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Molecular Phylogenies of Fig Wasps: Partial Cocladogenesis of Pollinators and Parasites

Carlos Lopez-Vaamonde,* Jean Yves Rasplus,† George D. Weiblen,‡ and James M. Cook*,[1]

*Department of Biology & NERC Centre for Population Biology, Imperial College, Silwood Park, Ascot, Berkshire SL5 7PY, United Kingdom; †INRA, Centre de Biologie et de Gestion des Populations, Campus International de Baillarguet, CS 30016, 34988 Montferrier-sur-Lez, France; and ‡Department of Zoology, 203 Natural Science Building, Michigan State University, East Lansing, Michigan 48824

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Figs (*Ficus* spp., Moraceae) and their pollinating wasps form an obligate mutualism, which has long been considered a classic case of coevolution and cospeciation. Figs are also exploited by several clades of nonpollinating wasps, which are parasites of the mutualism and whose patterns of speciation have received little attention. We used data from nuclear and mitochondrial DNA regions to estimate the phylogenies of 20 species of *Pleistodontes* pollinating wasps and 16 species of *Sycoscapter* nonpollinating wasps associated with *Ficus* species in the section *Malvanthera*. We compare the phylogenies of 15 matched *Pleistodontes/Sycoscapter* species pairs and show that the level of cospeciation is significantly greater than that expected by chance. Our estimates of the maximum level of cospeciation (50 to 64% of nodes) are very similar to those obtained in other recent studies of coevolved parasitic and mutualistic associations. However, we also show that there is not perfect congruence of pollinator and parasite phylogenies (for any substantial clade) and argue that host plant switching is likely to be less constrained for *Sycoscapter* parasites than for *Pleistodontes* pollinators. There is perfect correspondence between two terminal clades of two sister species in the respective phylogenies, and rates of molecular evolution in these pairs are similar.

Key Words: **Agaonidae; *Pleistodontes; Sycoscapter; Ficus*; fig wasps; *Malvanthera*; coevolution; cospeciation; 28S; ITS2; cytochrome *b*.**

INTRODUCTION

The speciation of parasites is often thought to be driven by the speciation of their hosts, such that, when an ancestral host species splits into two daughter species, its parasite also speciates. This assumption underpins the idea (Fahrenholz's rule) that host and parasite phylogenies should be mirror images (Lyal, 1986). Although cospeciation is not a new idea, the opportunity offered by molecular techniques to test it has led to a burst of interest and activity in this area (Hafner *et al.*, 1994; Hafner and Page, 1995; Moran *et al.*, 1995; Page, 1996a; Farrell and Mitter, 1998; Peek *et al.*, 1998; Clark *et al.*, 2000). Molecular markers may permit the estimation of phylogenies for parasites and their hosts by use of genes that (unlike some morphological traits) are not coadapted to the species interaction. Further, if cospeciation is inferred, molecular data can be used to compare rates of evolution in host and parasite lineages (Hafner and Nadler, 1990; Hafner *et al.*, 1994; Hafner and Page, 1995; Moran *et al.*, 1995; Page, 1996a; Paterson *et al.*, 2000).

To date few cospeciation studies have produced molecular phylogenies for both sets of taxa and insights are limited to a few systems. One interaction in particular—that between pocket gophers (geomyid rodents) and their chewing lice—has dominated both methodological (Page, 1990, 1993a,b, 1994) and biological (Nadler *et al.*, 1990; Demastes *et al.*, 1998) debates about cospeciation. The pioneering studies based on allozyme (Hafner and Nadler, 1988) and DNA (Hafner *et al.*, 1994) data provided evidence for a high degree of cospeciation in the gopher–louse assemblage. However, both studies are based on small phylogenies and may not be typical of mammals and lice, or other ectoparasites, in general (Taylor and Purvis, 2001). A recent study of pinworms and primates (Hugo, 1999) suggests that at least some endoparasites have undergone considerable cospeciation with their hosts. More predictably, high cospeciation levels have been inferred between invertebrates and endosymbiotic algae or bacteria that contribute significantly to host metabolism (Peek *et al.*, 1998; Funk *et al.*, 2000). Finally, whereas endogenous retroviruses generally track host phylogeny, occasional host shifts do occur, even between mammals and birds (Martin *et al.*, 1999).

[1] To whom correspondence and reprint requests should be addressed. Fax (+44) 207 594 2339. E-mail: j.cook@ic.ac.uk.

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Lice, endosymbiotic bacteria, retroviruses, and fig wasps are not expected to have the same levels of cospeciation as they encompass a range of interactions, with different transmission mechanisms. However, the same methods can be used to analyze these different systems, and, as case studies accumulate, it should become possible to identify general features of interactions (e.g., transmission mode) that promote or limit cospeciation (Clayton *et al.*, 2001).

Phytophagous insects can be regarded as parasites of their host plants (Price, 1977) and may be generalists or specialists. Some specialists attack just a single plant species, whereas many others feed on a few closely related species. Interaction and coevolution have probably been important during the radiations of plant lineages and their insect herbivores, with plant chemistry playing a key role (Ehrlich and Raven 1964); however, strict sense cospeciation seems generally unlikely (Farrell and Mitter, 1990, 1998). More specific interactions occur between certain groups of plants and their pollinators and perhaps the two best-known cases are figs and fig wasps (Herre, 1996; Anstett *et al.*, 1997) and yuccas and yucca moths (Pellmyr and Leebens-Mack, 1999). Both of these systems are mutualisms but also involve conflicts of interest, principally over whether female flowers nourish developing seeds or insect larvae.

Figs (*Ficus* spp.) and their pollinating wasps (family Agaonidae *sensu* Rasplus *et al.*, 1998) provide a classic example of an obligate mutualism. Most *Ficus* species have their own unique pollinating wasp species, whose larvae feed on the fig seeds. Each partner relies on the other to complete its life cycle and many morphological characters of the partners are thought to be coadapted (Herre, 1989; Van Noort and Compton, 1996). The biology of figs and their pollinators has led to a general acceptance of the idea that they have coevolved and cospeciated extensively. However, rigorous testing of this idea has been hindered by the fact that the pollinator taxonomy has developed under the influence of existing fig taxonomy and a strong cospeciation assumption (Ramirez, 1974, 1977; Wiebes, 1982b).

Fig-based invertebrate communities lend themselves to cospeciation studies because, in addition to pollinating wasps, there are diverse nonpollinating wasps, mites, drosophilids (Harry *et al.*, 1996), and parasitic nematodes (Poinar and Herre, 1991). Many (perhaps most) of these taxa are highly host-specific parasites of the figs or each other, suggesting that phylogenetic studies may identify key differences that are associated with cospeciation. Informative genes have been found for both pollinating (Herre *et al.*, 1996; Machado *et al.*, 1996, 2001; Kerdelhue *et al.*, 1999) and nonpollinating (Machado *et al.*, 1996; Rasplus *et al.*, 1998) wasps but there has been less success with closely related *Ficus* species (Weiblen, 2000; C. Lopez-Vaamonde *et al.*, unpublished). Initial molecular phylogenetic studies of Panamanian pollinating and associated nonpollinating wasps (Machado *et al.*, 1996) and of figs and pollinators in general (Herre *et al.*, 1996), were consistent with high levels of cospeciation.

Australasia harbors great *Ficus* diversity with at least 130 species in New Guinea alone (Wiebes, 1982a). The section *Malvanthera* Corner comprises 22 species, 14 of which are endemic to Australia and 8 to New Guinea (Corner, 1965). There are two distinct groups: a lineage of stranglers and free-standing trees found in rainforests of eastern Australia and New Guinea and a clade of lithophytic shrubs and trees found in more arid parts of Australia. *Malvanthera* species are all pollinated by wasps in the genus *Pleistodontes* and are exploited by a range of nonpollinating wasps (Boucek, 1988; Cook and Power, 1996), of which *Sycoscapter* species are the most prevalent. *Sycoscapter* larvae are thought to be either true parasitoids or phytophagous kleptoparasites of the pollinators (Boucek, 1993; Compton *et al.*, 1994). Although both are referred to as fig wasps, *Pleistodontes* and *Sycoscapter* are distantly related genera from different families of chalcid wasps (Rasplus *et al.*, 1998).

In this paper, we use nuclear and mitochondrial DNA sequences to infer phylogenies for 20 *Pleistodontes* and 16 *Sycoscapter* species from *Malvanthera* figs. We test two null hypotheses of host–parasite associations (Fig. 1; see Huelsenbeck *et al.*, 1997, 2000; Clark *et al.*, 2000). H1 is that host and parasite phylogenies have been produced by independent random-branching processes. Acceptance of H1 infers that cospeciation has been rare or absent, whereas rejection of H1 suggests that cospeciation (or another process imparting congruence) has occurred. In the latter case we can then test H2 that *Pleistodontes* and *Sycoscapter* data sets are consistent with the same underlying tree topology (Peek *et al.*, 1998; Clark *et al.*, 2000; Huelsenbeck *et al.*, 2000, 2001; Johnson *et al.*, 2001). Acceptance of H2 would provide stronger evidence for strict cospeciation and permit a test of H3 that the rates of molecular evolution and speciation times are the same in the two clades.

MATERIAL AND METHODS

Taxon Sampling

In taxonomic terms, *Malvanthera* figs and their pollinators represent one of the best studied groups of figs and wasps. The Australian *Malvanthera* species have been revised recently with the description of five new species (Dixon, 2001a,b; Dixon *et al.*, 2001). Meanwhile, we are currently revising the taxonomy of *Pleistodontes* pollinating wasps with the description of six new species (C. Lopez-Vaamonde *et al.*, unpublished). The taxonomy of *Sycoscapter* (like most genera of nonpollinating fig wasps) is less advanced, with only two

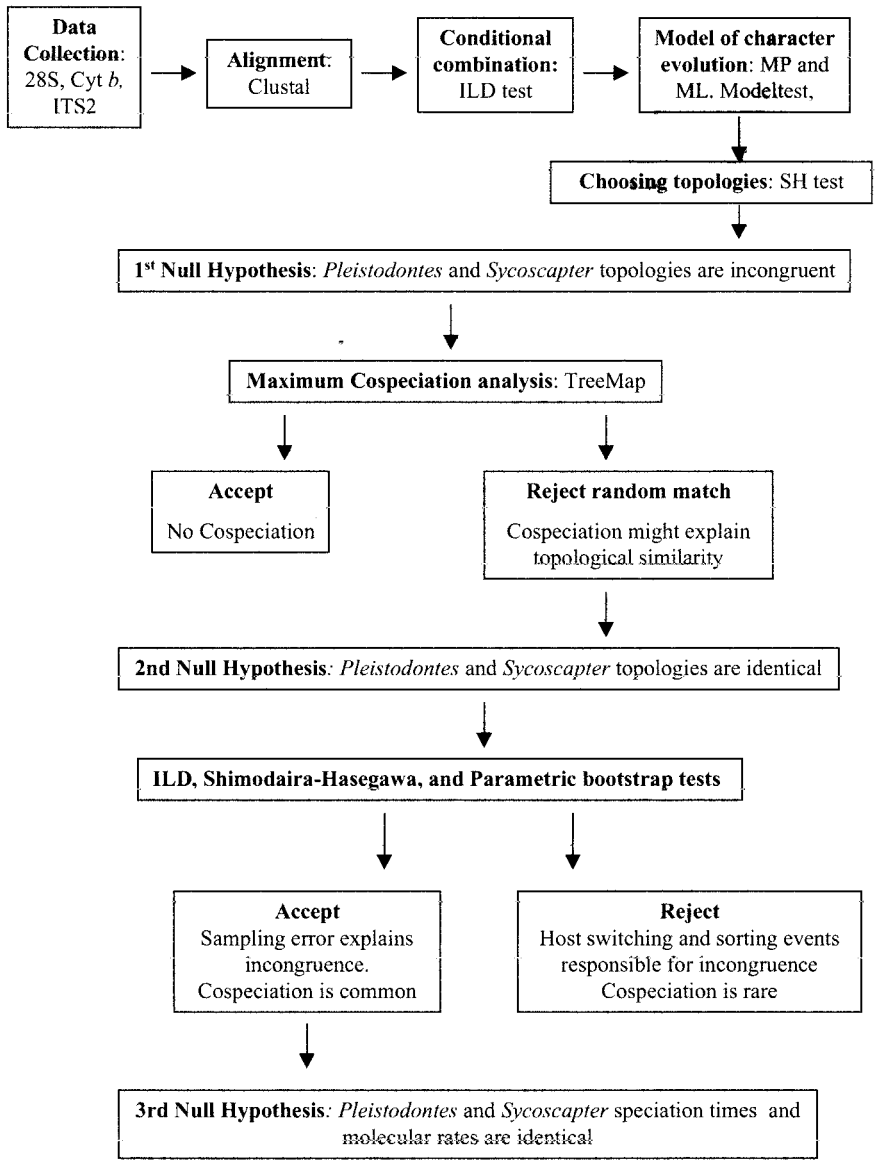

FIG. 1. Flow chart of data analysis procedures.

Malvanthera-feeding species described formally (Boucek, 1988). *Sycoscapter* wasp samples were collected from the same tree (and usually fruit) as the pollinators and were assigned to morphospecies.

Page (1996b) highlighted the importance of exhaustive phylogenetic sampling in host–parasite studies. We obtained specimens of *Pleistodontes* and *Sycoscapter* wasps from all but one Australian *Malvanthera* species and from three of the eight species from New Guinea (Table 1). Voucher wasp specimens are deposited at the Natural History Museum, London.

Molecular Procedures

We estimated phylogenies using data from three different genes: the mitochondrial cytochrome *b* (Cyt *b*), the nuclear 28S rDNA (28S), and the internal transcribed spacer (ITS2) of the eukaryotic ribosomal DNA (rDNA) transcription unit. These regions all evolve at sufficiently high rates to provide phylogenetic information at lower taxonomic levels in the Hymenoptera (Stone and Cook, 1998; Kerdelhue *et al.*, 1999). We sequenced a total of 1802 bp (length for aligned Cyt *b*, 28S, and ITS2 sequence data) for *Pleistodontes* species and 1168 bp (Cyt *b* and 28S) for *Sycoscapter* species. We decided to sequence a third gene (ITS2) only for *Pleistodontes*. This was because, whereas Cyt *b* and 28S produced well-resolved *Sycoscapter* trees, the pollinator phylogenetic signal required further augmentation.

TABLE 1

Specimens Used in This Study

Species name	Host *Ficus*	Collection site	Code[a]	Coll[b]	Accession Nos. 28S/cyt *b*/ITS2
Family Agaonidae					
Pleistodontes athysanus nom. provis	*F. brachypoda* nom. provis.	Western Australia	357 (470)	DD	AJ275069/AJ298423/AF284589
Pleistodontes xanthocephalus nom. provis.	*F. obliqua G. Forst*	Cooktown, Queensland, Australia	361	JMC	AJ275070/AJ298424/AF284590
Pleistodontes cuneatus Wiebes, 1990	*F. platypoda* (Miq.)	Kununurra, Kimberley, Western Australia	362 (455)	DD	AJ275071/AJ298425/AF284587
Pleistodontes greenwoodi (Grandi, 1928)	*F. obliqua* G. Forst	Hayman Island, East coast of Australia	363 (188)	IGC	AJ275072/AJ298426/AF284591
Pleistodontes macrocainus nom. provis.	*F. cerasicarpa* nom. provis.	Mount Isa, Australia	365 (405)	DD	AJ275073/AJ298427/AF284585
Pleistodontes proximus Wiebes, 1990	*F. lilliputiana* nom. provis.	Lake Argyle, Western Australia	367 (449)	DD	AJ275074/AJ298428/AF284588
Pleistodontes astrabocheilus nom. provis.	*F. subpuberula* Corner	Kununurra, Twin Pools, Western Australia	369 (442)	DD	AJ275075/AJ298429/AF284586
Pleistodontes regalis Grandi, 1952	*F. pleurocarpa* F. Muel.	Mount Sorrow, Queensland, Eastern Australia	371	CLV	AJ275076/AJ298430/AF284601
Pleistodontes schizodontes nom. provis.	*F. triradiata* Corner	Mount Windsor, Atherton Tablelands, Australia,	372 (354)	DD	AJ275077/–/AF284592
Pleistodontes nitens (Girault, 1915)	*F. crassipes* F. M. Bailey	Upper Cooper Creek, Queensland, Australia	374	CLV	AJ275078/AJ298431/AF284593
Pleistodontes blandus Wiebes, 1963	*F. glandifera* Summerh.	Keravat, East New Britain, Papua New Guinea (CLV01)	376	CLV	AJ275079/–/AF284594
Pleistodontes spec. nov. 1	*Ficus sp. aff. xylosycia* Diels	Raunsepna, East New Britain, Papua New Guinea (CL V10)	378	CLV	AJ275080/AJ298432/AF284599
Pleistodontes rieki Wiebes, 1963	*F. xylosycia* Diels	Ohu Conservation area, Madang, Papua New Guinea	427	BI	AJ275087/AJ298433/AF284597
		Raunsepna, East New Britain, Papua New Guinea (CLV11)	381	CLV	AJ275081/–/–
Pleistodontes near blandus	Unknown at light	Guadalcanal, Solomon Islands,	384/385	JL	AJ275082/AJ298434/AF284595
Pleistodontes rigisamos Wiebes, 1991	*F. destruens* F. Muel.	Gordonvale, Australia	386	JMC	AJ275083/AJ298435/
Pleistodontes nigriventris (Girault, 1915)	*F. watkinsiana* F. M. Bailey	Kairi, Queensland, Australia	412	JMC	AJ275084/AJ298436/AF284598
Pleistodontes froggatti Mayr, 1906	*F. macrophylla* Desf. ex Pers.	Sydney, Australia	425	DB	AJ275085/AJ298437/AF284600
Pleistodontes plebejus Wiebes, 1963	*F. hesperidiiformis* King	Ohu Conservation area, Madang, Papua New Guinea	429	BI	AJ275088/AJ298438/AF284596
Pleistodontes immaturus Wiebes, 1963	*F. sterrocarpa* Diels	Haia, Crater Mountain Wildlife Management Area, Madang, Papua New Guinea	GW 715	GW	AJ298404/–/–
Pleistodontes imperialis Saunders, 1883	*F. rubiginosa* Desf. ex Vent.	Pallerenda, Townsville, Australia	3	CLV	AJ298405/AJ298439/AF284584
Outgroup					
Family Agaonidae					
Platyscapa soraria Wiebes, 1980	*F. ingens*	South Africa	23	CM	AJ298406/AJ298440/AF284602
Family Pteromalidae					
Subfamily Sycoryctinae					
Sycoscapter sp. 1.	*F. brachypoda* nom. provis.	Ross Highway, East Alice Spring, Australia	406 (PhD 479)	DD	AJ298387/AJ298407/–
Sycoscapter sp. 2.	*F. obliqua* G. Forst	Cooktown, Queensland, Australia	413	JMC	AJ298388/AJ298408/–
Sycoscapter sp. 3	*F. platypoda* (Miq)	Kununurra, Western Australia	405(PhD 436)	DD	AJ298389/AJ298409/–
Sycoscapter sp. 4	*F. obliqua* G. Forst	Atherton Tablelands, Queensland, Australia	434 (PhD 335)	DD	AJ298390/AJ298410/–
Sycoscapter sp. 5	*F. cerasicarpa* nom. provis	Mount Isa, Australia	399 (PhD 405)	DD	AJ298391/AJ298411/–
Sycoscapter sp. 6	*F. lilliputiana* nom. provis	Lake Argyle, Western Australia	400(PhD 449)	DD	AJ298392/AJ298412/–
Sycoscapter sp. 7	*F. subpuberula* Corner	Kununurra, Twin Pools, Western Australia	403(PhD 442)	DD	AJ298393/AJ298413/–
Sycoscapter sp. 8	*F. pleurocarpa* F. Muel.	Upper Cooper Creek, Daintree, Queensland	402	CLV	AJ298394/AJ298414/–
Sycoscapter sp. 9	*F. triradiata* Corner	Mount Windsor, Atherton Tablelands, Australia	401(PhD 354)	DD	AJ298395/AJ298415/
Sycoscapter sp. 10	*F. crassipes* F. M. Bailey	Upper Cooper Creek, Daintree, Queensland	407	CLV	AJ298396/AJ298416/–
Sycoscapter sp. 11	*F. glandifera* Summerh.	Keravat. East New Britain, Papua New Guinea	411(CLV01)	CLV	AJ298397/AJ298417
Sycoscapter sp. 12	*F. xylosycia* Diels	Raunsepna, East New Britain, Papua New Guinea	410(CLV11)	CLV	AJ298398/AJ298418/–
Sycoscapter sp. 13	*F. watkinsiana* F. M. Bailey	Kairi, Queensland, Australia	409	JMC	AJ298399/–/–
Sycoscapter australis	*F. macrophylla* Desf. ex Pers.	Sydney, Australia	418	DB	AJ298400/AJ298419/–
Sycoscapter sp. 14	*F. hesperidiiformis* King	Ohu Conservation area, Madang, Papua New Guinea	430	BI	AJ298401/AJ298420/–
Sycoscapter sp. 15	*F. rubiginosa* Desf. ex Vent.	Pallerenda, Townsville, Australia	404	CLV	AJ298402/AJ298421/–
Outgroup					
Family Pteromalidae					
Subfamily Sycoryctinae					
Philotrypesis sp.	*F. rubiginosa*	Pallerenda, Townsville, Australia	416	CLV	AJ298403/AJ298422/–

[a] Figures between parentheses correspond to the plant voucher specimen deposited in Dale Dixon's herbarium at James Cook University, Townsville, Australia.

[b] coll, collectors; CLV, Carlos Lopez-Vaamonde; CM, Carlos Machado; JMC, James Cook; DD, Dale Dixon; GW, George Weiblen; IGC, Irene G. Champion; JL, John Lattke; DB, Daniel Bean; BI, Brus Isua.

Total genomic DNA was extracted from single individuals in 50 μl of an extraction buffer containing 2% Chelex 100 Resin (Bio-Rad) (Singer-Sam *et al.*, 1989), as described by West *et al.* (1998). Double-stranded PCR amplifications were performed with Ready-to-go PCR beads (Amersham, Pharmacia Biotech) with 2 μl of DNA extract and 10 pmol of each primer in a 25-μl reaction volume. The primers used in both amplification and sequencing were CB1 (5′ TAT GTA CTA CCA TGA GGA CAA ATA TC) and CB2 (5′ ATT ACAC CTC CTA ATT TAA TTA GGA AT) for the cyt *b* gene (Jermiin and Crozier, 1994), ITSF (5′ ATT CCC GGA CCA CGC CTG GCT GA) and ITSR (5′ CGC CTG ATC TGA GGT CGT C) for the ITS2 gene (Campbell *et al.*, 1993), and D1F (5′ ACC CGC TGA ATT TAA GCA TAT) (Harry *et al.*, 1996) and D3R (5′ TAG TTC ACC ATC TTT CGG GTC) for the 28S gene. PCR conditions were 40 cycles at 94°C for 1 min, an annealing step at 47°C for 1 min, and an extension step at 72°C for 90 s for the cyt *b* gene; 3 min at 93°C followed by 30 cycles of 98°C (15 s), 50°C (30 s), and 72°C (40 s) and a final extension of 72°C for 3 min for the ITS2 gene; and 3 min at 93°C followed by 35 cycles of 98°C (15 s), 57°C (30 s), and 72°C (40 s) and a final extension of 72°C for 3 min for the 28S rDNA gene. All PCR products were purified with the GFX PCR DNA and gel band purification kit (Amersham, Pharmacia Biotech) and then sequenced directly with an ABI PRISM BigDye terminator cycle sequencing ready reaction kit (Perkin–Elmer Biosystems) on an ABI 3700 automated sequencer. All products were sequenced in both directions. Chromatograms were edited and sequences (not including primers) compiled with Sequencher 3.0 (Gene Codes Corp., Ann Arbor, MI) and submitted to GenBank (Table 1).

Intragenomic variation in ITS2 is known to exist in insects (Wesson *et al.*, 1992) and divergent paralogous sequences can seriously confound species phylogenies (Buckler *et al.*, 1997). We therefore investigated ITS2 sequence variation within three species (*Pleistodontes rigisamos, P. imperialis,* and *P. froggatti*) by repeated PCR amplification and direct sequencing both of the same individual and of multiple individuals of each species.

Sequence Alignment and Phylogenetic Analyses

Cyt *b* sequences were all 381 bp (*Pleistodontes*) or 417 bp (*Sycoscapter*) long. They could be aligned by eye unambiguously, and all had full open reading frames. On the other hand, both 28S and ITS2 showed substantial variation in length across species: 28S (1019–1038 bp in *Pleistodontes,* 742–751 bp in *Sycoscapter*) and ITS2 (291–413 bp in *Pleistodontes*). Distance-based sequence alignments were performed with Clustal W (Higgins and Sharp, 1988) with default settings (Open Gap Penalty = 10.0; Extend Gap Penalty = 5.0; Delay Divergent = 40%; Transitions: weighted). Alignments are available from TreeBASE (http://www.herbaria.harvard.edu/treebase/console.html).

All phylogenetic analyses were conducted with PAUP* 4.0b2a (Swofford, 1999). Each gene was first analyzed independently and the signals from different genes were then compared by ILD tests. All parsimony-uninformative characters were removed before the test was used (Cunningham, 1997), which was run with 1000 replicates and 50 random stepwise additions of taxa (the "partition homogeneity test" option in PAUP*). Subsequently, congruent data sets were combined for further analysis (Bull *et al.*, 1993; Huelsenbeck *et al.*, 1996a).

Both maximum-parsimony (MP) (Farris, 1970) and maximum-likelihood (ML) (Felsenstein, 1981) analyses were performed. Wherever possible, MP trees were reconstructed with the branch-and-bound search method (Hendy and Penny, 1982). When these proved too computer intensive, we used heuristic searches, involving TBR branch swapping with 1000 random stepwise additions of taxa. Gaps were treated as a fifth state and all character transitions were given equal weighting. MacClade v. 4 (Maddison and Maddison, 2000) was used to calculate the average nucleotide frequencies and the number of Ts and Tv at each Cyt *b* codon position.

For the ML analyses, the best model of DNA substitution was selected by Modeltest3.0 (Posada and Crandall, 1998), which uses two criteria, the ML ratio test and the Akaike information criterion (AIC; Akaike, 1974). When the criteria favored different models, we compared the resulting topologies using the Shimodaira–Hasegawa test (SH) (Shimodaira and Hasegawa, 1999; Goldman *et al.*, 2000), implemented in SHTest version 1.0 using the RELL model and 1000 bootstrap replicates (Rambaut, 2000). A ML heuristic search (options: ASIS and TBR branch-swapping) was then run under the likelihood settings (empirical base frequencies, Ts/Tv ratio, proportion of invariable sites, and gamma shape parameter) estimated with Modeltest 3.0.

Support for individual nodes was evaluated by nonparametric bootstrapping (BV) (Felsenstein, 1985) with either 300 (ML) or 1000 replicates. We also measured the skew (g1) in the distribution of cladogram lengths (DCL) (Huelsenbeck, 1991) for each gene, based on 10,000 randomly generated trees, as an indicator of phylogenetic signal. Finally, the leaf stability (Thorley and Wilkinson, 1999) of each taxon was estimated with RadCon version 1.0.0 (Thorley and Page, 1999).

Species from several fig wasp genera were assessed for use as outgroups. These were *Ceratasolen (C) arabicus* (Mayr, 1906), *Pegoscapus gemellus* Wiebes, 1995, *Tetrapus costaricanus* (Grandi, 1925), *Wiebesia punctatae* Wiebes, 1992, and *Platyscapa soraria* Wiebes, 1980 for pollinating wasps and *Robertsia* sp., *Dobun-*

abaa sp., *Tenka* sp., *Watshamiella* sp., and *Philotrypesis* sp. for nonpollinating wasps. An initial MP analysis was performed with *T. costaricanus* and *Robertsia* sp. as outgroups to identify which of the other fig wasp genera was an appropriate outgroup for *Pleistodontes* and *Sycoscapter*, respectively.

Cospeciation Tests

To test for parallel cladogenesis we followed the procedure illustrated by the flow chart in Fig. 1 (see also Huelsenbeck *et al.*, 2001). First, we tested the null hypothesis (H1) that *Pleistodontes* and *Sycoscapter* trees are not more congruent than two independent phylogenies generated under a random branching model, using a maximum cospeciation analysis implemented in the Treemap 1.0 package (Page, 1995). The *Sycoscapter* phylogeny was mapped onto the *Pleistodontes* phylogeny to maximize the number of cospeciation events in the absence of host switching (Page, 1994; Page and Charleston, 1998). The probability of obtaining the observed number of cospeciation events was then calculated by randomization of both the *Sycoscapter* and the *Pleistodontes* trees 1000 times to generate a null frequency distribution (and hence test statistic). *P* values were corrected for problems with the randomization routine (see http://taxonomy.zoology.gla.ac.uk/rod/treemap.html for details) with the proportional-to-distinguishable model instead of the Yule (Markovian) model to generate random trees. The percentage of cospeciating nodes is simply the number of cospeciating nodes inferred by Treemap, divided by the total number of nodes in the parasite phylogeny, and then multiplied by 100. Only fully resolved host and parasite trees were compared (a limitation of Treemap).

SH tests were used to compare alternative MP and ML topologies derived from the Clustal alignment. If no significant difference was found, the ML topology alone was used; otherwise all topologies were investigated.

When H1 was rejected, we used the parsimony-based ILD (Johnson *et al.*, 2001; Johnson and Clayton, 2001) and the likelihood-based SH tests to test whether pollinator and parasite data sets were consistent with complete congruence (H2).

RESULTS

Characterization of Nucleotide Patterns

Sycoscapter species show slightly higher levels of uncorrected nucleotide divergence for both 28S D2-D3 (1–6% compared to 0.01–4.6% for *Pleistodontes*) and Cyt *b* (4–23% compared to 3–19% for *Pleistodontes*). These differences in the level of divergence were statistically significant (28S: $t = -8.063$, $df = 208$, $P > 0.0001$; Cyt *b*: $t = -2.540$, $df = 208$, $P > 0.05$) by *t* tests (see Discussion).

PCR amplification of ITS2 yielded single bands, and uncorrected p distances between *Pleistodontes* species varied from 3 to 62%. The generally high genetic distances were primarily due to a number of microsatellite loci near the 3′ end of ITS2. The presence of microsatellites in ITS2 has been documented in other Chalcidoidea (Campbell *et al.*, 1993) and, to control for distorting effects (Harris and Crandall, 2000), phylo-

TABLE 2

Nucleotide and Amino Acid Patterns

	A	C	G	T	*P*	Ts/Tv[a]	*n*	nv	ic
Pleistodontes									
28S D1 + D3	26.62	23.73	28.92	20.73	—		1038	249	95
Cyt *b* total sequence	33.3	13.5	9.8	43.5		0.81–0.82	381	155	105
Cyt *b* (1st)	32.24	15.47	17.14	35.15	—	2.03	127	37	25
Cyt *b* (2nd)	25.20	18.99	11.21	44.60	—	0.60–0.69	127	21	7
Cyt *b* (3rd)	42.47	5.97	0.93	50.62	*	0.62–0.64	127	97	73
Amino acids							127	38	22
ITS2	19.94	24.70	30.56	24.79	—		413	298	210
Sycoscapter									
28S D2 + D3	23.01	25.22	30.18	21.58	-		751	133	70
Cyt *b* total sequence	31.6	14.3	9.2	44.9		0.87–0.88	417	203	139
Cyt *b* (1st)	33.40	14.88	16.39	35.33	—	1.26–1.28	139	65	36
Cyt *b* (2nd)	23.36	20.82	9.92	45.89	—	2.7–2.88	139	21	10
Cyt *b* (3rd)	39.46	5.04	1.33	54.17	*	0.65–0.67	139	117	93
Amino acids							139	45	31

Note. A, C, G, T, average nucleotide frequencies; *P*, χ^2 test of homogeneity of base frequencies across taxa: $*P \leq 0.05$; —, not significant ($P > 0.05$); Ts/Tv, transition transversion ratio; *n*, total number of positions; nv, number of variable positions; ic, number of parsimony informative characters.

[a] The numbers of Ts and Tv were estimated as changes reconstructed on the MP tree and were not merely calculated from pairwise distances (Madison and Madison, 2000).

TABLE 3

Summary of Tree Statistics for Each Data Set under Unweighted MP Analysis and ML

	Trees							
	No.	S	T	CI	RI	g1	Model	−Ln likelihood
Pleistodontes								
28S rDNA	20	395	1	0.81	0.63	1.814	TrN + I + G	2970.91
Cyt *b*	17	440	16	0.48	0.39	0.43	TVM + I + G	2338.45
ITS2	19	1683	4	0.59	0.51	0.79	TVM + G	365374.87
Cyt *b* + 28S	17	737	2	0.59	0.39	0.75	GTR + I + G	5114.29
28S + ITS2	19	1978	1	0.62	0.51	1	TVM + I + G	8350.58
Cyt *b* + 28S + ITS2	18	2307	2	0.61	0.43	0.70	GTR + I + G	10454.66
Sycoscapter								
28S rDNA	16	230	18	0.71	0.70	0.69	GTR + I + G	2060.92
Cyt *b*	15	430	2	0.57	0.46	0.55	TVM + I + G	2812.26
Cyt *b* + 28S	15	728	6	0.62	0.52	0.85	GTR + I + G	4925.47

Note. No., number of ingroup taxa; S, length of most parsimonious cladogram; T, number of most parsimonious trees; CI, consistency index; RI, retention index; g_1, frequency distribution of cladograms length skewness; −Ln likelihood, score of best tree found; TrN, Tamura–Nei model (Tamura and Nei, 1993); I, proportion of invariable sites; G, shape parameter of the gamma distribution; TVM, submodel of the general time reversible model (Yang, 1994); GTR, general time reversible model (Rodriguez *et al.,* 1990).

genetic analyses were carried out with and without these regions. Comparisons of multiple sequences from the same individual, or from two different specimens of the same species (tested for *P. rigisamos*, *P. imperialis*, and *P. froggatti*), showed <5% sequence divergence, suggesting that paralogues were not highly divergent or not amplified. These sequences varied in the number of repeats within the repetitive motif regions near the 3′ end of the fragment.

Average nucleotide frequencies are shown in Table 2. There is strong A-T bias in Cyt *b*,as noted in other fig wasps (Kerdelhue *et al.,* 1999) and Hymenoptera in general (Jermiin and Crozier, 1994). This is reflected in the overall excess of Tv over Ts (Ts/Tv = 0.8) and is especially marked at 3rd codon positions (Ts/Tv = 0.6). In contrast, a higher Ts/Tv ratio occurs at the 1st codon position in *Pleistodontes* (2.03) and at the 2nd codon position in *Sycoscapter* (2.7–2.9) due to several T to C transitions.

In both *Pleistodontes* and *Sycoscapter*,the majority of phylogenetic information occurred at the 3rd, followed by the 1st and 2nd codon positions. Comparison of p distances with TVM corrected distances revealed minimal saturation at 1st and 2nd codon positions but high saturation at 3rd codon positions. Plots of Ts/Tv against uncorrected p distances for each codon confirmed this pattern and that Ts/Tv ratios are also higher between closely related species. There was also significant heterogeneity in nucleotide composition at 3rd codon positions of Cyt *b*.

Phylogenetic Analyses

Modeltest 3.0 identified the general time reversible model (GTR; Rodriguez *et al.* 1990; Yang *et al.*, 1994) as appropriate for most (but not all) data sets (Table 3). *Pleistodontes* and *Sycoscapter* trees from (conditionally) combined analyses under different optimality criteria are shown in Figs. 2–5. Summary statistics for MP and ML analyses of each data set are given in Table 3.

Pleistodontes phylogeny. MP analyses of individual genes yielded poorly resolved trees with few clades supported by BV >50%. The only clade that was consistently highly supported (>90%) was a group of four western Australian species (*P. cuneatus* + *P. proximus* + *P astrabocheilus* + *P. spec. nov*. 2). Phylogenetic signal (g1) was lowest for Cyt *b*,consistent with the poor signal of this region in the fig-pollinating genus *Ceratosolen* (Kerdelhue *et al.,* 1999). Removal of the ITS2 microsatellite region did not yield a significantly different topology but reduced bootstrap support for most nodes. Consequently we retained this region in combined analyses.

The Cyt *b* data set showed significant conflict with ITS2 but there was no conflict between 28S and ITS2 (Table 4). Consequently, we combined 28S + ITS2 as our preferred data set and also 28S + Cyt *b*. Not surprisingly, the 28S + Cyt *b Pleistodontes* phylogeny was poorly resolved with few well-supported nodes and low leaf stability values for most species (Fig. 2). The clade of *P. froggatti* and *P. regalis* (both species with a mandibular appendage that bears ventral rows of small teeth instead of ventral lamellae) was recovered. Another interesting clade is *P. greenwoodi* and *P. xanthocephalus* (both pollinators of *F. obliqua*) which appeared in the 28S MP tree and was recovered in Cyt *b* only when Ts were removed from 3rd codon positions. There was no significant difference between MP and ML topologies (Table 5).

On the other hand, the 28S + ITS2 topology was completely resolved and many nodes were well sup-

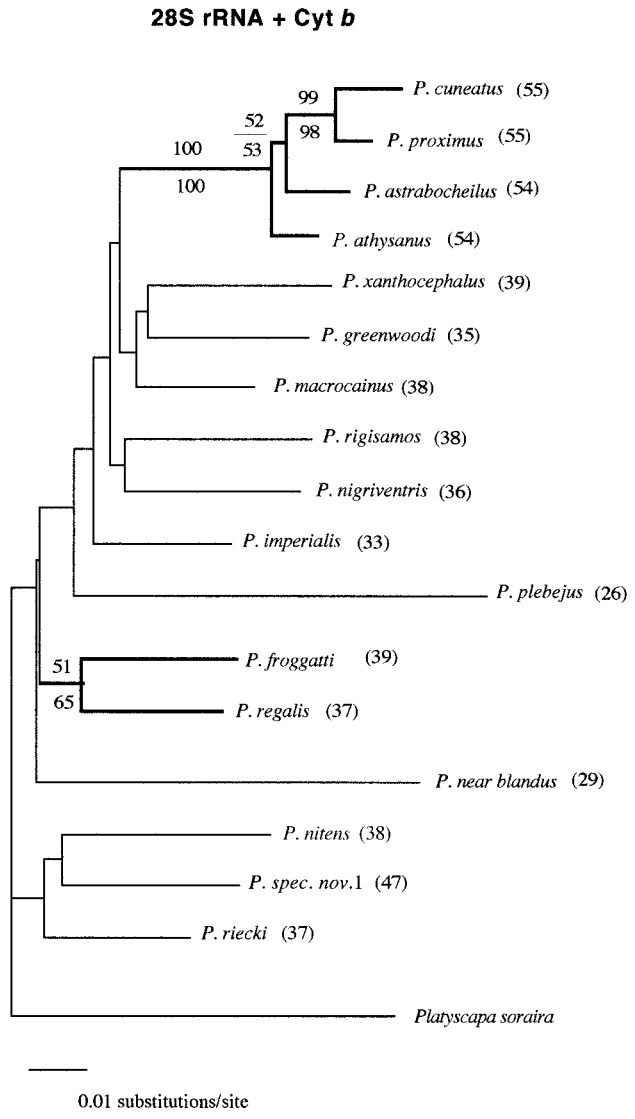

FIG. 2. ML tree based on combined 28S and Cyt *b* sequence data for 17 *Pleistodontes* species (13 Australian and 4 New Guinean). *P. schizodontes*, *P. immaturus*, and *P. blandus* have not been sequenced for Cyt *b* and are not included. -In likelihood= 5130.27. Model parameters: empirical base frequencies with rate heterogeneity; gamma shape parameter = 0.5827; proportion of invariable sites = 0.6007; six rate categories; GTR + I + G model with transformation parameters [A-C] = 1.2896, [A-G] = 5.3805, [A-T] = 12.4806, [C-G] = 1.8212, [C-T] = 17.2534, [G-T] = 1.00. Branch lengths are proportional to lengths estimated under the ML model. Boldface lines show clades present in both MP and ML analyses. Percentage bootstrap support (1000 replications) is indicated above branches for ML and below branches for MP. Leaf stability values are given between parentheses next to the terminal taxa.

ported. The MP (Fig. 3) and ML topologies (Fig. 4) were not significantly different (Table 5). We nevertheless retained both topologies for subsequent Treemap analyses, to examine the sensitivity of our cospeciation analysis to different optimality criteria. The western Australian clade is recovered in both MP and ML trees, as is the *P. greenwoodi–P. xanthocephalus* clade. The species *P. regalis* is basal due largely to the extreme divergence of its ITS2 sequence. There was no conflict between well-supported clades (BV >50%) in the 28S + Cyt *b* and 28S + ITS2 topologies.

Sycoscapter phylogeny. There was no conflict between 28S D2-D3 and Cyt *b* data partitions (Table 4), and total phylogenetic structure (g1) increased when the genes were combined (Table 3). The *Sycoscapter* phylogeny resulting from the combined analysis was completely resolved and well supported (Fig. 5), and the MP and ML topologies were not significantly different (Table 5). We therefore present only the combined ML tree (Fig. 5). *Sycoscapter* ex *F. macrophylla* was the only species to lack good leaf stability and its

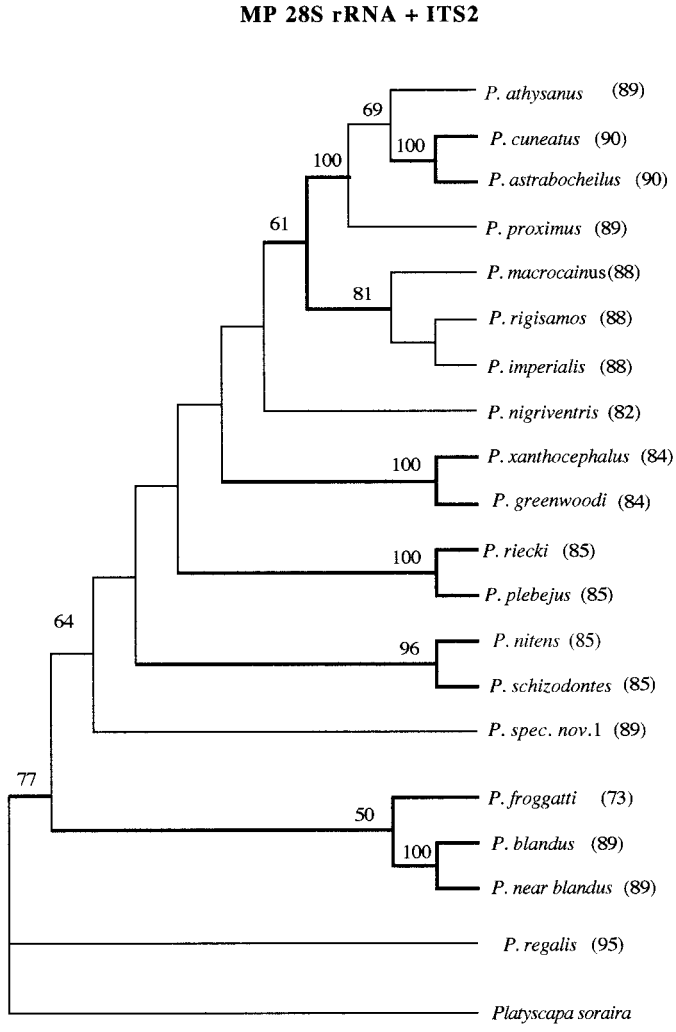

FIG. 3. Most-parsimonious tree of 19 *Pleistodontes* species (14 Australian and 5 New Guinean) for which both 28S and ITS2 sequence data were available. Boldface lines show clades present in both MP and ML analyses. Leaf stability values are given between parentheses next to the terminal taxa. Unlabeled branches had bootstrap support lower than 50%.

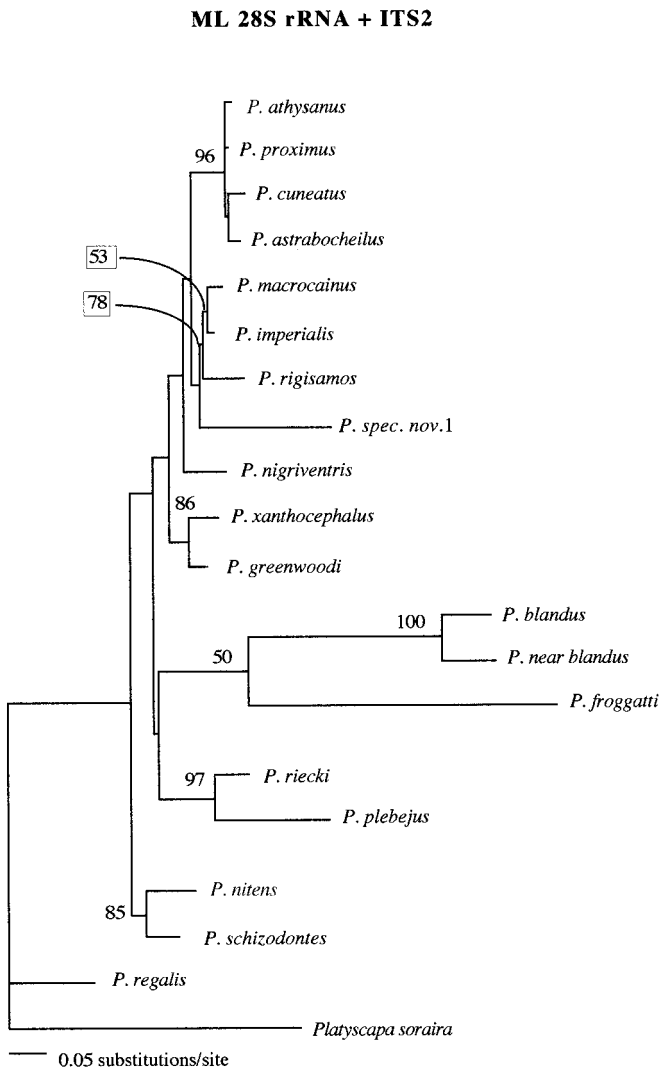

FIG. 4. ML tree based on combined 28S and ITS2 for the same 19 *Pleistodontes* species as those in Fig. 3. −ln likelihood = 8350.58. Model parameters: empirical base frequencies with rate heterogeneity; gamma shape parameter = 0.365; proportion of invariable sites = 0.3648; six rate categories; TVM + I + G model with transformation parameters [A-C] = 0.8134, [A-G] = 2.7801, [A-T] = 1.6023, [C-G] = 0.6608, [C-T] = 2.7801, [G-T] = 1.00. Branch lengths are proportional to lengths estimated under the ML model. Bootstrap values >50% are shown.

position depended on the outgroup. When *Philotrypesis* (the sister genus of *Sycoscapter*) was used as the outgroup, *S.* ex *F. macrophylla* appears as sister to the rest of *Malvanthera*-feeding *Sycoscapter* species. However, when African *Sycoscapter* species were used as outgroups, it appeared as the sister group to the western Australian species.

Are Pleistodontes and Sycoscapter Topologies Independent?

Treemap was used to compare the *Sycoscapter* ML phylogeny with three *Pleistodontes* trees: (a) the ML tree derived from 28S + Cyt *b* (ML1), (b) the 28S + ITS ML tree (ML2), and (c) the 28S + ITS MP tree (MP). The level of cospeciation estimated ranged from 50 to 64% and was significant for ML2 and MP trees but not for the poor-quality 28S + Cyt *b* tree (Table 7).

The highest estimate of cospeciation was obtained with the MP *Pleistodontes* topology (Fig. 3); an exact search in Treemap identified 17 optimal reconstructions, each with nine cospeciating nodes, but differing in duplication (1–3), host switching (2–5), and sorting events (11–18). Comparison with randomized trees (Fig. 7) allows us to reject H1 with $P = 0.001$ and with $P = 0.008$ with a conservative correction suggested by Taylor and Purvis (2001).

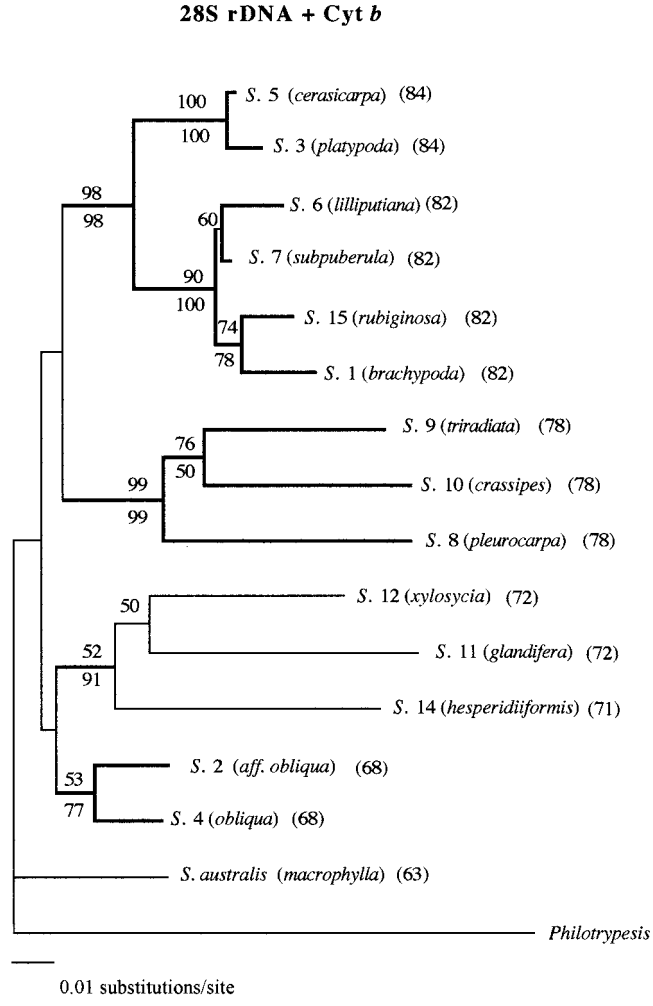

FIG. 5. ML tree based on the combination of 28S + Cyt *b* ML for 15 *Sycoscapter* species (12 Australian and 3 New Guinean). −ln likelihood = 4925.46682. Model parameters: empirical base frequencies with rate heterogeneity; gamma shape parameter = 0.7437; proportion of invariable sites = 0.4514; GTR + I + G model with transformation parameters [A-C] = 2.5474, [A-G] = 8.7791, [A-T] = 12.6510, [C-G] = 2.1232, [C-T] = 18.4266, [G-T] = 1.00. Conventions as in Fig. 4.

TABLE 4

Results of the ILD Congruence Test between Different Data Sets

Data sets compared	*P* value
28S *Pleistodontes* vs cyt *b Pleistodontes*	**0.077**
28S *Pleistodontes* vs ITS2 *Pleistodontes*	**0.182**
cyt *b Pleistodontes* vs ITS2 *Pleistodontes*	0.001
28S *Sycoscapter* vs cyt *b Sycoscapter*	**0.510**
28S *Pleistodontes* vs 28S *Sycoscapter*	0.001
cyt *b Pleistodontes* vs cyt *b Sycoscapter*	0.001
28S *Pleistodontes* vs cyt *b Sycoscapter*	0.001
cyt *b Pleistodontes* vs 28S *b Sycoscapter*	0.001
ITS2 *Pleistodontes* vs 28S *Sycoscapter*	0.001
ITS2 *Pleistodontes* vs cyt *b Sycoscapter*	0.001

Note. The incongruence threshold is $P < 0.05$ and the combinable data sets are highlighted in boldface.

Do Pleistodontes and Sycoscapter Datasets Support the Same Topology?

With H1 rejected, we can consider H2, that the underlying *Pleistodontes* and *Sycoscapter* phylogenies are identical. If H2 is accepted, the level of incongruence found in the Treemap analysis could be attributed to phylogenetic uncertainty (weakly supported conflicting nodes). On the other hand, rejection of H2 would suggest that incongruence is based on real differences in speciation patterns. ILD tests (Table 4) and SH tests (Table 6) both indicated highly significant incongruence between all pairwise combinations of *Pleistodontes* and *Sycoscapter* data sets. The rejection of H2 precludes testing of H3 on comparative rates of molecular evolution and timing of cospeciation events (see Fig. 1), as this requires perfect concordance of clade or subclade topologies (Hafner *et al.* 1994; Huelsenbeck *et al.*, 1997).

DISCUSSION

Partial Cocladogenesis of Pleistodontes and Sycoscapter

This study demonstrates partial congruence of the molecular phylogenies of *Pleistodontes* pollinating wasps and their ecologically associated *Sycoscapter* nonpollinating wasps. Congruence is significantly greater than that expected by chance but significantly less than that expected under perfect cospeciation. Overall, the level of sequence variation was appropriate to resolve the interacting phylogenies but the degree of resolution and support was far better for *Sycoscapter* than for *Pleistodontes*. The *Pleistodontes* 28S + ITS2 tree, and especially the single best *Sycoscapter* tree, represent robust estimates of phylogeny upon which to base tests of cospeciation.

There are no major clades in the two phylogenies that correspond exactly. In fact, perfect correspondence is seen only between two terminal pairs of pollinator and parasite sister species. The first of these is *P. xanthocephalus*/S.2 + *P. greenwoodi*/S.4 and the second is *P. schizodontes*/S.9 + *P. nitens*/S.10. Whereas these might represent two recent cospeciation events, overall the partial congruence level observed generates too many optimal reconstructions to infer, with confidence, particular cospeciation, host switching, duplication, or sorting events (see also Clark *et al.*, 2000). Nevertheless, the overall percentage (50–64%) of cospeciating nodes falls within the range of values observed for other putatively coevolved interactions (Taylor and Purvis, 2001). For example, analysis of the textbook example of pocket gophers and lice identified 62.5% cospeciating nodes (Hafner *et al.*, 1994), and a broader survey of mammals and chewing lice yielded estimates of 20–75% for different clades (Taylor and Purvis, 2001).

Simple demonstration that there is a greater than random match between two sets of taxa does not *per se* demonstrate cospeciation, since many different ecological and biogeographical scenarios can result in partial congruence (Clark *et al.*, 2000). For example, resource tracking (Kethley and Johnston, 1975) and sequential evolution (Jermy, 1976) models both predict partial phylogenetic congruence of interacting clades, without implying synchronous speciation. In addition, concordant speciation may be caused by extrinsic factors such as vicariance, although this is unlikely for the fig wasps in question.

TABLE 5

Shimodaira–Hasegawa Test Results: Statistical Difference between ML and MP Topologies Obtained from Same Dataset

Trees	LnL	Delta	*P* (delta)
Pleistodontes			
28S + Cyt *b*			
MP	−5233.742038	3.276152	0.430
ML1	−5230.465886	0.000000	1.000
ML2	−5230.465886	0.000000	1.000
28S + ITS2			
MP	−8427.769935	5.445047	0.293
ML	−8422.324888	0.000000	1.000
Sycoscapter			
28S + Cyt *b*			
MP1	−4986.192497	8.742769	0.272
MP2	−4984.841368	7.391640	0.338
MP3	−4984.416198	6.966470	0.392
MP4	−4987.106189	9.656460	0.247
MP5	−4985.754901	8.305173	0.301
MP6	−4985.355363	7.905635	0.335
ML	−4977.449728	0.000000	1.000

Note. LnL, −Ln likelihood, score of best tree found; Delta, difference between the likelihoods of trees; *P* (delta), the probability of getting an equal or larger delta if the null is true. This is obtained from a distribution of deltas obtained by bootstrapping.

TABLE 6

Results of Shimodaira–Hasegawa Test

Dataset	Tree	Ln (L)	Delta	*P*(delta)
Pleistodontes 28S + Cyt *b*	*Pleistodontes* 28S + Cyt *b*	−4613.921450	0.000000	1.000000
	Pleistodontes 28S + ITS2	−4667.350733	53.429283	0.014000
	Sycoscapter 28S + Cyt *b*	−4772.833530	158.91208	<0.000001
Pleistodontes 28S + ITS2	*Pleistodontes* 28S + ITS2	−7159.508394	0.000000	1.000000
	Pleistodontes 28S + Cyt *b*	−7265.083200	105.57481	<0.000001
	Sycoscapter 28S + Cyt *b*	−7354.990855	195.48246	<0.000001
Sycoscapter 28S + Cyt *b*	*Sycoscapter* 28S + Cyt *b*	−9349.680092	0.000000	1.000000
	Pleistodontes 28S + Cyt *b*	−9174.360082	175.32001	<0.000001
	Pleistodontes 28S + ITS2	−9194.231292	155.44880	<0.000001

Note. Ln likelihoods of *Pleistodontes* and *Sycoscapter* trees under alternative datasets. Each data set was tested against each ML tree generated from successive data sets, where only the taxa in common to each pair of data sets being compared were used. *P* values represent the probability that the score of the maximum-likelihood tree for a given dataset is significantly higher than that of alternative topologies. LnL, −Ln likelihood, score of best tree found; Delta, difference between the likelihoods of trees; *P* (delta), the probability of getting an equal or larger delta if the null is true. This is obtained from a distribution of deltas obtained by bootstrapping.

Our study generated estimates of cospeciation levels (50–64%) very similar to those of the only other study on associated genera of pollinating and nonpollinating fig wasps (62.5%), by Machado *et al.* (1996). The partial concordance of *Pleistodontes* and *Sycoscapter* phylogenies may be mediated through their common use of *Malvanthera* fig plants, rather than through their own direct interaction, with the sequential radiation of *Sycoscapter* across *Malvanthera* species following a pattern broadly similar to that of the radiation of *Pleistodontes*. For this reason, we urgently need a robust phylogeny for the *Malvanthera* to compare with the phylogenies of both wasp genera. We also need a better understanding of the larval ecology of *Sycoscapter* (and indeed other nonpollinating wasp) species. Are they parasitoids that feed directly on wasps (pollinators and/or other nonpollinators) or kleptoparasites reliant upon *Pleistodontes* to initiate galls that produce plant material on which both wasp taxa feed? Studies of other sycoryctine fig wasps favor the latter case (Abdurahiman and Joseph, 1978a,b) but hardly constitute conclusive evidence.

Among the possible explanations for incongruence between *Sycoscapter* and *Pleistodontes* phylogenies, perhaps the most interesting is host plant switching by either group. Some aspects of host plant biology (e.g., geographic distribution) apply to both groups of wasps, but others influence one group but not the other. For example, *Pleistodontes* females enter the fig inflorescence through a narrow tunnel (the ostiole) to reproduce, whereas *Sycoscapter* females use their long ovipositors to lay eggs through the wall of the inflorescence without entering it. Since morphological features of pollinating wasps (especially head shape) appear to be highly adapted for entering their host fig (Van Noort and Compton 1996), physical barriers to host shifting are more likely to apply to pollinators.

Another important factor is chemical signaling by figs. Pollinating wasps rely heavily on chemical cues to locate receptive figs. Recent studies have shown that they respond to the ratios of a few plant volatiles and suggest that chemistry may play an important role in host specificity (M. Hossaert-McKey, pers. comm.). To date, nobody has investigated host plant location by nonpollinating wasps. However, we do know that *Sycoscapter* wasps lay eggs into relatively mature *Malvanthera* figs, some weeks after they have been pollinated (J.M.C., pers. obs.). The chemical signals of older figs are likely to be different from those of receptive figs, perhaps increasing the probability of "mistakes" by nonpollinating wasps. Furthermore, whereas selection on figs favors attraction of the specific pollinators

TABLE 7

Results of the Treemap Cospeciation Analysis Using Different Datasets

Pleistodontes tree	*Sycoscapter* tree	No. of taxa	Max.	Cosp	%	Observed *P* value	Corrected *P* value
28S + Cyt *b* ML	28S + Cyt *b* ML	13	12	6	50	0.082	0.225
28S + ITS2 ML	28S + Cyt *b* ML	15	14	8	57.14	**0.011**	0.055
28S + ITS2 MP	28S + Cyt *b* ML	15	14	9	64.28	**0.001**	**0.008**

Note. Max, the maximum possible number of cospeciation events; Cosp, the number of cospeciation events observed; %, the percentage of cospeciating nodes detected (% = 100 × Cosp/Max); *P* value, the "corrected" *P* values (see text) obtained by randomization of both trees 1000 times with the proportional-to-distinguishable model. The significant results ($P \leq 0.05$) are highlighted in boldface.

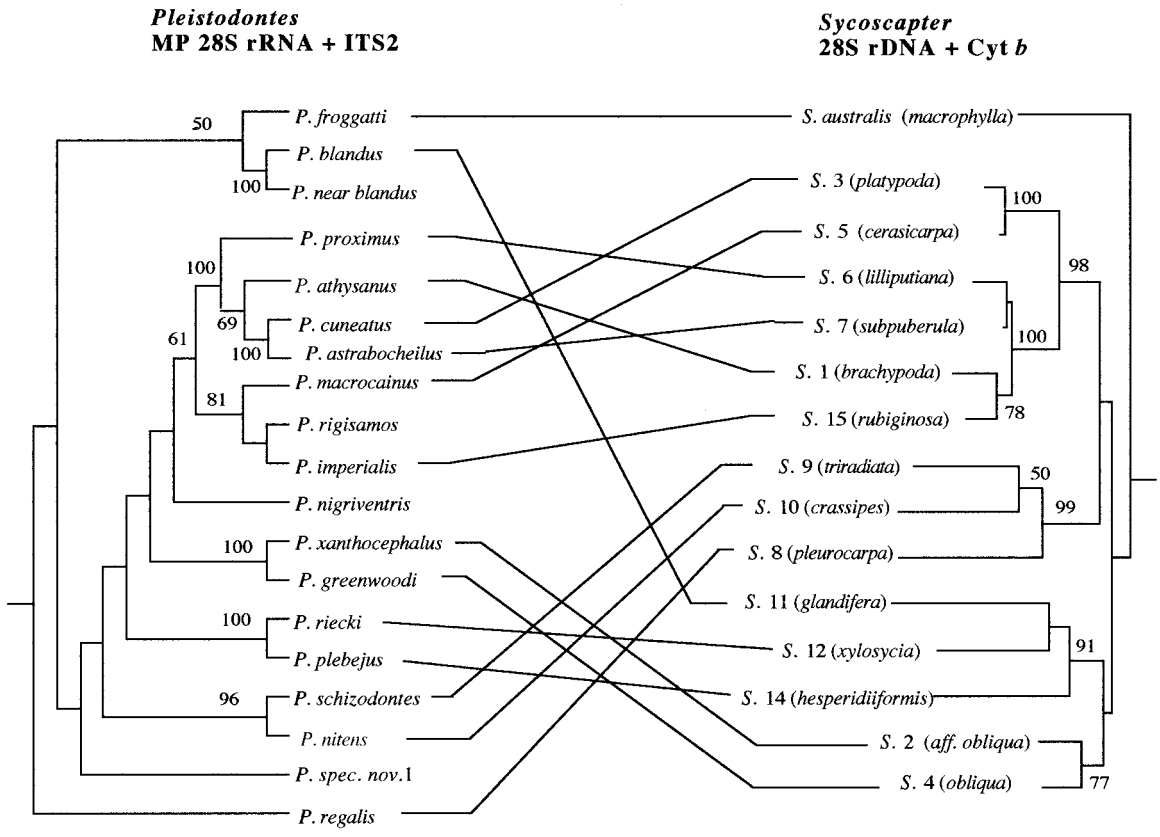

FIG. 6. Phylogenies for *Pleistodontes* and their associated *Sycoscapter* species drawn as cladogram and phylogram, respectively. The *Pleistodontes* MP tree is based on partial sequences of 28S + Cyt *b*; the *Sycoscapter* ML tree is based on 28S + ITS2. Lines connect pollinating wasps with their specific associated nonpollinating wasps. Numbers on branches are bootstrap values for parsimony searches.

to receptive fruits, it should oppose attraction of nonpollinating wasps to more mature figs.

As more studies are carried out on the phylogenies of fig wasp communities, we will discover which taxa have high degrees of cospeciation and should be able to relate variation in cospeciation levels to biological features of species interactions. One possibility is that nonpollinating wasps that lay eggs in relatively mature fig fruits may host shift more frequently than those that oviposit in receptive or recently pollinated fig fruits, as a result of the chemical signals available.

Methodological Issues

Methods of reconstructing the history of association between hosts and parasites such as the one used in this paper, maximum cospeciation analysis (Page, 1995), and others (Brooks, 1981; Ronquist, 1996, 1998; Charleston, 1998) have been criticized recently for the assumption that host and parasite phylogenies are estimated without error (Huelsenbeck *et al.*, 2000; Johnson *et al.*, 2001). Indeed, the level of support for cospeciating nodes is not taken into account when tree topologies are simply compared (Clark *et al.*, 2000). Huelsenbeck *et al.* (2000) proposed a new method based on a stochastic model of host switching that used Bayesian inference, which allows estimation of the host switching rate, without assuming that the phylogeny is error-free. In other words, the DNA sequences, rather than the topologies, are compared.

Support for cospeciating nodes is an important issue, which we return to below; however, no method is error-free, given that models of DNA substitution may be oversimplified (Posada and Crandall, 1998), and errors can be made in sampling or character scoring. In addition, the approach of Huelsenbeck *et al.* (2000) has other limitations, such as assuming that host switching is the only cause of incongruence between phylogenies.

The absence of perfect concordance between phylogenies can result from differences in data quality (Clark *et al.*, 2000), as illustrated by our suboptimal 28S + Cyt *b* phylogeny (Fig. 2), which reduces the estimate of cospeciation to borderline significance. The availability of a general "cospeciation index" to compare between studies is highly desirable and, to date, the maximum percentage of cospeciating nodes (as estimated by Treemap) is the only one to have been advocated (Taylor and Purvis, 2001). Unfortunately, in itself, this value may not tell us a great deal, and this is illustrated by

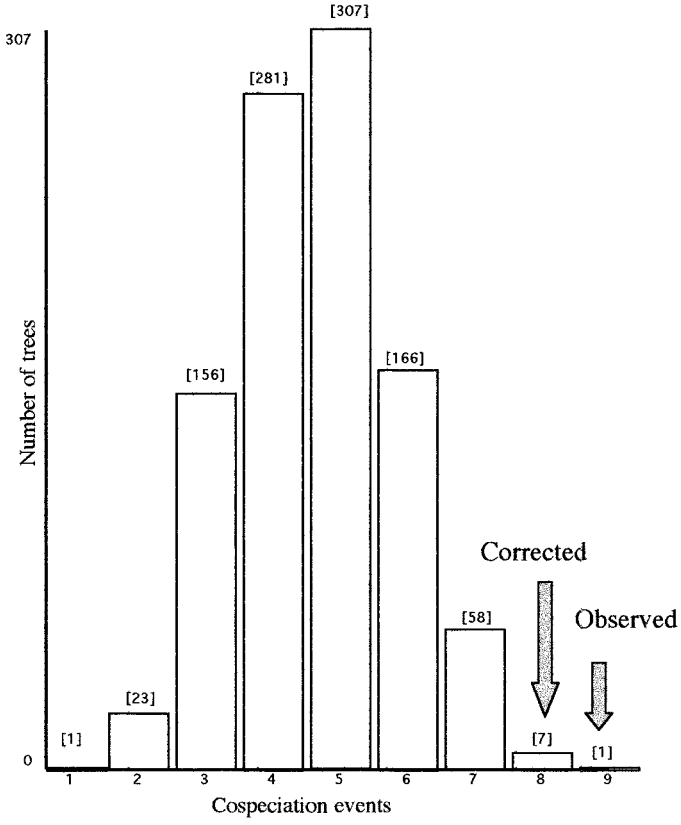

FIG. 7. Distribution of the maximum number of cospeciation events inferred from 1000 randomizations of the *Pleistodontes* and *Sycoscapter* trees generated by the proportional-to-distinguishable model. Arrows show the observed (9) estimates of the maximum number of cospeciation events and a conservative corrected estimate (8).

contrasting our study with that of Clark *et al.* (2000). Their overall findings are consistent with a history of strict cospeciation of aphids and endosymbionts. In fact, surprisingly, congruence among different aphid mitochondrial genes was no greater than that between aphid and symbiont genes (Clark *et al.,* 2000). However, the overall percentage of cospeciation was estimated at only 50–56.3%. In contrast, in our study, there is strong evidence for incongruence (ILD and SH tests) between pollinator and parasite phylogenies and yet the estimate of cospeciation is about the same (50–64%). Thus, both the distribution of, and the support for, may be as important as the overall percentage of cospeciating nodes.

In our study, genes in the same organisms were generally congruent (except Cyt *b* and ITS2 in *Pleistodontes*) but phylogenies of pollinators and parasites were incongruent. However, this does not necessarily mean that cospeciation is uncommon, since a small number of host switches in well-resolved trees can result in incongruence. In addition, recent studies have emphasized that the ILD is not an ideal test of whether two data sets support the same underlying topology because the test result is also influenced by the rate of evolution and the level of homoplasy. If one of the data sets (such the *Pleistodontes* Cyt *b* data set) is noisier than the other, this could result in a spurious significant result due to noise alone (Dolphin *et al.,* 2000). However if a nonsignificant result is obtained (in our case only between data sets from the same organism), it is probably safe to say that the data sets are congruent. On the other hand, the SH test and other likelihood-based tests of topologies, such as parametric bootstrapping (Huelsenbeck *et al.,* 1996b), seem appropriate for testing the null hypothesis that molecular data collected for *Pleistodontes* and *Sycoscapter* are consistent with the same underlying phylogeny, since these tests compare all members of a given set of *a priori* possible topologies (Goldman *et. al.,* 2000). Nevertheless, the results of Clark *et al.* (2000) suggest that some maximum-likelihood-based tests (they used KH and likelihood ratio tests) may reject the null hypothesis of perfect matching even if it is true, especially if the number of taxa is fairly large. They also suggest that having multiple genes from the same taxon, especially from nonrecombining mitochondrial regions, is one way to assess whether random matching is being falsely rejected. A related point is that, as the number of taxa increases, it becomes extremely unlikely that even an exceptionally well-planned and intensive study will actually generate the two "true" trees, so significant incongruence may generally increase with the number of taxa considered.

These methodological issues will clearly require further development and debate as more and larger phylogenies of interacting taxa become available for comparison. However, for now, we conclude that the partial matching of phylogenies showed by Treemap analysis and the significant levels of incongruence revealed by both SH and ILD tests support a history of significant but incomplete cocladogenesis between *Pleistodontes* and *Sycoscapter* wasps. Furthermore, their independent transmission processes and nonidentical phylogenies suggest a likely role for host switching in the evolutionary history of this association.

Molecular and Morphological Evolution

Dramatic examples of the "uncoupling" of rates of molecular and morphological evolution are found in several lineages, including African cichlid fishes (Sturmbauer and Meyer, 1992), Pacific scincid lizards (Bruna *et al.,* 1996), and horseshoe crabs (Selander *et al.,* 1970). Whereas *Pleistodontes* species display considerable morphological differentiation (C. Lopez-Vaamonde *et al.,*unpublished), *Sycoscapter* species (especially the females) are extremely similar in appearance (unpublished data). Although the differences in rates of molecular divergence are less dramatic, it is nevertheless *Sycoscapter* that shows the greater divergence (*t* test

results), suggesting that molecular and morphological evolution are not tightly coupled.

The greater genetic distances between *Sycoscapter* species than between their associated pollinators suggest that *Sycoscapter* may have the higher rate of molecular evolution. Another possibility is that *Sycoscapter* species are older. However, since all *Malvanthera* figs are pollinated by *Pleistodontes* species, and these two clades have presumably radiated over the same time scale, this would require that many of the *Sycoscapter* species originated on other fig species (not *Malvanthera*) and subsequently underwent host shifts. Such a scenario seems unlikely, given evidence for significant levels of cospeciation between *Malvanthera*-associated *Pleistodontes* and *Sycoscapter* species.

We must be cautious in our interpretation of relative rates of evolution since our use of the *t* test assumes that all pairwise distances are independent. Clearly they are not since each species is used several times and tree structure is ignored. However, these tests give us a crude index in the absence of fully congruent pollinator and parasite clades for formal comparison (see Hafner and Nadler, 1990). In addition, we can compare the 28S ML branch lengths for the two pairs of potentially cospeciated terminal sister taxa (i.e., *P. xanthocephalus*/S.2 + *P. greenwoodi*/S.4 and *P. schizodontes*/S.9 + *P. nitens*/S.10). The branch lengths were quite similar in both cases (0.010/0.016 and 0.005/0.006 for the first pair and 0.009/0.012 and 0.013/0.003 for the second pair), with the parasite having the longer branch in three cases and the pollinator in one (but this one had a larger difference). These data are far from conclusive but, for the moment, we conclude that the *t* tests and limited branch length comparisons suggest that there are not consistent major differences in genetic distances between species in the two genera.

We can also turn the question around and ask why females of different *Pleistodontes* species clearly show much greater morphological differentiation than females of the associated *Sycoscapter* species. There is an obvious (but unproven) answer to this question, which concerns their respective interactions with the fig inflorescence (syconia). The female pollinating wasps must enter the syconia of their host figs, and this involves adaptation to the anatomical structure of the fig and especially to the task of entering it via the tight and narrow ostiolar tunnel. The influence of syconium anatomy on wasp head morphology has been demonstrated by analysis of the parallel morphological adaptations of pairs of African pollinator wasp species and their associated members of an unusual clade of (sycoecine) fig-parasitic wasps that also enter the fig fruits (Van Noort and Compton 1996). We expect a high degree of coadaptation of the morphology of *Pleistodontes* females and their host fig syconia. In contrast, *Sycoscapter* females oviposit only through the fig wall and seem more likely to respond to syconial evolution largely in terms of their ovipositor length and form. Given that the mean diameters of ripe *Malvanthera* syconia vary from about 0.5 cm (*F. obliqua*) to over 5 cm (*F. sterrocarpa*), and in shape from spherical to elongate cylindrical with an extended ostiole, there is great scope for coadaptation of pollinator morphology in this group. In contrast, *Sycoscapter* species are also known from a wide range of non-*Malvanthera* fig species in Asia and Africa, and the female wasps generally show rather limited morphological variation.

CONCLUSION

The intimate invertebrate communities found within fig syconia provide excellent models for the study of cospeciation and host switching. Each *Ficus* species is typically host to one pollinating and many different nonpollinating wasp species (from different genera), in addition to mites and nematodes (Herre, 1996). Exploration of the phylogenetic relationships within and between these different groups, in tandem with studies of their trophic ecology, should help us better understand the interplay between ecology and cospeciation. One cannot overemphasize the importance of good alpha taxonomy to underpin cospeciation studies, and the *Malvanthera* (Dixon, 2001a,b; Dixon *et al.*, 2001) and *Pleistodontes* (C. Lopez-Vaamonde *et al.*, unpublished) are the only fig section and corresponding pollinating wasp genus to be subjected to recent, intensive, taxonomic revision.

Our study shows significant but imperfect correspondence between the phylogenies of associated pollinating and nonpollinating fig wasp genera. At present we cannot be sure whether this is due to their direct interaction or whether it is mediated through other processes (e.g., resource tracking of figs) leading to partial phylogenetic congruence. Consequently, there is an urgent need to generate a phylogeny of the *Malvanthera* figs for comparison with both wasp phylogenies. Additional data on the ecology and taxonomy of the nonpollinating wasps will also be important to our understanding, and morphological studies of *Malvanthera* fig syconia and the wasps that inhabit them will soon permit tests of ideas about the morphological coadaptation of these interacting taxa.

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