One fig to bind them all: host conservatism in a fig wasp community unraveled by cospeciation analyses among pollinating and nonpollinating fig wasps.

Emmanuelle Jousselin, Simon van Noort, Vincent Berry, Jean-Yves Rasplus, Nina Rønsted, Christoff Erasmus, Jaco Greeff

doi:10.1111/j.1558-5646.2008.00406.x

ONE FIG TO BIND THEM ALL: HOST CONSERVATISM IN A FIG WASP COMMUNITY UNRAVELED BY COSPECIATION ANALYSES AMONG POLLINATING AND NONPOLLINATING FIG WASPS

Emmanuelle Jousselin,[1,2,3] Simon van Noort,[4] Vincent Berry,[5] Jean-Yves Rasplus,[1] Nina Rønsted,[6] J. Christoff Erasmus,[7] and Jaco M. Greeff[7]

[1]*Institut National de la Recherche Agronomique, Centre de Biologie et de Gestion des Populations, Campus International de Baillarguet, CS-30 016, 34 988 Montferrier sur Lez, France*

[2]*E-mail: jousseli@supagro.inra.fr*

[4]*Natural History Division, South African Museum, Iziko Museums of Cape Town, PO Box 61, Cape Town 8000, South Africa*

[5]*Département Informatique, LIRMM- CNRS, 161, rue Ada 34392 Montpellier Cedex 5, France*

[6]*Jodrell Laboratory, Royal Botanic Gardens, Kew, TW9 3DS Richmond, Surrey, United Kingdom*

[7]*Department of Genetics, University of Pretoria, Pretoria 0002, South Africa*

Received February 28, 2008
Accepted April 1, 2008

The study of chalcid wasps that live within syconia of fig trees (Moraceae, *Ficus*), provides a unique opportunity to investigate the evolution of specialized communities of insects. By conducting cospeciation analyses between figs of section *Galoglychia* and some of their associated fig wasps, we show that, although host switches and duplication have evidently played a role in the construction of the current associations, the global picture is one of significant cospeciation throughout the evolution of these communities. Contrary to common belief, nonpollinating wasps are at least as constrained as pollinators by their host association in their diversification in this section. By adapting a randomization test in a supertree context, we further confirm that wasp phylogenies are significantly congruent with each other, and build a "wasp community" supertree that retrieves *Galoglychia* taxonomic subdivisions. Altogether, these results probably reflect wasp host specialization but also, to some extent, they might indicate that niche saturation within the fig prevents recurrent intrahost speciation and host switching. Finally, a comparison of ITS2 sequence divergence of cospeciating pairs of wasps suggests that the diversification of some pollinating and nonpollinating wasps of *Galoglychia* figs has been synchronous but that pollinating wasps exhibit a higher rate of molecular evolution.

KEY WORDS: **Community ecology, fig wasps, host utilization, mutualism, phylogeny, randomization, specialization, supertree.**

When organisms are tightly bound in interspecific interactions over long evolutionary times, the diversification of the partners is rarely independent. The symbiotic partner (i.e., the "host-associated" organism that lives part or its entire life cycle on another organism) is often constrained by the speciation of its hosts. There are numerous examples of phytophagous insects and parasites that specialize and phylogenetically track their host (Ehrlich and Raven 1964; Janz and Nylin 1998; Lopez-Vaamonde et al.

[3]Present address: Institut National de la Recherche Agronomique, Centre de Biologie et de Gestion des Populations, Campus International de Baillarguet, CS-30 016, 34 988 Montferrier sur Lez, France

2003; Percy et al. 2004; Kergoat et al. 2005), or even speciate simultaneously with them (Weiblen and Bush 2002; Hafner et al. 2003; Degnan et al. 2004), a diversification mode known as cospeciation. These codiversification processes can have a major impact on the composition of ecological communities. If host-associated lineages constituting a community are all similarly stranded on their host, their speciation patterns will be similarly affected by their host association and they will all diversify in parallel. This will result in replicate communities: that is, communities associated with closely related hosts will encompass related species and have a very similar structure (Johnson and Clayton 2003; Abrahamson and Blair 2007; McLeish et al. 2007).

However, host-associated organisms often show major ecological differences and respond independently to their host diversification. For instance, some parasites have long dispersal abilities that favor the occurrence of host switching throughout their evolution (Clayton and Johnson 2003; Johnson and Clayton 2003). The ability to use different ecological niches of some parasite lineages might also break down cospeciation patterns by favoring duplication events (i.e., speciation on the hosts) (Johnson and Clayton 2004). Ecological interactions between host-associated organisms could also influence their diversification process. For instance, competitive exclusion between parasites could cause parasite lineage extinction; specialized trophic interactions between associates might lead to codivergence between them separately from a cospeciation with their hosts. Hence, investigating dissimilarities in the cospeciation patterns of several lineages associated with the same hosts may reveal important information on the ecology of host-associated organisms and give insight into the processes behind a community structure and composition.

The community of wasps (Hymenoptera, Chalcidoidea) associated with figs represents an ideal system in which comparing cospeciation patterns of different lineages could improve our understanding of the construction and persistence of the ecological communities. Fig wasps communities can be very diverse, with up to 30 wasp species inhabiting the syconia of one host tree species (Boucek et al. 1981). The establishment of these communities is believed to follow a general codiversification pattern: during speciation of the host tree, wasps speciate along with their hosts. This view is supported by the fact that closely related fig species host related wasp fauna; that is, a pool of species related to the community associated with a closely related fig species (Berg and Wiebes 1992; Compton and Van Noort 1992; Kerdelhué et al. 2000). Hence, each fig wasp assemblage seems to be an ecological replicate of the community associated with a closely related fig species. The presence of such replicates suggests that all fig wasp lineages are highly specialized on their host figs, which precludes host shifts during the course of their evolution. Additionally, fig wasp communities may be saturated and hence offer limited opportunity for the existence of new ecological niches and consequently for wasp speciation on their hosts (duplication) and/or host colonization by a new wasp (but see Hawkins and Compton 1992). However, this proposed host conservatism of fig wasp communities has not been tested and there are still few comparative studies of the codiversification of wasps using different ecological niches within a fig (Weiblen and Bush 2002; Jackson 2004; Marussich and Machado 2007; Silvieus et al. 2008).

Most attention in fig/fig wasp codiversification studies has been focused on the plant–pollinator interaction because of the interest in understanding the mutualism stability (Cook and Rasplus 2003), but also probably because people generally assume that mutualists are more specialized and thus more likely to speciate along with their host plants than parasites (Weiblen and Bush 2002; Althoff et al. 2007; Marussich and Machado 2007). Fig pollinators (Agaonide family sensu Rasplus et al. 1998) all lay their eggs at fig receptivity by entering the fig cavity (closed cavity lined with uniovulate flowers) through a slit formed by bracts situated at the apex of the fig (called the ostiole). They then lay their eggs in the fig flowers and their larvae complete their development in galled flowers. Pollinating wasps have long been thought to be very specific to their host figs and reciprocally each fig species was believed to shelter a single species of pollinating wasps (Janzen 1979). Although this view still holds for most sampled species of figs, contemporary taxonomic and molecular studies are revealing an increasing number of exceptions to the reciprocal specificity of the host/pollinator interaction (Lopez-Vaamonde et al. 2001; Cook and Rasplus 2003; Molbo et al. 2003; Machado et al. 2005; Haine et al. 2006). Furthermore, a recent study of Neotropical figs, that is, within section *Americana*, shows that, at such a fine scale, there is no evidence of cospeciation (Machado et al., 2005). However, at a broad taxonomic level, wasp/fig association and cospeciation studies confirm that pollinating fig wasp diversification is largely constrained by the host affiliation (Herre et al. 1996; Machado et al. 2001; Weiblen 2001; Weiblen and Bush 2002; Jousselin et al. 2003b; Jackson 2004; Rønsted et al. 2005).

Few studies have attempted to unravel the history of nonpollinating fig wasp diversification. These wasps are classified in four subfamilies and more than 60 genera (http://www.figweb.org). A phylogeny including species belonging to different fig wasp subfamilies and some Chalcidoidea not associated with figs suggests that fig wasps did not originate from a common ancestor but that different lineages of Chalcids have colonized *Ficus* independently (Rasplus et al. 1998). All nonpollinating fig wasps complete most of their life cycle on their hosts. They lay their eggs, develop, and for most species mate within the *Ficus* inflorescence. Such intertwined life cycles led to the idea that the nonpollinating wasp/fig associations are, like pollinating wasp/fig associations, very specific (Berg and Wiebes 1992; Jousselin et al. 2006). Following this assumption, taxonomic descriptions of nonpollinating wasps often mention a single host fig species per wasp species

(http://www.figweb.org). However, the life cycle characteristics (e.g., the developmental stage of the fig at which wasps lay their eggs) of nonpollinating wasps, their reproductive strategies, and their population sizes are very different (Compton et al. 1994; West et al. 1996). Their feeding habits also differ, nonpollinating fig wasps can be flower gallers, inquilines, or even parasitoids of pollinating wasps or other flower gallers (Compton and Van Noort 1992; Kerdelhué et al. 2000; Weiblen 2002; Marussich and Machado 2007). All these differences may play a role in both the degree of specificity of the wasps toward their host plants and the opportunities for occupying new ecological niches within a fig, and hence influence their diversification patterns. A review of current taxonomic studies (http://www.figweb.org; Berg and Wiebes 1992; Compton et al. 1994) and of the few published molecular phylogenies (Machado et al. 1996; Lopez-Vaamonde et al. 2001; Weiblen 2002; Jousselin et al. 2006) suggests at least a trend toward host specialization in most nonpollinating fig wasp genera studied. Most formal cospeciation studies conducted to date only consider one or two genera of nonpollinating wasps at a time (but see Marussich and Machado 2007; Silvieus et al. 2008). Moreover, they often do not address the question of fig/fig wasp cospeciation by comparing the phylogenies of nonpollinating fig wasps to that of figs. Instead, they compare nonpollinator phylogenies with those of pollinating wasps or other nonpollinating wasps (Machado et al. 1996; Lopez-Vaamonde et al. 2001; Jousselin et al. 2006; Marussich and Machado 2007). This is because of the lack of resolution of fig phylogenies below the taxonomic level of the section. These studies generally suggest that nonpollinating wasp speciation is not independent of their host association (but see Marussich and Machado, 2007). Nevertheless, the only formal fig/nonpollinating wasp cospeciation tests concluded that cospeciation of nonpollinating wasps with their host figs was not significant (Weiblen and Bush 2002, Silvieus et al. 2008).

In the Afrotropical section of *Ficus*, that is, *Galoglychia*, fig wasps seem to follow an unusual cospeciation scenario: pollinators seem to have recurrently switched hosts through the course of evolution whereas nonpollinating wasp diversification has been more constrained by their host association. The *Galoglychia* section currently comprises 72 species that are further subdivided into six subsections (Burrows and Burrows 2003). It is the only section that is pollinated by several genera of Agaonid wasps, as there usually is a one-to-one association between fig sections and wasp genera. Furthermore, wasp genera are not restricted to a single fig subsection, and wasp species within a genus sometimes pollinate fig species that are scattered into two subsections (Berg and Wiebes 1992; Erasmus et al. 2007). Recent phylogenetic studies showed that Galoglychia pollinating wasp genera were monophyletic (Erasmus et al. 2007) and fig subsections were also monophyletic (Rønsted et al. 2007), thus necessarily implying that the pollinator/fig evolution deviates from a cospeciation scenario. The community of nonpollinating wasps associated with Galoglychia figs is very diverse (Compton et al. 1994), but there are still few descriptions of these insects below the genus level and basic biological information such as larval feeding habits are lacking. Wasps belonging to the subfamily Otitesellinae (Pteromalidae) are among the most common nonpollinating wasps found in Galoglychia figs. Similar to pollinators, these wasps lay their eggs at fig receptivity, but do so by inserting their ovipositor through the fig wall; their larvae then develop in galled flowers (Van Noort and Compton 1988). Phylogenetic analyses revealed that Otitesellinae wasps associated with Galoglychia figs are in fact divided into two distinct clades: the *Otitesella* "*uluzi*" species group (hereafter called *uluzi*) and the *Otitesella* "*sesquinianellata*" group (hereafter called *sesqui*). *Uluzi* and *sesqui* exhibit differences in their ovipositor length and probably lay their eggs at slightly different times of fig development (Van Noort and Compton 1988; Jousselin et al. 2006). Such shift in the timing of oviposition might have a role in the maintenance of the two forms on the same figs (Weiblen and Bush 2002; Jousselin et al. 2006). These two groups of species form two parallel radiations whose phylogenetic patterns follow Galoglychia fig taxonomy (Jousselin et al. 2006). Species belonging to the genus *Philotrypesis* (Sycoryctinae) also occur frequently in Galoglychia figs (Vincent, 1991). These wasps lay their eggs late in the fig development (Kerdelhué et al. 2000) by inserting their very long ovipositor through the swollen fig wall. Biological information on these wasps is scarce but it has been suggested that they were parasitoids of the pollinators or of other flower gallers (Joseph 1959). The phylogeny of *Philotrypesis* has also been shown to reflect Galoglychia fig taxonomy (Jousselin et al. 2004).

This study expands from previous work conducted on these wasp lineages associated with *Galoglychia*. Here, we formally investigate the fig/fig wasp cospeciation patterns in each lineage by comparing fig wasp phylogenies to the recent phylogeny of *Galoglychia* figs (Rønsted et al. 2007). More particularly, we ask whether nonpollinating wasps are more likely to switch hosts than pollinating wasps. We also conduct pairwise cospeciation tests between wasp phylogenies to compare sequence divergence in cospeciating wasps and help establish a temporal framework of the evolution of this community. This is particularly relevant to our model system as the association patterns observed in *Galoglychia* figs could easily be due to differences in the timing of speciation in pollinating and nonpollinating wasp lineages. Pollinators possibly represent an older radiation on *Galoglychia* figs and their current association patterns might be influenced by old extinction events that have erased many of the initial cospeciation patterns (Erasmus et al. 2007).

To test for fig/fig wasp cospeciation and wasp parallel divergence, we used both tree-based (TreeMap [Page 1994]) and distance-based (ParaFit [Legendre et al. 2002]) methods and

compared their adequacy. However, cospeciation tests are aimed at comparing pairs of host and/ parasite phylogenies and not at testing whether several host-associated lineages diversify in parallel (Lopez-Vaamonde et al. 2005). We therefore, in addition to pairwise cospeciation tests, adapted a randomization test in a supertree context (Lapointe and Rissler 2005) and explicitly assess the global congruence of the set of fig wasp phylogenies. This method allows the incorporation of species that were not always collected on the same host figs in a global analysis.

Material and Methods

SAMPLING AND PHYLOGENETIC RECONSTRUCTIONS

Galoglychia figs are restricted to the Afrotropical region (Burrows and Burrows 2003). We sampled wasp fauna associated with 23 fig species in a variety of localities throughout Africa. Altogether, we sampled in eight countries and 25 localities. The collecting sites were scattered from Senegal to the extreme south of the African continent (Cape Town, RSA), with the main collecting sites being situated in southern Africa. Although, for each wasp lineage considered, the numbers of species sampled might only represent a third or a quarter of the species that are associated with *Galoglychia* figs, our sampling is quite representative of the diversity of the section as it encompasses specimens associated with all subsections of the fig taxonomy. Whenever possible, wasp specimens were all collected from the same crop (i.e., from the same fig tree) to avoid mistakes due to erroneous fig identification. A list of species sampled and their associated host figs, as well as locality information for wasps are given in Table 1. All wasps were identified by two recognized fig wasp taxonomists J. Y. Rasplus and/or S van Noort.

Molecular phylogenies for the four wasp lineages studied (*uluzi*, *sesqui*, *Philotrypesis*, and pollinators) based on these collections have been previously published (Jousselin et al. 2006; Erasmus et al. 2007), sequences are accessible in the GenBank database. Reconstruction of the phylogenetic relationships of pollinators of *Ficus* section Galoglychia was based on the combined analysis of one ribosomal gene (28S) and the internal transcribed spacer (ITS2) and analyses of ITS2 alone for which we had a denser taxon sampling (Erasmus et al. 2007). These markers worked better on this group of pollinating wasps than the cytochrome oxidase (COI) DNA fragment that is usually used in fig pollinating wasp phylogenies: we failed to find COI primers that consistently succeeded in amplifying all the templates and COI sequences generally showed little variation (Erasmus et al. 2007). The phylogeny of Afrotropical Otitesellinae was also based on analyses of ITS2 (Jousselin et al. 2006). The only addition to the published phylogenetic reconstruction is the inclusion of the ITS2 sequence for a wasp associated with *Ficus ovata* (O sp. 44, GenBank accession number EU 683611). The less variable ribosomal gene, 28S, had also been used on a subset of taxa in the 2006 study to confirm the deep nodes of the phylogeny. As stated in the Introduction, this study revealed that Otitesellinae wasps associated with *Galoglychia* figs were divided into two distinct clades: the *uluzi* group (that includes only *Otitesella* species) and the *sesqui* group (that include species of the *Philosycus* and *Otitesella* genera). We treated *uluzi* and *sesqui* as separate lineages in this article. The phylogenies of *Philotrypesis* used here were based on the combined analysis of ITS2 and Cytochrome b (Cytb) (Jousselin et al. 2004), and the analyses of ITS2 alone.

All wasp phylogenies were initially reconstructed using several specimens per species to detect potential morphological misidentifications and/or cryptic species. For instance, *Elisabethiella stuckenbergii* and *E. socotrensis* are both associated with *Ficus natalensis* and *F. burkei*. Although our identifications revealed only two species, in both cases, molecular studies revealed significant divergence between wasps associated with the two different fig species (Erasmus et al. 2007). Hence, each host-associated wasp population was considered as a separate species in the present study.

The phylogeny of associated host figs is based on a study of *Galoglychia* figs by Rønsted et al. (2007), which was based on two nuclear DNA fragments (ETS and ITS). The fig phylogeny is therefore based on different individuals than those sampled for wasps, but fig identifications have been conducted by the same team of authors.

COSPECIATION TESTS

We tested the congruence of the fig phylogeny with the phylogenies of each wasp lineage (pollinators, *sesqui*, *uluzi*, and *Philotrypesis*) using cospeciation analyses. Several methods to estimate the importance of cospeciation in the history of interspecific interactions have been proposed (see Paterson and Banks, 2001; Johnson and Clayton 2004; Hughes et al. 2007; for recent reviews). We chose the most widely used method, known as reconciliation analysis (Page 1994), as implemented in TreeMap 1 and TreeMap 2.02β and the more recent method developed by Legendre et al. (2002), implemented in ParaFit.

Reconciliation analyses aim at finding optimal reconstructions of the history of a host–parasite association by mapping the parasite tree onto the host tree (Page 1994). The probability of obtaining the observed number of cospeciation events is then estimated by randomizing the parasite trees or both host and parasite trees and generating a null frequency distribution. We used both TreeMap 1 and TreeMap 2.02 β (an updated version of TreeMap 1) (Charleston 1998). TreeMap 1 uses parsimony to reconstruct codiversification scenarios and aims at maximizing cospeciation events. Its major weakness is that it does not allow for host switches in the reconstruction but adds them a posteriori. We used heuristic searches to find optimal solutions in TreeMap

Table 1. Host figs and associated wasp species used in this study, collecting sites are indicated for wasps, voucher numbers are indicated in parentheses, *Ficus* subsections are indicated in the first column in bold. See Rønsted et al. (2007) for host fig collection details and voucher numbers.

Ficus species	Locality and collectors	Nonpollinating wasp species			Pollinating wasp species
		Philotrypesis species	*uluzi* species	*sesqui* species	
Galoglychia					
lutea	RSA, Mpumalanga, Nelspruit, EJ and JP			*O*. sp. 3 (013-EJ)	
	RSA, Louis Trichardt. JG	*P*. sp. 2 (LT99.1)			
	RSA, Kwazulu Natal, Durban, CE				*Alltotriozoon heterandromorphum*
Platyphyllae					
stuhlmannii	RSA, Mpumalanga, Nelspruit, EJ and JP	*P*. sp. 14 (NS1JM)	*O*. sp. 8.2 (O2)		*Alfonsiella binghami*
	Tanzania, Mkomazi Game Reserve, SVN			*O*. sp. 9.1 (FMK21 D)	
abutilifolia	RSA, Gauteng, Pretoria Bot. gardens, EJ	*P*. sp. 3 (PBG-01)			*Elisabethiella comptoniella*
	Burkina Faso, SVN				*Nigeriella fusciceps*
trichopoda	RSA, Kwazulu-Natal, Sodwana, EJ and JP	*P*. sp. 22 (SB1JM)	*O*. sp 14.1 (O5EJKW)		
	RSA, Kwazulu-Natal, Ballito, CE				*Elisabethiella bergi*
glumosa	Tanzania, Mkomazi Game Reserve, Ibaya, SVN	*P*. sp. 4 (FMK1)	*O*. sp. 6.1 (KW99F09L)	*O*. sp. 7.1 (KW99F09K)	
	RSA, Gauteng, Pretoria, EJ				*Elisabethiella glumosae*
tettensis	RSA, Louis Trichardt. JG			*O*. sp. 13 (O13)	
	RSA, Makhado, JG				*Nigeriella excavata*
Chlamydodorae					
craterostoma	RSA, Limpopo, Soutspanberg, CE & JG	*P*. sp. 21 (SPB49)	*O*. sp. 18 (SPB11)	*O*. sp. 19 (SPB11)	*Alfonsiella pipithiensis*
burtt-davyi	RSA, Eastern Cape, Grahamstown, EJ and JP	*P*. sp. 27 (GHS03 1)	*O*. *uluzi* (O31)	*O*. *sesqunianellata* (O27)	*Elisabethiella baijnathi*
ilicina	RSA, Eastern Cape, Springbock, SVN	*P*. sp. 8 (NA97-F6)		*Philosycus* sp.1 (NA97-F6)	
	Namibia, Namib-Nankluft park, SVN				*Elisabethiella enriquesi*

Continued.

Table 1. Continued.

Ficus species	Locality and collectors	Nonpollinating wasp species			Pollinating wasp species
		Philotrypesis species	*uluzi* species	*sesqui* species	
burkei	RSA, Mpumalanga, Krokodilpoort, SVN	*P*. sp. 7 (Kw99-F49)			
	RSA, Pretoria, Botanical gardens, EJ		*O*. sp. 46 (Oth2/3)	*O*. sp. 26. *3* (Oth2/3)	
				O. sp. 26. 2 (O20)	
	Zimbabwe, Macheke, AW		*O*. sp. 47. *5*(O36/37)		
	Tanzania, Mayo Valley, JYR				*Elisabethiella stuckenbergi*
	RSA, Kwazulu-Natal, Durban, CE				*Elisabethiella socotrensis*
petersii	RSA, Mpumalanga, Nelspruit, EJ and JP	*P*. sp. 13 (P13)	*O*. sp. 29 (Kw99F36M)	*O*. sp. 30 (Kw99-F36N)	*Alfonsiella binghami*
natalensis	RSA, Kwazulu-Natal, Durban, SB	*P*. sp. 16 (SB1–2002)	*O*. sp. 21 (SBD 2002)	*O*. sp. 9. 3 (SBD 2002)	*Elisabethiella. socotrensis*
					Elisabethiella stuckenbergi
Caulocarpae					
bizanae	RSA, Mkambati, Game reserve, SVN	*P*. sp. 11 (KN98-F6)			*Courtella* sp. *n*.
sansibarica	RSA, Mpumalanga, Krokodilpoort, SVN	*P*. sp. 12 (Kw99-F55)			
	Southa Africa, Mpumalanga, Kruger park, SVN				*Courtella armata*
	Tanzania, Pongwe, JYR		*O*. sp. 42 (JYR557)		
ovata	Côte d'Ivoire, Lamto, JYR		*O*. sp. 44 (JYR 370)	*Philosycus monstruosus* (JYR443)	
	Senegal, SM	*P*. sp. 23 (JYR 03)			
bubu	Tanzania, Mkomazi Game Reserve, SVN		*O*. sp. 43 (FMK31 C)	*Philosycus* sp. 15 (FMK31A)	*Courtella michaloudi*
ottoniifolia	Tanzania, Amani, JYR		*O*. sp. 45 (JYR2000		
Cyathistipulae					
scott eliotii	Côte d'Ivoire, Lamto, JYR			*Philosycus* sp. 9 (JYR344)	*Agaon* sp.
sagittifolia	Côte d'Ivoire, Lamto, JYR			*Philosycus* sp. 11 (JYR399)	
Cyathistipuloides	Côte d'Ivoire, Lamto, JYR			*Philosycus* sp. 7 (JYR541)	
Crassicostae					
elasticoides	Gabon, Monts Doudou, SVN		*O*. sp. 31 (GA00-F03 I)	*Philosycus* sp. 2 (GA00-F03 C)	*Elisabethiella articulata*
usambarensis	Tanzania, Pongwe, JYR			*O*. sp. 32 (JYR557)	*Elisabethiella* sp.
louisii	Gabon, Monts Doudou, SVN		*O*. sp. 33 (GA00-F03 H)	*O*. sp. 34 (GA00-F03 G)	*Paragaon josephi*

Collectors SVN, Simon van Noort; JG, Jaco M. Greeff; JYR, Jean-Yves Rasplus; EJ, Emmanuelle Jousselin; CE, Christoff Erasmus; AW, Anthony Watsham; SB, Snowy Bajnath; JP, Jason Pienaar; SM, Serge Meusnier.

1 (using the proportional to distinguishable model, with 10,000 searches). TreeMap 2.02β uses Jungles to infer codiversification scenarios (Charleston 1998). The Jungle algorithm allows users to explore all possible mappings of one tree onto another, assigning different costs to the diversification events, and finds optimal (i.e., yielding minimal costs) solutions. We used the default cost settings (0 for cospeciation, 1 for host switching, duplications, and losses) for our search of optimal solutions. Unfortunately, for several cospeciation tests using TreeMap 2.02 β, we reached calculation limitations (the program is currently limited in terms of size of datasets that can be computed due to the algorithm complexity), so we often had to limit the number of host switches in our tests.

ParaFit (Legendre et al. 2002) tests the global null hypothesis that the diversification of hosts and parasites has been independent. The phylogenies of the host and parasites are described by their respective matrices of patristic distances (the distance between two taxa is represented by the sum of the lengths of the branches connecting those taxa). The associations are also described by a matrix of absence/presence of a parasite on a host. Each matrix representing parasites and hosts are transformed into a matrix of principal coordinates. The association is then described by a matrix that crosses both matrices of principal coordinates and the matrices of association. A trace statistic, called ParaFit Global, is then computed. The null hypothesis is tested through a permutational procedure: host/parasite associations are permuted to obtain a null distribution of the statistic "ParaFit Global." Each individual link can also be tested to see whether it contributes significantly to the fit of the two phylogenies. This is done by computing the trace statistics with and without the link. Contrary to TreeMap, ParaFit can be used with trees presenting polytomies and is not affected by the presence of multiple parasites per host or multiple hosts per parasite. However, it does not yield any codiversification scenarios, it merely tests whether there is significant cospeciation but does not identify cospeciation and host switching events on the phylogenies.

We know from a large morphological survey on Otitesellinae (Jousselin et al. 2006) and an unpublished Thesis on *Philotrypesis* (Vincent 1991) that most fig species in *Galoglychia* shelter different morphospecies of *uluz*i and *sesqui* wasps and a species of *Philotrypesis*. This indicates that our sampling was not exhaustive, that is, we did not always manage to collect the pollinator, the *Philotrypesis* species, and both *uluzi* and *sesqui* wasps associated with a specific fig species, and reciprocally, some fig species, which wasp fauna was sampled, were not always included in the published fig phylogeny. Thus, for each cospeciation test, any taxon that did not have its correspondent in the other species group was pruned from the tree, as these situations would be interpreted as extinction events in cospeciation tests, although they often merely reflect the absence of a species in our sampling. These faunistic lists, based on wasp morphological identification, also suggest that we do not overestimate the level of wasp specificity in our datasets by omitting fig species that shelter several nonpollinating fig wasps of the *uluzi*, *sesqui*, and *Philotrypesis* groups.

For each lineage and each cospeciation test, we derived the topology to be tested from the ML tree with the denser species sampling by pruning taxa in TreeEdit (Rambault and Charleston 2001; http://evolve.zoo.ox.ac.uk). We thought this was preferable to rebuilding phylogenetic trees from a subset of taxa for each test, as the best topologies are likely to be those obtained with the denser taxon sampling (Rannala et al. 1998; Zwickl and Hillis 2002). ML trees were thus derived from: the ETS-ITS fig ML tree based on 56 taxa published in Rønsted et al. (2007), the *uluzi* and *sesqui* ITS2 trees derived from the *Otitesella* ML tree published in Jousselin et al. (2006) (based on 15 *uluzi* species and 20 *sesqui* species), the *Philotrypesis* ITS2-Cytb ML topology based on 16 species published in Jousselin et al. (2004), and both ITS2 and 28s-ITS2 pollinator ML topologies established on 26 species published in Erasmus et al. (2007).

Patristic distances to represent the phylogenies for ParaFit tests were computed from pruned ML trees using TreeEdit (Rambault and Charleston 2001; http://evolve.zoo.ox.ac.uk) and principal coordinates calculated using the R V.4.0 package for multidimensional and spatial analyses (Casgrain and Legendre 2001). Tests of random association in ParaFit were performed with 9999 permutations globally across both phylogenies (Desdevises et al. 2002).

COMPARISON OF WASP SEQUENCE DIVERGENCE

Knowledge of the relative divergence of lineages can help establish temporal congruence between phylogenies (true cospeciation as opposed to phylogenetic tracking, Percy et al. 2004) and also distinguish between the various explanations of incongruence (such as duplication and host switch) (Light and Hafner 2007). The very low variation observed in the ITS2 *Ficus* data (often zero for species in the same subsection) prevented us from comparing sequence divergence between figs and cospeciating wasps. On the other hand, all wasp phylogenies were partly based on ITS2, establishing a common scale for comparing the relative amounts of divergence in the four wasp lineages. Under the hypothesis that fig wasps are all equally constrained by their host in their diversification, they should also codiversify. We thus first tested cospeciation between wasp lineages and then, restricting our attention to pairs of phylogenies where significant amounts of cospeciation were detected, we compared sequence divergence between cospeciating wasps.

These cospeciation analyses between wasps can also provide useful information on the evolution of the community (Lopez-Vaamonde et al. 2001; Marussich and Machado 2007; Silvieus

et al. 2008). First, such comparisons give an indirect estimate of fig/fig wasp cospeciation that is not dependent on a well-resolved fig phylogeny. Additionally, as our tests involved wasps whose host figs were not necessarily included in the Galoglychia phylogeny of Rønsted et al. (2007), conducting pairwise comparisons between wasp phylogenies increases the sampling density (i.e., host coverage) over which fig/fig wasp cospeciation is tested. Finally, the detection of a cospeciation pattern between wasp lineages that would be uncoupled from fig/fig wasp cospeciation might reveal a specific ecological interaction between wasps, such as parasitoid/galler relationships between *Philotrypsesis* and the wasps they feed on (Marussich and Machado 2007).

All cospeciation methods postulate a host and a parasite lineage, which is not applicable to our pairs of wasp lineages. Therefore for these comparisons, we tested each wasp lineage against each other, first assuming one wasp lineage was "the host" and the other was "the parasite" and then inverting their respective roles. ML topologies used to derive trees were based on the same reconstructions as detailed in the previous section.

We then tested the presence of a molecular clock for each relevant ITS2 dataset (pollinators, *uluzi*, *sesqui*, *Philotrypesis*) using ML models of evolution selected by Modeltest (Posada and Crandall 1998) and a likelihood ratio test (Swofford et al. 1996): the difference between likelihood scores with a clock enforced and without a clock was used in a chi-square test using number of taxa minus two degrees of freedom. When the molecular clock was not rejected, we reconstructed ML ITS2 ultrametric trees using Paup* (Swofford 2002) (using heuristic searches, the TBR swapping algorithm with a molecular clock enforced), for each phylogenetic tree needed. Following Page (1990, 1991, 1996), we then used these new trees and plotted "host" divergence, against "parasite" divergence for cospeciating nodes inferred by TreeMap1. This divergence represented the "depth" of the cospeciating nodes in the phylogeny, that is the sum of branch lengths along the path from each node to any of its descendants. We assessed the correlation coefficient between the two variables. As branch lengths in ultrametric trees are not independent, we tested the significance of the correlations using the randomization test implemented in TreeMap: the observed R^2 value is compared to the distribution of R^2 obtained with 10,000 randomized trees. We then employed ordinary least square linear regression but also reduced major axis (RMA) regression to estimate the slope and intercept of the regression lines. RMA is more appropriate than ordinary least square regressions as the x and y variables involved here are equally subject to measurement errors (Sokal and Rohlf 1995). We used the RMA program for Java (Bohonak and van der Linde 2004). We recorded the 95% CI for the intercept using both the standard linear approximation and 1000 bootstrap replicates.

GLOBAL CONGRUENCE OF THE WASP PHYLOGENIES AND THE COMMUNITY SUPERTREE

To compare the phylogenetic trees representing the different wasp lineages associated with the same host figs in a global analysis, we used the method developed by Lapointe and Rissler (2005) for comparative phylogeography. This approach aims at combining several phylogeographic trees, exhibiting partially overlapping geographical regions. First, the global congruence of the source phylogenies is tested by a randomization procedure based on the size of Maximum Agreement Subtrees (Finden and Gordon 1985). When the source trees are shown to be more congruent than expected by chance alone, they are amalgamated using MRP (Matrix Representation with Parsimony), a common supertree construction method. If we replace geographical areas by "hosts," we typically have trees bearing a different number of leaves representing overlapping hosts (instead of regions) that can be synthesized into a supertree. We thus adapted the method of Lapointe and Rissler, and calculated a global congruency index of the wasp phylogenetic trees and proposed a test of its significance. This method has three advantages for our fig wasp dataset: (1) it does not presuppose host/parasite relationships, (2) it allows a test of the global congruence of multiple phylogenies instead of conducting pairwise tests, (3) it explicitly allows the incorporation of missing data; that is we can include all wasp species even when they were not collected on the same host figs.

For each wasp lineage, each species was labeled according to its host fig name. When two or more wasp species from the same species group were associated with the same host fig species, the fig name was duplicated (for instance, in the *uluzi* species group, *O*. sp. 46 and *O*. sp. 47.5 were coded as *Ficus burkei* 1 and *F. burkei* 2, respectively). We used MRP, the most common supertree construction method (Bininda-Emonds and Sanderson 2001; Salamin et al. 2002; Bininda-Emonds 2004) to reconstruct a "community supertree." MRP consists of a binary matrix representation of each tree, where each node is represented by a column. Taxa that are derived from a given node are scored as 1 in the corresponding column, those that are not but are present in the tree are scored as 0, and the other taxa are scored as missing data. These binary matrices are combined into a single matrix and leaves that are not in a given tree are coded as missing data in the corresponding matrix element. This combined matrix is then analyzed through Maximum Parsimony to reconstruct the supertree. The method is very similar to Brooks Parsimony Analysis (BPA) (Brooks 1981; Brooks and McLennan 2003) which translates a parasite tree into a binary matrix that is nearly identical to an MRP matrix (although, in BPA, columns of the matrix represent branches and not nodes of the parasite trees). However, with BPA, the matrix is not necessarily analyzed phylogenetically but treated as a character matrix optimized onto the host phylogenetic tree, and character homoplasy is then interpreted in terms of host switching and losses.

We used Rainbow (Chen et al. 2004) to generate the MRP matrix and Paup* (Swofford 2002) with TBR branch swapping and 1000 replicates to reconstruct the supertree. One of the most surprising results of our study is that, contrary to what is commonly believed, the pollinator phylogeny showed less congruence with the hosts than nonpollinating wasp phylogenies. To more accurately assess this result, we checked whether including the pollinator phylogeny in the congruence analysis decreased the global level of congruence between the different phylogenetic trees. We thus conducted two congruence analyses, one excluding the pollinator phylogenetic tree and one including it. The pollinator topology used was that obtained from ITS data.

Congruence of a set of source trees was assessed through MAST (Maximum Agreement Subtree) scores for all pairs of source trees. A MAST is the largest tree compatible with a given pair of trees. The MAST score of a pair of source trees is the number of leaves in their MAST. Trees of a pair to be compared were first pruned from taxa that did not appear in both source trees. Then MAST between the two trees restricted to the same set of leaves were computed in Paup*. As trees of different sizes (number of leaves) were compared, we computed normalized MAST scores, that is each MAST size was divided by the number of leaves appearing in the two compared trees. A congruence value for a set of more than two source trees is obtained by computing the average normalized MAST score over all pairwise comparisons of source trees. We first applied this to our wasp phylogenies, obtaining two such values, that is one excluding and one including the pollinator phylogeny.

To test the significance of these average normalized MAST scores we modified the randomization test suggested by Lapointe and Rissler (2005). A thousand sets of three trees (for comparison of the nonpollinator phylogenies only) and four trees (for comparisons including the pollinator phylogeny) were generated by shuffling taxa names on the original trees a thousand times using a Perl script. For each set, pairwise MAST scores were computed between trees restricted to their common leaves and the average normalized MAST scores were calculated. From the 1000 values obtained, for each test, a distribution of the normalized MAST scores was built. The original set of trees are thus considered more congruent than expected by chance if the observed average normalized MAST score is greater that that obtained for 95% of the random sets. Compared to the test described by Lapointe and Rissler (2005), where trees of similar size to the original set of trees are generated, but not necessarily with the same taxa and with the same topological structure, each generated tree in our tests had the exact same taxa as the tree it represented in the observed set. Thus, our shuffling procedure ensures that the overlap between generated trees is identical to that of the actual source trees, and also that the tree topologies are respected. This latter condition has been shown to have an impact on the computation of MAST scores (Bryant et al. 2003). This randomization procedure was repeated twice for each test to check the variability of the *P* value. Although runs gave very similar distributions, we conservatively report the largest *P* value. Scripts used to implement the test are available upon request to V. Berry.

Results

COSPECIATION BETWEEN FIGS AND ASSOCIATED WASPS

All *sesqui* ITS2 trees and *Philotrypesis* ITS2-Cytb trees used for the comparisons were resolved (i.e., there were no polytomies). For the fig phylogeny, the pollinator 28S and 28S-ITS2 phylogenies, and for the *uluzi* phylogeny, some of the nodes in the ML phylogenetic trees were unresolved or poorly supported. We thus tested alternative topologies in TreeMap. For the fig phylogeny, the ambiguities concerned a couple of shallow nodes and the relative placement of the closely related *Ficus craterostoma*, *F. natalensis*, and *F. burtt-davyi*. For the *uluzi* phylogeny, the ambiguity concerned a deeper node, that is the relative placement of wasps associated with subsection Caulocarpae. For the pollinator phylogenies, ambiguities mainly concerned closely related *Elisabethiella* wasps.

The results of cospeciation tests between *Galoglychia* figs and their pollinators varied according to the pollinator phylogenetic reconstruction tested. When the pollinator ITS2 phylogeny was tested against the fig phylogeny, the cospeciation hypothesis was rejected by both TreeMap 1 and ParaFit (Fig. 1A, Table 2), the maximum number of cospeciation events inferred by TreeMap 2.02 β was higher than that inferred by TreeMap 1 but randomization (constrained to a maximum number of three host switches due to computational limitations), again, indicated nonsignificant cospeciation. When the pollinator phylogenetic tree based on the combined analysis of 28S and ITS2 was considered, all methods detected significant cospeciation (Fig. 1B, Table 2). The main difference between the two pollinator reconstructions concerned the number of taxa rather than the tree topology, the only topological difference being the relative placement of the genera *Alfonsiella* and *Nigeriella* (Erasmus et al. 2007). There were less species included in the combined pollinator tree, because we obtained less 28S sequences than ITS2 sequences.

TreeMap1 suggested significant cospeciation between Galoglychia figs and their nonpollinating wasps of the *uluzi* species group, and between Galoglychia figs and the *sesqui* group, regardless of the fig and wasp topologies tested (Figs. 1C,D, Table 2). The optimum codiversification scenarios inferred with TreeMap 2.02β again gave higher numbers of cospeciation events for both comparisons, and the randomization test indicated significant cospeciation. The distance-based method, ParaFit, suggested similar codiversification scenarios (Table 2). All analyses

(Figs. 1B,C) suggested that there has been ancestral cospeciation at the node separating species associated with the *Caulocarpae* subsection and the rest of the wasp species, a couple of host switches have occurred between wasps associated with figs belonging to the *Chlamydodorae* and *Platyphyllae* subsections and associations between *sesqui* and *uluzi* wasps and figs of the *Crassicostae* subsection have resulted from several host switches.

The results obtained for the comparison of *Philotrypesis* and their host fig phylogeny varied according to the fig topology tested. The maximum number of cospeciation events inferred by TreeMap 1 between *Philotrypesis* and their host figs was seven (Fig. 1D), which was significant, but alternative fig topologies yielded nonsignificant results. When tested with ParaFit and TreeMap 2.02β, there was significant cospeciation between the two lineages. Again, the links between wasps associated with subsection Caulocarpae and their hosts all made the codivergence test results significant.

COMPARISONS OF WASP DIVERGENCE

Mean *p* genetic distances for each wasp lineage are given in Table 3. The average 28S genetic distance in Agaonidae associated with section *Galoglychia* was about five times that found

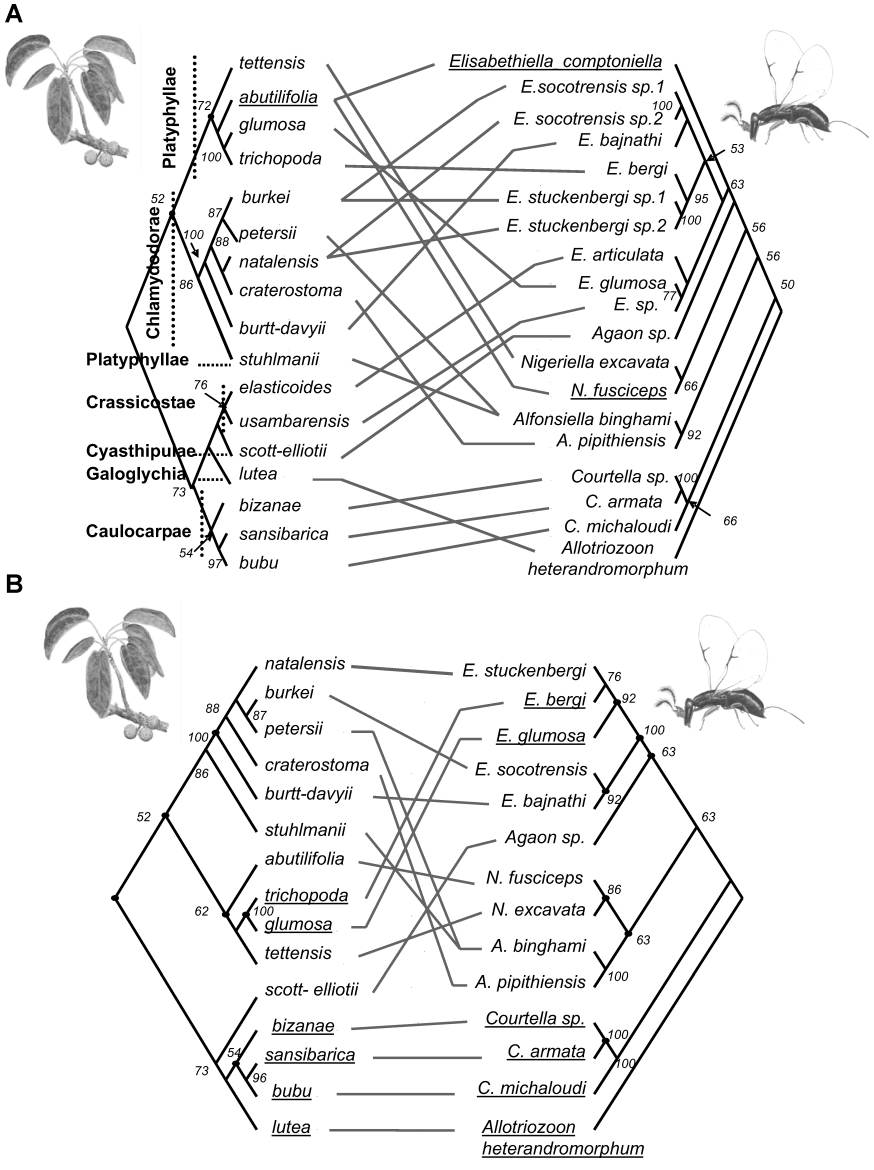

Figure 1. **Comparison of figs and fig wasps phylogenies. Underlined species represent significant links in ParaFit tests: (A) *Galoglychia* figs versus pollinator ITS2 ML phylogenetic reconstruction; (B) *Galoglychia* figs versus pollinator 28S-ITS2 ML phylogenetic reconstruction; (C) *Galoglychia* figs versus *sesqui* ITS2 ML phylogeny; (D) *Galoglychia* figs versus *uluzi* ITS2 ML phylogenetic reconstruction, (E) *Galoglychia* figs versus ITS2-Cytb ML *Philotrypesis* phylogenetic reconstruction. Node bootstrap supports are reported from Jousselin et al. 2004, 2006; Erasmus et al. 2007; and Rønsted et al. 2007.**

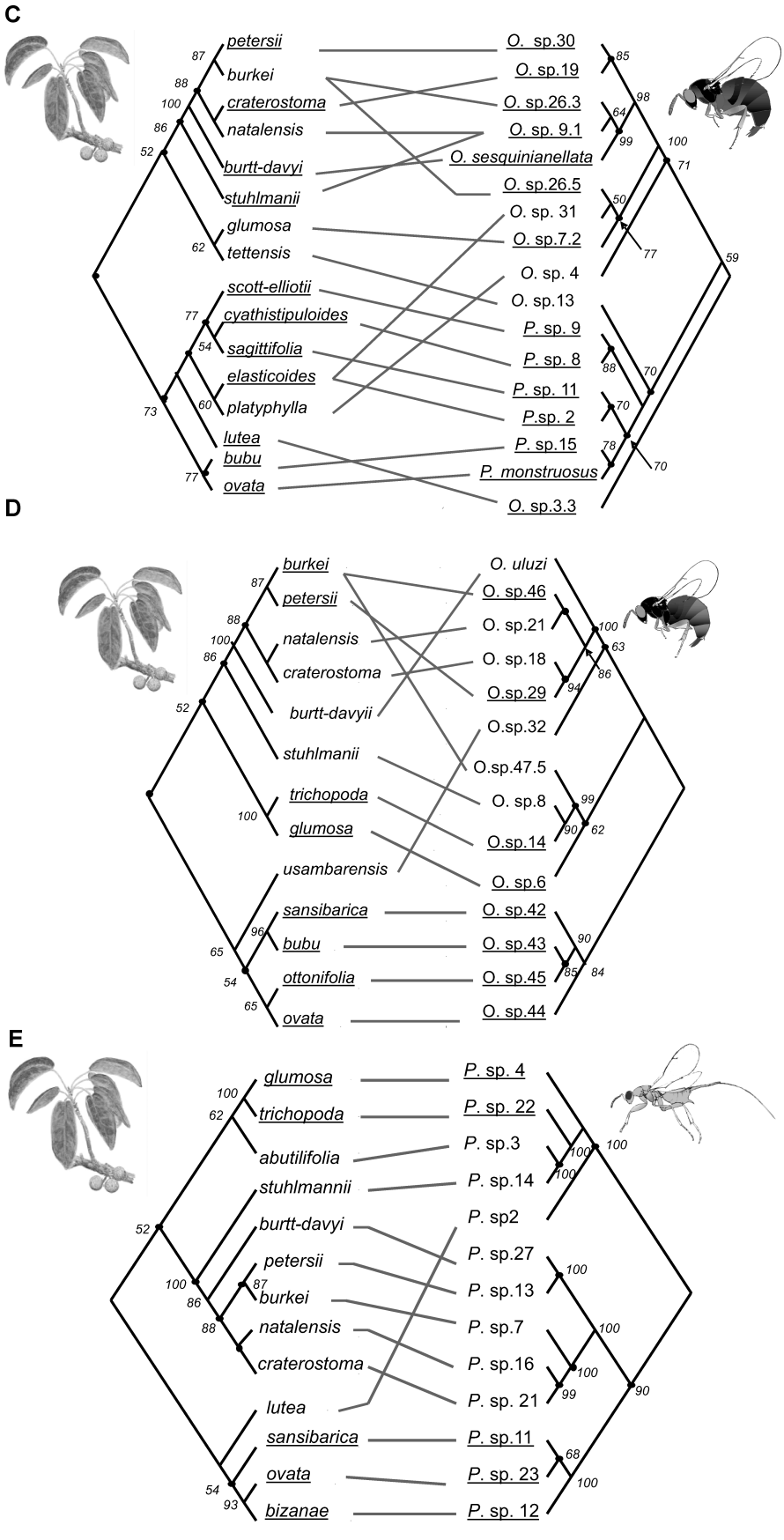

Figure 1. Continued.

Table 2. Results of cospeciation tests between figs and their associates using TreeMap1, TreeMap 2.02β, and ParaFit. *P* values in bold are significant at the 5% level. Numbers of host switches in bold for TreeMap 2.02β results, were limited to the value indicated. *P* values for TreeMap 2.02 β correspond to randomizations for reconstructions resulting in the highest numbers of cospeciation events and lowest cost. Numbers of links in ParaFit results refer to numbers of significant links. C, cospeciation; D, duplication; S, host switches; L, lineage extinction.

Wasp lineage		TreeMap 1		TreeMap 2. 02β		ParaFit		
	N^{br} *Ficus* sp./N^{br} of wasp sp.	N^{br} of cosp.	*P*	N^{br1} of opt.[1]	Codiversification scenari[1]	*P*	N^{br} of links	*P*
Uluzi	13/14	6–7	**0.02–0.03**	14	14<C<16, 10<D<12	<**0.01**	8	**0.003**
					S<5, 6<L<20			
Sesqui	16/17	8–9	<**0.001- 0.012**	29	14<C<20, 12<D<18	<**0.01**	14	**0.001**
					S<5, 18<L<37-			
Philotrypesis	13/13	6–7	**0.09–0.04**	25	6<C<14,10<D<18	<**0.01**	5	**0.02**
					S<5, 20<L<35			
Pollinator ITS2/28S	13/14	6–7	**0.02**	30	10<C<16, 10<D<16	<**0.01**	6	**0.003**
					S<5, 8<L<29			
Pollinator ITS2	17/19	6–7	0.20	11	10<C<14, 18<D<22	0.11	2	0.29
					S<3, 27<L<53			

[1]Opt, optimal solutions found by TreeMap 2.02 β.

in nonpollinating wasps. When we considered a single pollinating genus, for example *Elisabethiella*, 28S, *p* distances were only about twice those found in Otitesellinae. Wasps in the *sesqui* group were generally more divergent than those in the *uluzi* group. Similar patterns were observed for ITS2 sequences. Genetic distances in pollinators were twice or three times greater than in nonpollinating wasp lineages. Again when we considered pollinating wasps belonging to a single genus, *Elisabethiella*, the level of ITS2 sequence divergence was less than twice that found in *sesqui*, and about twice the level found in *uluzi* and *Philotrypesis*.

Several fig wasp ITS2 phylogenies comparisons showed significant cospeciation. As shown in Jousselin et al. (2006), *sesqui* and *uluzi* have diversified in parallel, and the cospeciation tests were significant, with all methods, irrespective of whether *sesqui* or *uluzi* were considered as hosts or parasites (Table 4, Fig. 2A). Cospeciation tests between *Philotrypesis* and *uluzi* were also significant with all methods, irrespective of whether *Philotrypesis* were considered as hosts or parasites (Table 4, Fig. 2B). Conversely, the comparison of *sesqui* with *Philotrypesis* was not significant (Table 4, figure not shown). For comparisons involving pollinating wasps, the only tests that gave marginally significant to significant results were comparisons between the *uluzi* phylogeny and pollinator ITS2 phylogenies, whether pollinators were considered as hosts or parasites (Table 4, Fig. 2C). For *sesqui*/pollinator (Fig. 2D) and *Philotrypesis*/pollinator comparisons (figure not shown), the cospeciation test results differed according to: the method used (ParaFit, TreeMap1 or TreeMap 2;02β) and whether pollinators were considered as hosts or parasites (Table 4). Overall, it seemed that inverting the role of hosts and parasites could change TreeMap test results dramatically. Reconciliation analyses aim at optimizing the "parasite tree" onto the "host tree," doing the reverse sometimes yielded very different diversification scenarios.

For comparisons that consistently showed significant numbers of cospeciation events (i.e., the comparisons of *sesqui* and *uluzi* trees and the comparisons of *Philotrypesis* and *uluzi* trees), we tested the presence of a molecular clock for each tree. As none of the ITS2 phylogenetic trees used in these comparisons rejected the existence of a molecular clock (*sesqui* tree : $\chi^2 = 14.19$, df $= 14$, $P = 0.33$; *uluzi* tree: $\chi^2 = 9.03$, df $= 12$, $P = 0.449$; *Philotrypesis tree*: $\chi^2 = 4.21$, df $= 9$, $P = 0.36$), we built ultrametric trees and plotted equivalent branch lengths (coalescence times at cospeciation nodes).

The equation for the RMA linear regression fitted between *sesqui* and *uluzi* divergence is shown in Figure 3A. The

Table 3. Mean *p* genetic distances (min-max values) among fig wasps for different lineages.

	Among pollinating wasp		Among nonpollinating wasps		
	All pollinating wasps	*Elisabethiella* genus	*uluzi* group	*sesqui* group	*Philotrypesis*
28S	0.067 (0.014–0.135)	0.023 (0–0.051)	0.008 (0.001–0.028)	0.014 (0.001–0.027)	
ITS2	0.254 (0.049–0.52)	0.126 (0.025–0.20)	0.051 (0.003–0.102)	0.082 (0.01–0.129)	0.062 (0–0.098)

Table 4. Results of cospeciation tests between fig wasp lineages using TreeMap 1, TreeMap 2.02 β, and ParaFit. All wasp lineages were tested against each other, first assuming one wasp lineage was "the host" and the other was "the parasite" and then inverting their respective roles. Numbers in parentheses in the first and second columns indicate numbers of species included in each lineage for each test. *P* values in bold are significant at a level of 5%. Numbers of host switches in bold for TreeMap 2.02β results, were limited to the value indicated. *P* values for TreeMap 2.02 β correspond to randomizations for reconstructions resulting in the highest numbers of cospeciation events and lowest cost. Numbers of links in ParaFit results refer to numbers of significant links. C, cospeciation; D, duplication; S, host switches; L, lineage extinction.

"Host" lineage	"Parasite" lineage	TreeMap 1		TreeMap 2.02β		ParaFit		
		N^{br} of cosp.	*P*	N^{br} of opt.[1]	Codiversification scenari[1]	*P*	N^{br} of links	*P*
Sesqui (10)	*uluzi* (11)	7	**0.01–0.001**	10	6<C<14, 6<D<14	<**0.01**	5	**0.021**
					0<L<16, **S<5**			
Uluzi	*sesqui*	4–6	**0.07–0.01**	63	2<C<12, 6<D<14	<**0.01**	7	**0.01**
					0<L<18, 0<S<8			
Philotrypesis (10)	*sesqui*(10)	3	0.30	119	0<C<10, 8<D<18	0.46	1	0.53
					0<S<9, 0<L<26			
Sesqui	*Philotrypesis*	4	0.15	119	0<C<10, 8<D<18	0.37	0	0.56
					0<L<26, 0<S<9			
Philotrypesis (10)	*uluzi* (11)	5–6	**0.003–0.009**	60	4<C<12, 8<D<16	**0.04**	8	**0.007**
					0<L<17, 0<S<8			
Uluzi	*Philotrypesis*	6	**0.001**	28	4<C<14, 4<D<14	<**0.01**	9	**0.004**
					0<L<11, 0<S<7			
Pollinator ITS2 (16)	*Philotrypesis* (14)	6	**0.006**		8<C<12, 14<D<18	0.11	4	0.16
					S<4, 22<L<45			
Philotrypesis	Pollinator ITS2	6	0.22	14	12<C<16, 18<D<22	0.33	2	0.10
					S<4, 26<L<52			
Pollinator ITS2 (13)	*uluzi* (13)	4–5	0.15–**0.02**	45	10<C<16, 8<D<14	<**0.01**	6	0.07
					0<S<6, 3<L<31			
Uluzi	Pollinator ITS2	3	0.5	24	8<C<14, 12< D<18	<**0.01**	4	**0.025**
					0<S<6, 0<L<23			
Pollinator ITS2 (15)	*sesqui* (15)	5	0.06	17	10<C<14, 14<D<18	0.08	1	0.36
					S<4, 8<L<40			
Sesqui	Pollinator ITS2	4	0.57	33	12<C<18, 14<D<22	**0.01**	0	0.26
					S<4, 14<L<35			

[1]Opt, optimal solutions found by TreeMap 2.02 β.

correlation was significant based on the randomization test described in Page (1996) ($r = 0.8163$, $P = 0.04$, 10,000 randomizations). Ordinary least square regression gave a line ($y = 0.4116x - 0.0003$) with a y intercept that was not significantly different from 0 ($F_{1,5} = 0.003$, $P = 0.99$). The 95% confidence interval from bootstrap [−(0.0229) − 0.0088] for the y intercept estimated from the RMA regression includes zero and also suggests that the diversification of these two lineages was probably synchronous. The slope of the regression suggested that ITS2 in *sesqui* evolved approximately twice as fast as in *uluzi*. Though TreeMap1 indicated significant cospeciation between *Philotrypesis* and *uluzi*, the correlation between *Philotrypesis* and *uluzi* was not significant according to the TreeMap1 randomization test; this was mainly due to the difference in branch lengths in the group of wasps associated with subsection *Caulocarpae* (*F. sansibarica* and *F. ovata*) (Fig. 3B).

As seen above, cospeciation between pollinators and the different nonpollinating wasps was not consistently significant. However, the cospeciation between *uluzi* and pollinators was almost always significant and there were some nodes in the pollinator phylogeny that had their obvious correspondent in the *uluzi* and *sesqui* phylogenies. For instance, the genus *Courtella* formed a monophyletic group associated with subsection *Caulocarpae* and in nonpollinating wasp phylogenies most wasps associated with subsection *Caulocarpae* also formed a clade. Similarly, some nonpollinating wasps that were presumably sister species were associated with pollinating wasps that were also closely related. Silvieus et al. (2008) have shown that comparing congruent nodes in phylogenies could be more relevant than comparing nodes retrieved in reconciliation analyses when studying cospeciation. Therefore, in an attempt to compare evolutionary rates and timing of speciation events between pollinators and nonpollinating wasps, we

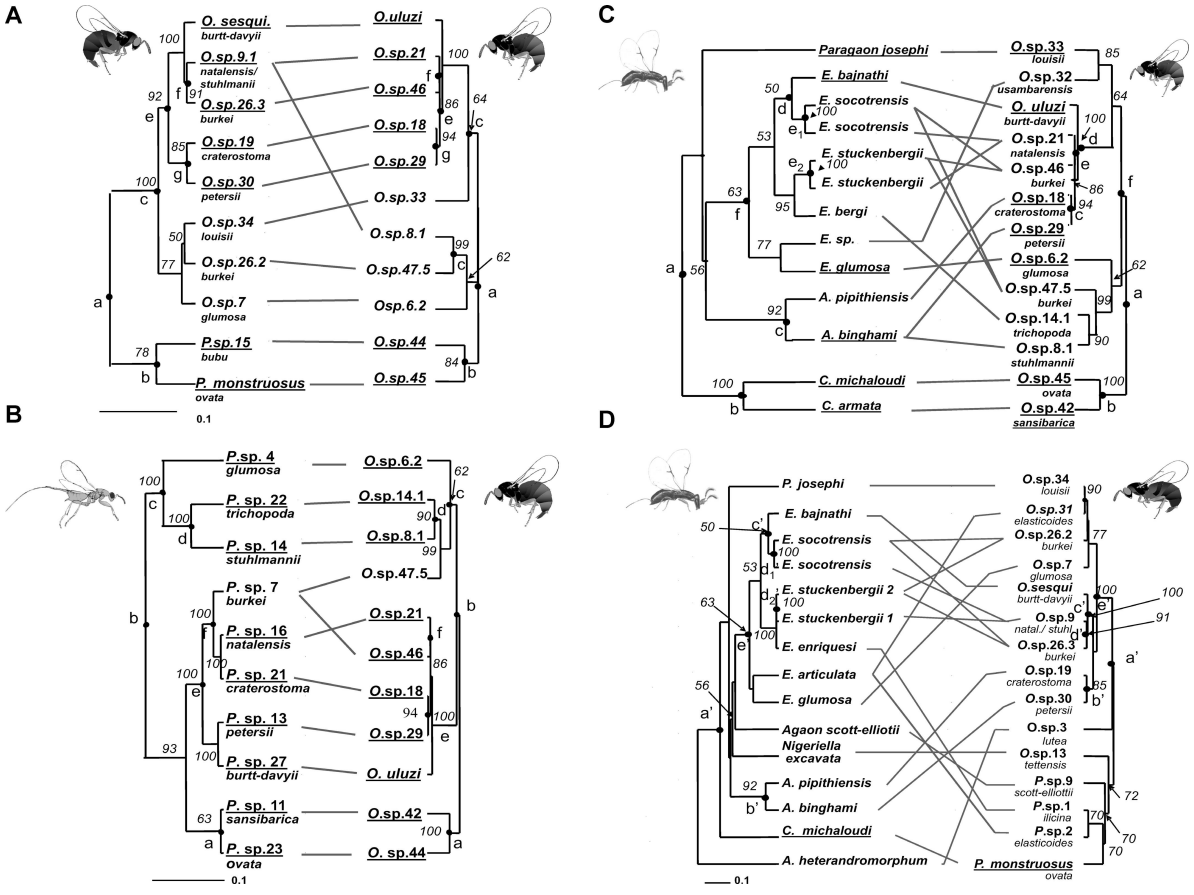

Figure 2. **Pairwise comparisons of ITS2 fig wasps phylogenies (based on ML analyses): (A) *sesqui* versus *uluzi* phylogeny; (B) *Philotrypesis* versus *uluzi* phylogeny; (C) pollinator versus *uluzi* phylogeny; (D) pollinator versus *sesqui* phylogeny. Nodes used for branch length comparisons between wasp lineages are indicated on each figure. Underlined species represent significant links in ParaFit tests. Node bootstrap supports (1000 replicates) have been reevaluated using ML reconstructions with a molecular clock enforced (using Paup *).**

first checked that the pollinator ITS2 trees used in the comparison did not reject the existence of a molecular clock (pollinator: $\chi^2 = 25.08$, df $= 16$, $P = 0.07$) and then compared coalescence times on ultrametric trees at several nodes. For instance, we compared the depths of nodes separating wasps associated with *Caulocarpae* from the rest of the wasps, in *sesqui*, *uluzi*, and pollinator phylogenies (i.e., pollinating wasps belonging to the *Courtella* genus). We also compared the node separating all *Elisabethiella* sp. associated with *Chlamydodorae* and the Platyphylla *Ficus* subsection from other pollinators with the nodes separating *uluzi* and *sesqui* wasps associated also with *Chlamydodorae* and *Platyphylla* from the rest of the wasps. The node separating the two pollinator sister species (*A. pipithiensis*, *A. michaloudi*) associated with *F. craterostoma*, and *F. petersii*, with the nodes separating the two *sesqui* sister species and the two *uluzi* sister species also associated *F. craterostoma* and *F. petersii*, was also included in the analyses. Hence, within the pollinator phylogeny, the nodes used were only nodes defining phylogenetic relationships within pollinator genera (*Elisabethiella* and *Alfonsiella*) except for the node separating the genus *Courtella* from the other pollinating wasps, and they were often retrieved via reconciliation analyses (most TreeMap 2.02β reconstructions) even when cospeciation was not significant.

These results all suggested a strong correlation between nonpollinating wasp divergence and pollinator divergence, as indicated by the R^2 values (Fig. 3C). Both R^2 values were significant according to the branch length randomization tests (*sesqui* $P = 0.04$, *uluzi* $P = 0.02$). For both least square linear regressions, the *y* intercept was not significantly different from 0 ($P = 0.60$, $P = 0.99$). The 95% confidence intervals from bootstrap for the *y* intercept for RMA regressions also suggests that the lines passed through 0 (*y* intercept: *uluzi* vs. pollinators [(−0.0229) − 0.00883], *sesqui* vs. pollinators [(−0.0093) − 0.01]).

GLOBAL CONGRUENCE OF THE WASP PHYLOGENIES AND FIG WASP COMMUNITY SUPERTREE

The average normalized MAST score for the set of nonpollinator phylogenies was 0.567, whereas, the set of trees containing both nonpollinator and pollinator phylogenies was a bit lower, with a value of 0.520 (Table 5). For both sets of trees, the distributions

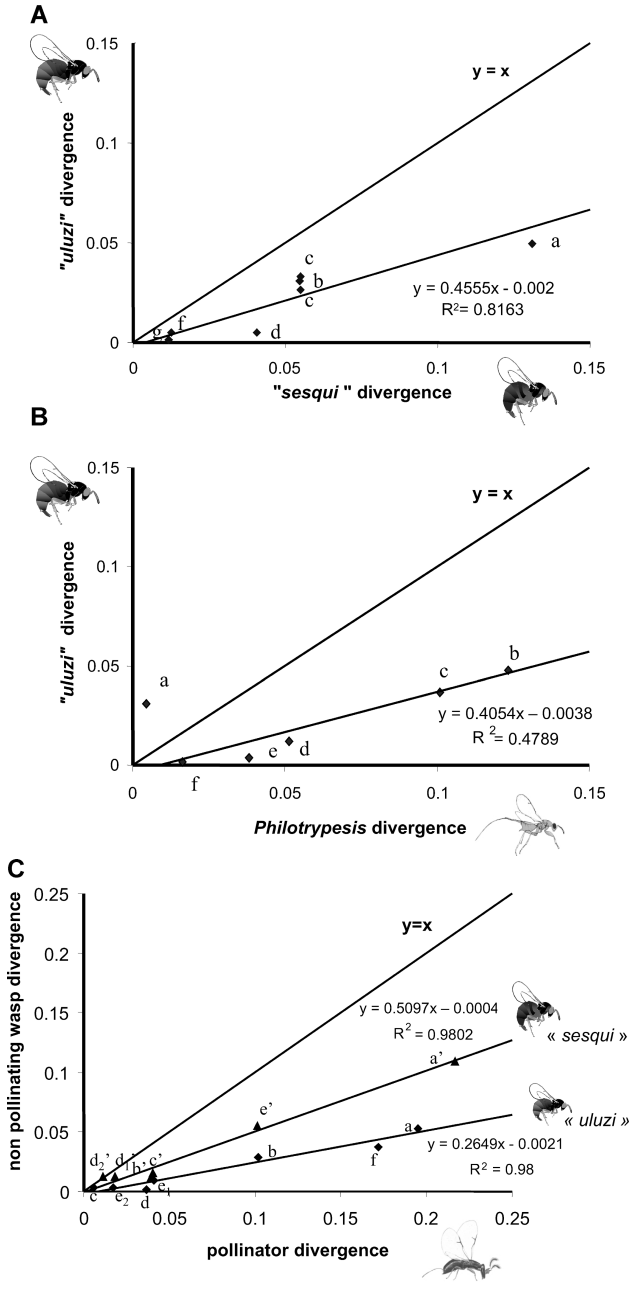

Figure 3. Plots of ITS2 sequence divergence for cospeciating nodes. Sequence divergence refers to the "depth" of the nodes of ultrametric ML ITS2 phylogenetic trees from Figure 2. The reduced major axis regression lines are drawn for each comparison. (A) *uluzi* against *sesqui* divergence for cospeciating nodes indicated in Figure 2A; (B) *uluzi* against *Philotrypesis* divergence for cospeciating nodes indicated in Figure 2B; (C) *uluzi* and *sesqui* divergence against pollinator divergence for nodes indicated in Figures 2C,D.

obtained by randomizing trees indicated that these values were significant (for the three nonpollinating wasp trees: $0.0049 < P < 0.005$; for the nonpollinating wasp trees plus the pollinator tree, $0.0001 < P < 0.0002$). It is thus appropriate to combine nonpollinating wasp trees into a supertree, and also to combine

Table 5. The values in the upper triangle are MAST (Maximum Agreement Subtree) scores for each wasp phylogeny pairwise comparison. The values in the lower triangle are the numbers of host fig species in common between two trees.

	sesqui	*uluzi*	*Philotrypesis*	Pollinators
Sesqui		0.54	0.55	0.5
Uluzi	11		0.60	0.46
Philotrypesis	9	10		0.46
Pollinators	14	13	13	

nonpollinator phylogenies and the pollinator phylogeny into a supertree. These tests suggest that in both cases the original sets of trees are more congruent than expected by chance and thus that wasp lineages share the same phylogenetic history. Surprisingly, although the normalized MAST score obtained for the parasite phylogenies was higher than that obtained for the pollinator and nonpollinating wasp trees, the *P* value obtained was better in the second test. This suggests that the number of trees compared might influence the randomization test results. Simulations might be necessary to test the power of the randomization test and the influence of the number of trees being compared on Type I error.

Both wasp supertrees obtained were in agreement with the *Galoglychia* fig phylogenetic reconstruction in several places (Fig. 4). In the supertree including only the nonpollinating wasp phylogenies, the clades defined corresponded closely to the different *Galoglychia* fig subsections. However, in the supertree including the pollinator phylogeny, several *Ficus* subsections appeared to be polyphyletic. These results probably reflect switches between distantly related host figs and/or duplication events during pollinator diversification.

Discussion

DIVERSIFICATION PATTERNS OF FIG WASPS: ARE POLLINATORS MORE CONSTRAINED BY THEIR HOST ASSOCIATION THAN NONPOLLINATING FIG WASPS?

Cospeciation analyses and a global test of congruence using a supertree construction method all suggest that nonpollinating wasp and pollinating wasp phylogenies are strongly structured with respect to their host fig phylogenies. Even when considering several wasp lineages with different life-history traits, the overall picture of fig wasps phylogenetic histories is one of concordance with the host fig phylogeny. This result is also confirmed indirectly by the parallelism of nonpollinating wasp phylogenies, which is probably partly driven by their common history with their host figs. Hence, in *Galoglychia* figs, codivergence in the diversification of the various fig wasp lineages has probably played a significant role in building the wasp community. Our results concerning

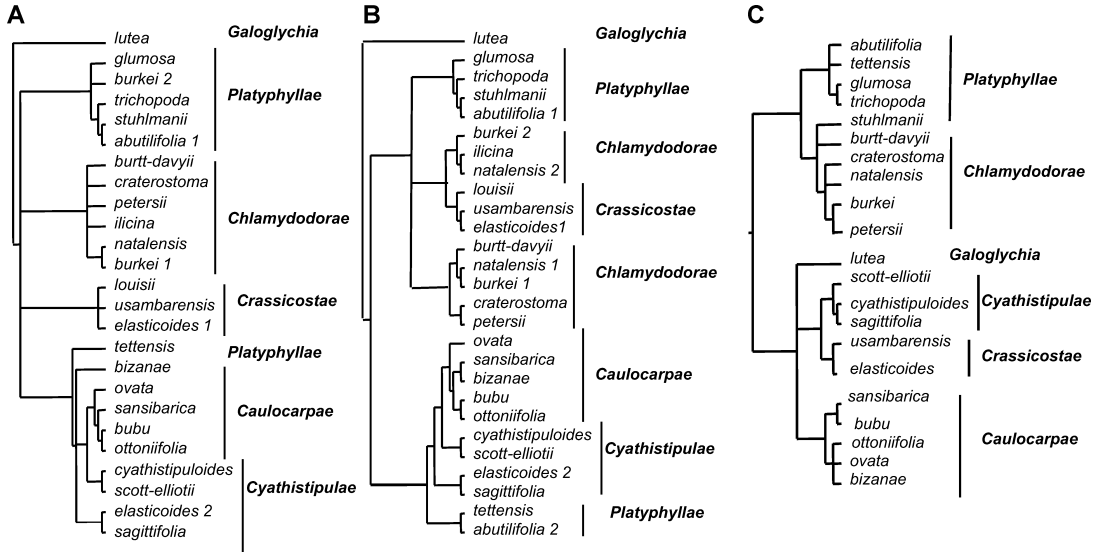

Figure 4. MRP supertree of the fig wasp phylogenies (each wasp species is labeled as its host fig species): (A) supertree excluding the pollinator phylogeny; (B) supertree including the pollinator phylogeny; (C) ML topology for the *Galoglychia* fig phylogeny (retrieved from Rønsted et al. 2007). *Ficus* subsections are indicated on the right inside of the figures.

Galoglychia figs also contradict the current consensus that pollinators show more codivergence with their host figs than nonpollinating wasps (Weiblen and Bush 2002).

These results must be considered provisional because they rely on a nonexhaustive sampling and it is know that missing data can have a major impact on codiversification analyses (Jackson 2004). This is exemplified by our cospeciation tests results between *Galoglychia* figs and their pollinators that varied according to the pollinator phylogenetic reconstruction tested (ITS2 vs. combined 28S-ITS2). Because the combined analysis (i.e., 28S-ITS2) included less species, fig species that normally host several pollinator species (sometimes species belonging to different genera) were erroneously associated with a single species in the cospeciation test. High host specificity tends to make recovery of a large number of cospeciation events more likely by chance through reconciliation analyses (Jackson 2004). Hence, excluding some species in the pollinators' phylogeny erased a number of mismatched species, resulting in partially artificial cospeciation. However, the main cospeciation signals in nonpollinating wasp phylogenies come from monophyletic groups of wasp species associated with a monophyletic subsection of figs. The lack of cospeciation between pollinators and their host figs relies on the monophyly of the pollinator genera and the lack of a one-to-one association between wasp genera and fig subsections. These results were strongly supported in our phylogenetic reconstructions and we believe that they would likely not change with an increase in wasp sampling.

The fact that nonpollinating wasps show congruence with their host phylogeny is actually not surprising. Nonpollinating wasps exhibit many characteristics that should favor their host fidelity and thus limit host switching. Whether they lay their eggs at fig receptivity, before fig receptivity, or during the fig development, the ovipositing process relies on strong adaptations with the host figs. Like pollinating wasps, nonpollinating wasps could use chemical cues to locate their hosts (Ware et al. 1993; Grison-Pigé et al. 2002), which could lead to host specialization. For "external" nonpollinating wasps, that is wasps that lay their eggs by inserting their ovipositor through the fig wall such as *Philotrypesis* and Otitesellinae, ovipositors have to be highly adapted to the structure and thickness of the fig wall. The galling process, necessary for successful development of Otitesellinae wasps, is also known to be highly specific (Cook et al. 2002; Shorthouse et al. 2005). Furthermore, all these nonpollinating wasps mate on their host fig (Greeff and Ferguson 1999), another feature that selects for host specificity. Hence, many factors could favor host conservatism throughout nonpollinating wasp evolution. Alternatively, the strong cospeciation signal detected between *sesqui* wasps and *Philotrypesis* could be indicative of a specialized host/parasitoid interaction. *Philotrypesis* could follow the radiation of *uluzi* wasps that diversified in parallel with their host figs. As underlined in the Introduction, crucial biological information for these fig associates are still missing to confirm this conclusion, and it is difficult to say whether the only reason behind the parallel diversification of the two wasp lineages is based upon sharing the same host figs.

The fact that pollinating wasps do not show more convergence with their host phylogeny is maybe more surprising at first. However, the occurrence of multiple pollinator species per fig (Machado et al. 2005, Haine et al. 2006) is an evidence of the ability of pollinators to switch host and/or duplicate (i.e., speciate) on their host. Pollinator host switches are actually not as unlikely

as previously thought. Contrary to what has long been believed, pollinators do not need to ensure efficient fertilization of the fig in which they lay their eggs to produce viable offspring (Galil and Eisikowitch 1971; Jousselin et al. 2003a). Hence, their fitness is not strictly dependent on their host fidelity. The long dispersal ability of pollinators (Nason et al. 1998; Harrison and Rasplus 2006) might also facilitate host switching in unusual ecological conditions. In situations in which fig trees remain unpollinated because climatic events have caused local pollinator extinction, long pollinator jumps between host species have actually been observed (Compton 1990; Bronstein and Hossaert-McKey 1995; Harrison and Rasplus 2006). Such host shifts are facilitated when the resident pollinator is absent, successful colonization and displacement of the locally adapted pollinator is probably a less likely event (Weiblen and Bush 2002).

Finally, our results may not accurately reflect differences in host fidelity and/or various responses to host isolation between wasp lineages. It is known that cophylogenetic analyses may identify cospeciation events if host shifts occur primarily between closely related hosts (Charleston and Robertson 2002). The parallel divergence of nonpollinating wasps with their host figs could thus merely reflect phylogenetic tracking and not synchronicity of speciation events. Conversely, the more haphazard host association exhibited by the pollinators associated with section *Galoglychia* may not be the result of multiple host switches. Rather, it could be the result of some ancient duplication of several pollinating wasp genera on Galoglychia figs followed by cospeciation and asymmetrical extinctions (Erasmus et al. 2007). This scenario is quite likely. As suggested by former studies (Weiblen and Bush 2002; Jackson 2004), in contrast to nonpollinating wasps that seem to shift to different ecological niches when speciating on their host fig (Weiblen and Bush 2002; Jousselin et al. 2006), pollinator speciation (duplication) could be easily followed by lineage extinction because of niche competition (but see Zhang et al. 2004). This scenario could actually apply to *Alfonsiella* and *Elisabethiella,* two well-diversified pollinator genera that are both associated with figs from two *Galoglychia* subsections, *Chlamydodorae* and *Platyphylla* (Burrows and Burrows 2003). This association pattern could result from an ancient duplication followed by asymmetrical lineage extinction. In other words, the occurrence of paralogous lineages (Jackson 2005) could influence our cospeciation tests between pollinators and figs. Hence, unraveling the real history of pollinating and nonpollinating fig wasps requires a test of the synchronicity of speciation events in wasp lineages.

AN ATTEMPT TO COMPARE TIMING OF SPECIATION EVENTS IN SEVERAL FIG WASP LINEAGES

By comparing sequence divergence in cospeciating pairs of nonpollinating wasps and pollinator wasps, we found significant linear regressions with an intercept of zero for regressions obtained between *uluzi* and *sesqui* and between pollinators and these two lineages. The linear regressions imply two things. First, they suggest that speciation of these wasps has been synchronous and second, that pollinating wasps exhibit faster rates of evolution. In addition, the cospeciating nodes used within the pollinator phylogeny were limited to intragenera relationship, our results thus suggest that the divergence observed within *Elisabethiella* was proportional to the divergence of both *uluzi* and *sesqui* wasps associated with the same figs in *Platyphyllae* and *Chlamydodorae Ficus* subsections, which suggests that the associations do not result from *Elisabethiella* host switches from one fig section to the other but rather that *Elisabethiella* has diversified in parallel with their host figs. These results must be interpreted with extreme caution because they rely on a single short DNA fragment (ITS2) and a few cospeciating nodes. Further tests of whether *Elisabethiella* and *Alfonsiella* represent two separate radiations on their host figs will necessitate extensive sampling within the *Alfonsiella* genus.

Nevertheless, the hypothesis of synchronous speciation in nonpollinating wasps and their host figs is not unlikely given our data. The occurrence of two sister clades of Otitesellinae wasps (*sesqui* and *uluzi*) that both show synchronized codivergence with figs from section *Galoglychia* strongly suggests that the common ancestor of these two groups of wasps had colonized *Galoglychia* figs prior to their diversification on the African continent (Jousselin et al. 2006). Assuming the alternative scenario, that is, that the colonization of African figs by Otitesellinae, and thus the split between *uluzi* and *sesqui*, postdates the diversification of *Galoglychia* figs, would imply that all *Galoglychia* fig species have captured a *sesqui* and an *uluzi* in a manner that mimics the fig phylogeny. And although phylogenetic tracking can easily lead to topological similarity between phylogenies (Charleston and Roberston 2002), to our knowledge it rarely leads to significant linear regression in divergence times, such as those observed here between pollinators and nonpollinating wasps and between the two nonpollinating wasp lineages *uluzi* and *sesqui*. This scenario of synchronous speciation could be confirmed if the phylogeny of Chalcidoidea (Rasplus et al. 1998; Campbell et al. 2000), that encompasses all fig wasp families, could be calibrated and used to compare timing of divergence of all fig wasps. Unfortunately, this phylogeny is still largely unresolved. Preliminary results suggest that Agaonidae (the pollinator family), are at the base of the tree, but also that the family is subtended by a very long branch of the tree (Rasplus et al. 1998). Hence, Agaonidae could be both older than other fig wasp families, but could also evolve faster. There are actually several factors that could favor a faster evolutionary rate in pollinating fig wasps comparatively to nonpollinating wasps. First, pollinators are highly inbred, which leads to a reduced effective population size and can make more mutations effectively neutral (Halliburton 2004). Second, pollinators have highly female biased sex ratios and can have multiple matings (Hamilton

1967; Herre 1987). Pollinators therefore must produce a lot of sperm (Murray 1990; Greeff and Ferguson 1999). Such intense sperm competition in pollinators might increase their mutation rates (Moller and Cuervo 2003), as it is actually the generation time of germinal lines that influences evolution rates and not the generation time per se. Differences in sequence divergence could thus reflect radically different reproductive strategies in pollinating and nonpollinating wasps.

ASSESSING THE CONGRUENCE OF MULTIPLE PHYLOGENIES OUTSIDE A HOST–PARASITE FRAMEWORK

Our study also addresses important methodological issues. It underlines the lack of specific methods aimed at investigating the congruence of two or more phylogenies of different lineages outside a host/parasite relationship framework and at testing whether they share diversification events. Such questions are frequently addressed through pairwise cospeciation tests (Lopez-Vaamonde et al. 2001, 2005; Jousselin et al. 2006; McLeish et al. 2007). We show here that the use of reconciliation analyses can be problematic in such a framework because results can change dramatically depending on which lineage is considered as the "host" or the "parasite," as the evolution model in reconciliation analysis is asymmetric. Moreover, inverting the "role" of the two lineages automatically changes the degree of parasite specificity and therefore influences the results of tree comparisons (Jackson 2004). Our results suggest that a method that tests a global congruence level, such as ParaFit, is more appropriate to the questions of parallel diversification of wasps, as cospeciation test results do not vary depending on which lineage is considered as the "host" or the "parasite." Comparatively to TreeMap 2.02β that is still hindered by computational limitations, it is also adapted to the most complicated association patterns and to large datasets.

In an attempt to improve methodological approaches in the field of cospeciation and community evolution, we adapted a randomization procedure previously used in a comparative phylogeography study (Lapointe and Rissler 2005) to test the congruence of a set of phylogenies having different taxa. We applied it to our wasp phylogenies. Given the high level congruence detected, we built a supertree in amalgamating trees from different wasp lineages. As discussed previously (Hall and Harvey 2002; Racheli 2004), supertree construction based on topological character matrices such as MRP matrix is quite similar to BPA. However, it is not interpreted in terms of lineage losses and host switches. The supertree approach aims at presenting a general picture of the community and at giving a global congruence level of the trees. This approach is more relevant for the question of community evolution. Moreover, MRP is easy to apply thanks to the availability of supertree construction software packages. The development of congruence index for supertree construction and a test of its significance, such as the one proposed here and in a recent study (de Vienne et al. 2007), provides new tools for measuring the congruence of sets of phylogenies and can be of future use in community phylogenetic studies. Furthermore, in addition to allowing the comparison of multiple trees, it also allows users to take unbalanced sampling in the phylogenetic trees into account. Indeed, the procedure we propose takes the varying numbers of species that pairs of tested phylogenies have in common into account.

Other new developments for comparative phylogeography, such as the use of coalescent simulations (Althoff et al. 2007), seem to open promising avenues for comparing diversification patterns in multiple lineages. Through this article, we want to encourage the bridging of gaps between the fields of comparative phylogeography and cospeciation studies that could use common analytical tools to investigate phylogeny congruence. More phylogenies are becoming available with the increasing use of molecular tools. Methods inferring codiversification scenarios such as reconciliation analyses are attractive, but as soon as the host–parasite association patterns are too complex or the phylogenies are too large, the multiplicity of the scenarios yielded is often overwhelming. We suggest that global fit methods such as ParaFit or the supertree method for multiple phylogenies might often be the only practical method to implement.

CONCLUSIONS AND PERSPECTIVES

This study confirms that construction of the *Galoglychia* fig wasp community is highly dependent on the codiversification of wasps with their host figs. This suggests that all fig wasps (pollinating and nonpollinating) are specialized on their host. It might also reflect a certain level of saturation of ecological niches within the fig that prevents recurrent host colonization by wasps and/or speciation on their hosts. Clearly, we also show that host switching and duplication (intrahost speciation) have occurred in both pollinator and nonpollinator diversification. However, the association patterns observed suggest that pollinator duplication in *Galoglychia* figs might have been followed by asymmetrical lineage extinction. On the other hand, congeneric nonpollinating wasps, such as *sesqui* and *uluzi*, seem to be able to coexist via the evolution of ecological differences when speciating on their host (Weiblen and Bush 2002; Jousselin et al. 2006). To formally test this hypothesis, information concerning the temporal framework of speciation in the different wasp lineages is needed. This will require phylogenies based on afar more exhaustive species sampling. Once this is acquired, the challenge will be to go beyond phylogenetic studies and get data not only on wasp ecological niches, their dispersal abilities, and population sizes but also on wasp ecological interactions. This is necessary if we want to understand the processes underlying the diversification patterns revealed by the phylogenies. Recent studies investigating the rules of natural community assembly strongly urge that the role of species diversification needs to be assessed

(Losos 1996; Gillespie 2004; Stephens and Wiens 2004; Kozak et al. 2005; Weiblen et al. 2006). The fig/fig wasp system, one of the classic models for studying coevolution, probably provides a unique opportunity to unravel the construction and maintenance of a community through phylogenetic studies.

ACKNOWLEDGMENTS

We thank the people who participated in the field work and helped in gathering wasp specimens: S. Bajnath, A. Watsham, S. Meusnier, and J. Pienaar. Many thanks to Y. Desdevises for numerous insightful discussions about cospeciation analyses and to G. Kergoat for very helpful comments on an early draft of the manuscript and for inspiration for the title. We also thank R. Page, M. Hafner, C. Machado, D. Percy, A. Paterson, J. Sullivan for very constructive comments on earlier versions of this manuscript. This material is based upon work supported by the National Research Foundation under Grant number 2053809 to JMG. EJ was supported by an NRF postdoctoral fellowship during most of this work. The article also benefited from collaborations supported by the "NICE Fig project" (ANR) funding from the NRF grant GUN 61497 to SVN for field collecting.

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Associate Editor: P. Tiffin