconium ⁴; I) Phase C2 observed in dissected syconia ³; J) Syconia ⁵; K) Syconium ⁶; L) Big and small syconia in phases D and, respectively ⁷; M) Syconium with ant coming to the cavity by the exit hole in the ostiole bracts ⁴; N) Syconium open showing “seeds”, empty galls and some male wasps ⁸; O) Syconium open showing “seeds” and empty flower galls ⁹; P) Syconium probably bitten by bats ¹. Number of locations: ¹ Todos Santos, Baja California Sur; ² Nacapule, Sonora; ³ Chilpancingo, Guerrero; ⁴ Juncalito, Loreto, Baja California Sur; ⁵ Nizanda, Oaxaca; ⁶ Izúcar de Matamoros, Puebla; ⁷ Tacámbaro, Michoacán; ⁸ San José del Cabo, Baja California Sur; ⁹ Tequila, Jalisco.