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SPECIAL PAPER

Out of Australia and back again: the world-wide historical biogeography of non-pollinating fig wasps (Hymenoptera: Sycophaginae)

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ABSTRACT

Aim Figs (*Ficus*, Moraceae) are exploited by rich communities of often host-specific phytophagous wasps. Among them, gall-inducing Sycophaginae (Hymenoptera, Chalcidoidea) may share a common history with *Ficus* and their mutualistic pollinators (Agaonidae). We investigate here, for the first time, the phylogeny and biogeographical history of Sycophaginae and compare the timing of radiation and dispersion of major clades with available data on *Ficus* and fig pollinators. Reconstructing the history of their host colonization and association over space and time is central to understanding how fig wasp communities were assembled.

Location World-wide.

Methods Maximum likelihood and Bayesian analyses were conducted on 4267 bp of mitochondrial and nuclear DNA to produce a phylogeny of all genera of Sycophaginae. Two relaxed clock methods with or without rate autocorrelation were used for date estimation. Analyses of ancestral area were also conducted to investigate the geographical origin of the Sycophaginae.

Results The phylogeny is well resolved and supported. Our data suggest a post-Gondwanan origin for the Sycophaginae (50–40 Ma) and two independent out-of-Australia dispersal events to continental Asia. Given palaeoclimatic and palaeogeographic records, the following scenario appears the most likely. The ancestor of *Idarnes+Apocryptophagus* migrated to Greater India through the Ninetyeast Ridge (40–30 Ma). The ancestor of *Anidarnes+Conidarnes* dispersed later via Sundaland (25–20 Ma). *Idarnes* and *Anidarnes* subsequently reached the New World via the North Atlantic land bridges during the Late Oligocene Warming Event. *Apocryptophagus* reached Africa *c.* 20 Ma via the Arabic corridors and returned to Australasia following the expansion of Sundaland tropical forests (20–10 Ma).

Main conclusions Sycophaginae probably invaded the fig microcosm in Australia *c.* 50–40 Ma after the origin of their host plant. Once associated with figs, they dispersed out of Australia and radiated together with their host fig and associated pollinator through the tropics. We recorded a good coincidence of timing between dispersal events of Sycophaginae and continental connections. Furthermore, fruit pigeons that disperse figs probably spread out of Australasia through the Indian Ocean via the Ninetyeast Ridge *c.* 38 Ma. Therefore, our study highlights the potential for combining molecular phylogenetics with multiple methods of dating of interacting groups to reconstruct the historical biogeography of plant–herbivore associations.

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Keywords
Biogeography, dispersal, divergence times, *Ficus*, fig wasps, gall-inducing insects, Ninetyeast Ridge, phylogeny.

INTRODUCTION

Gall-inducing insects are specialized herbivores that stimulate the development of, and feed on, modified plant tissues. Most of the gall inducers have colonized their host plants by host-shifts either soon or long after the diversification of their host plant (Weiblen & Bush, 2002; Nyman *et al.*, 2006; McLeish *et al.*, 2007; Stone *et al.*, 2009). Once they colonize a new plant resource, gall inducers track their host with a degree of fidelity that depends on the relative frequency of co-speciation and host-shift (Ehrlich & Raven, 1964; Janz & Nylin, 1998; Page & Charleston, 1998; Percy *et al.*, 2004). Therefore, inferring the phylogeny and the biogeographical history of galling lineages is of key importance for a better understanding of how galler communities were structured over space and time. To date only a few global analyses of the radiation and biogeography of galling lineages have been performed (Stone *et al.*, 2009). Here we propose the first world-wide historical biogeography of one major lineage of galling fig wasps.

The fig system is a well-known case of an intimate association between plants and numerous galling lineages (Weiblen, 2002; Cook & Rasplus, 2003; Herre *et al.*, 2008). Fig trees (*Ficus*, Moraceae) and their pollinating fig wasps (Agaonidae, Chalcidoidea) are inter-dependent for reproduction and are suspected to have coevolved, sharing a common evolutionary history, if not strictly co-speciated in a pairwise fashion (Jackson, 2004; Percy *et al.*, 2004; but see Jousselin *et al.*, 2008; and Machado *et al.*, 2005). Additionally, at least five other monophyletic groups of non-pollinating chalcids have colonized figs independently (West *et al.*, 1996; Kerdelhué *et al.*, 2000) so that fig inflorescences are host to a rich assemblage of chalcid wasps (up to 36 species). Most of the non-pollinating fig wasps (NPFW) are gall inducers or inquilines (i.e. wasps that develop on gall tissues and also devour the gall-inducer larvae) strictly associated with *Ficus* (e.g. Sycophaginae, Epichrysomallinae, Sycoecinae and Otitesellinae; Marussich & Machado, 2007). Only some species are functional parasitoids (larvae strictly developing on host-wasp tissues).

The NPFW assemblages differ among regions of the world and among groups of *Ficus*. Among the NPFW groups, the subfamily Sycophaginae occurs throughout the tropics and is associated with two unrelated subgenera of *Ficus*, namely *Urostigma* and *Sycomorus*. Within *Urostigma*, the Neotropical genera *Idarnes* and *Anidarnes* develop in *Ficus* of section *Americana* (pollinated by the genus *Pegoscapus*). The Australasian genus *Eukoebelea* is strictly associated with *Ficus* of subsection *Malvanthera* (pollinated by the genus *Pleistodontes*). In the Oriental region, one undescribed genus (*Conidarnes* nom. provis) is associated with subsection *Conosycea* (pollinated by the genera *Eupristina* and *Waterstoniella*). Except for two species of *Apocryptophagus* recently discovered in figs of *Ficus orthoneura* (subsection *Urostigma*) in South China, *Apocryptophagus* and *Sycophaga* species are strictly associated with subgenus *Sycomorus* (pollinated by the genus *Ceratosolen*) across the Old World (Table 1).

Few Sycophaginae species have been studied biologically but most are gall makers (Godfray, 1988; Kerdelhué & Rasplus, 1996). Sycophaginae use chemical mediation to locate their host plants (Proffit *et al.*, 2007). Most species oviposit through the fig wall using their elongated ovipositor (Wiebes, 1966). However, *Sycophaga*, and possibly also one

Table 1 Distribution, host figs and associated pollinators of Sycophaginae genera. The approximate ages estimated by Rønsted *et al.* (2005) for *Ficus* and Lopez-Vaamonde *et al.* (2009) for fig wasp are given in parentheses (95% confidence intervals if available).

Genera	Distribution	*Ficus* subgenus/(sub)section (crown group origin)	Genera of pollinators (crown group origin)
Anidarnes	Neo	*Urostigma Americana* (33.6–27.7 Ma)	*Pegoscapus* (calibration point: Dominican amber fossil 15–30 Ma)
Eukoebelea	Aus	*Urostigma Malvanthera* (41–35 Ma)	*Pleistodontes* (45–37 Ma)
Conidarnes	Ori	*Urostigma Conosycea* (*c.* 38 Ma)	*Waterstoniella, Eupristina* (52–48 Ma)
Idarnes	Neo	*Urostigma Americana* (33.6–27.7 Ma)	*Pegoscapus* (calibration point: Dominican amber fossil 15–30 Ma)
Apocryptophagus	Afr + Ori + Aus	*Sycomorus* (*c.* 48 Ma) + *Urostigma Urostigma* (*c.* 40 Ma)	*Ceratosolen* (68–62 Ma)
Pseudidarnes	Aus	*Urostigma Malvanthera* (41–35 Ma)	*Pleistodontes* (45–37 Ma)
Sycophaga	Afr	*Sycomorus* (*c.* 48 Ma)	*Ceratosolen* (68–62 Ma)

Afr, Afrotropical; Aus, Australia; Neo, Neotropical; Ori, Oriental.

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species of *Conidarnes* (J.-Y.R., pers. obs.), enter the fig through the ostiole and are consequently morphologically modified. Oviposition in a fig ovule induces rapid abnormal growth of the plant tissues on which the larvae feed. Each galler occupies a single ovule and thus reduces the fecundity of *Ficus* by one seed while negatively affecting pollinators by competing for access to flowers. Some species (*Idarnes carme* group and *Apocryptophagus* species with a long ovipositor) are either inquilines (cleptoparasitic) or parasitic on the pollinators or other NPFW (Elias *et al.*, 2008) and may even be facultative seed eaters (Pereira *et al.*, 2007a). Most Sycophaginae are host specific; however, some species can develop in closely related *Ficus* species and are then associated with two or more host fig species (e.g. *Apocryptophagus*, *Sycophaga*, *Idarnes*; Marussich & Machado, 2007; Silvieus *et al.*, 2007). With the exception of one species of *Apocryptophagus* inhabiting figs of *Ficus auriculata* in China (Peng *et al.*, 2005) and *Pseudidarnes minerva* associated with *Ficus rubiginosa* in Australia (Cook & Power, 1996), the majority of Sycophaginae species do not chew an exit hole through the fig wall to emerge from ripe figs and hence are dependent on the presence of pollinator males, who routinely chew holes, to complete their life cycle (Bronstein, 1991).

On each *Ficus* species, Sycophaginae form associations of one to six species (West & Herre, 1994; Kerdelhué & Rasplus, 1996; Kerdelhué *et al.*, 2000; Elias *et al.*, 2008; Wang & Zheng, 2008). These associations are structured by the development of the fig, the timing of oviposition and the feeding habits of the wasps (gallers versus cleptoparasites; Compton *et al.*, 1994). No complete biogeographic scenario has yet been proposed for the fig wasps associated with *Ficus*, although some partial hypotheses have been suggested for the world-wide diversification of the pollinating Agaonidae. Dating analyses have suggested that the fig–fig wasp mutualism diversified 60–100 Ma (Machado *et al.*, 2001; Datwyler & Weiblen, 2004; Rønsted *et al.*, 2005; Lopez-Vaamonde *et al.*, 2009). The area from which all extant fig trees originated is still largely unknown, but recent analyses suggested an origin of the Agaonidae fig wasp lineage in Asia or in East Gondwanaland (Lopez-Vaamonde *et al.*, 2009) and that dispersal played an important role in the evolution of the mutualism.

By unravelling the geography and phylogeny of fig wasp communities, historical biogeography provides a basis for answering questions such as: Where did the basal fig wasp and *Ficus* lineages originate? What is the age of the association of fig wasps with *Ficus*? Did NPFW colonize already-diversified hosts? Where did the different genera of fig wasps originate? Do specific genera of fig wasps share a common history of dispersal or vicariance with their host plants and other NPFW?

To answer these questions for the sycophagine fig wasps we establish here their phylogenetic relationships using multilocus data (three mitochondrial and one nuclear gene). The resulting phylogeny is based on 4.3 kbp of aligned mitochondrial and nuclear DNA sequences for all extant genera. We use the resulting phylogeny to estimate divergence times and infer ancestral areas. We also propose a biogeographic scenario for dispersal of the Sycophaginae across the world and compare the timing with the radiation and dispersal of *Ficus*, Agaonidae and non-pollinating Sycophaginae.

MATERIALS AND METHODS

Taxonomic sampling

We included 55 ingroup species representing all known genera of Sycophaginae, as well as most species-groups (Table 2). As the phylogenetic relationships within the Chalcidoidea superfamily are still unresolved, closer and more distant relatives were included as outgroups (Gibson *et al.*, 1999; Cruaud *et al.*, 2010). Five species belonging to the genera *Ceratosolen* (Agaonidae), *Odontofroggatia* (Epichrysomallinae), *Ficomila* (Eurytomidae) and *Megastigmus* (Torymidae) were used. All material was collected alive and fixed in 95% ethanol. Each time destructive extraction was used, vouchers were selected among specimens sampled from the same fig tree and the same fig after careful identification. Vouchers are deposited at CBGP, Montferrier-sur-Lez, France.

Laboratory protocols

The extraction protocol follows Cruaud *et al.* (2010). In the present study we combined one nuclear protein-coding gene, F2 copy of elongation factor-1α (EF1α, 516 bp), two mitochondrial protein-coding genes [cytochrome *c* oxidase subunit I (COI, 1503 bp), cytochrome *b* (cyt *b*, 728 bp)] and the ribosomal 28S rRNA (D2–D3 and D4–D5 expansion regions, 1520 bp). EF1α was amplified using F2-557F 5′-GAACGT GAACGTGGTTATYACSAT-3′ and F2-1118R 5′-TTACCT GAAGGGGAAGACGRAG-3′. The amplification protocol involved 3 min denaturation at 94 °C, then 35 cycles of 30 s denaturation at 94 °C, 1 min annealing at 58 °C, 1 min extension at 72 °C and a final extension of 10 min at 72 °C. Primer sequences and amplification protocols of other genes follow Cruaud *et al.* (2010). All the sequences are deposited in GenBank (Table 2).

Sequence alignment

Protein-coding genes and hypervariable regions were aligned using ClustalW 1.81 default settings (Thompson *et al.*, 1994). Alignments were translated to amino acids using MEGA 4 (Tamura *et al.*, 2007) to detect frameshift mutations and premature stop codons, which may indicate the presence of pseudogenes. Alignment of sequences encoding rRNA was based on secondary structure models (Gillespie *et al.*, 2006). The structural model of rRNA fragments and alignment details follow Cruaud *et al.* (2010).

Phylogenetic analyses

Phylogenetic trees were estimated using maximum likelihood and Bayesian methods. Analyses were conducted on a 150-core

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Table 2 List of Sycophaginae and outgroup species included in this study: voucher numbers, taxonomic information, host *Ficus* species, locality data and GenBank accession numbers. More information is available from the authors upon request.

Voucher	Genus	Species	Host *Ficus* species	Countries	COI	Cyt *b*	EF1α	rRNA 28S
0550_01w01a	*Apocryptophagus*	*comptoni*	*sycomorus*	Tanzania	HM770654	–	HM770497	HM770716
0659_21w01x	*Apocryptophagus*	*explorator*	*mauritiana*	La Réunion	HM770655	HM770556	HM770498	HM770717
0820_02w01a	*Apocryptophagus*	sp.	*prostrata*	China	HM770656	–	HM770499	HM770718
0857_11w012	*Apocryptophagus*	sp.	*squamosa*	China	HM770657	HM770557	HM770500	HM770719
1223_04w01a	*Apocryptophagus*	*stratheni*	*racemosa*	India	HM770658	HM770558	HM770501	HM770720
1259_03	*Megastigmus*	sp.	*virgata sessilis*	New Caledonia	GQ367876	GQ367971	HM770502	GQ367582
1360_05w01c	*Apocryptophagus*	*agraensis*	*racemosa*	India	HM770659	HM770559	HM770503	HM770664
1418_05w01x	*Pseudidarnes*	*minerva*	*rubiginosa*	Australia	HM770660	HM770560	HM770504	HM770665
1418_06w01e	*Eukoebelea*	sp.	*rubiginosa*	Australia	HM770661	HM770561	HM770505	HM770666
1422_03w01c	*Eukoebelea*	sp.	*obliqua*	Australia	HM770662	–	HM770506	HM770667
1426_01w013	*Apocryptophagus*	*testacea*	*racemosa*	Australia	HM770663	HM770562	HM770507	HM770668
1441_01w01c	*Apocryptophagus*	sp.	*sur*	Principe	HM770607	HM770563	HM770508	HM770669
1532_02w01c	*Sycophaga*	*sycomori*	*sycomorus*	South Africa	HM770608	HM770564	HM770509	HM770670
1604_02w01x	*Apocryptophagus*	sp.	*orthoneura*	China	HM770609	HM770565	HM770510	HM770671
1684_12w013	*Apocryptophagus*	sp.	*septica*	Taiwan	HM770610	HM770566	HM770511	HM770672
1767_02w013	*Idarnes*	sp.	*amazonica*	French Guiana	HM770611	HM770567	HM770512	HM770673
1767_03w01d	*Idarnes*	sp.	*amazonica*	French Guiana	HM770612	HM770568	HM770513	HM770674
1801_02w01a	*Idarnes*	sp.	*obtusifolia*	Mexico	HM770613	HM770569	HM770514	HM770675
1821_03w01c	*Apocryptophagus*	*randrianjohanyi*	*trichoclada*	Madagascar	HM770614	HM770570	HM770515	HM770676
1822_02w01a	*Apocryptophagus*	*labati*	*botryoides*	Madagascar	HM770615	HM770571	HM770516	HM770677
1866_02w01a	*Apocryptophagus*	sp.	*lepicarpa*	Malaysia	HM770616	HM770572	HM770517	HM770678
1910_02w01a	*Apocryptophagus*	sp.	*oligodon*	China	HM770617	HM770573	HM770518	HM770679
1947_02w01b	*Apocryptophagus*	*nesiotes*	*sakalavarum*	Madagascar	HM770618	HM770574	HM770519	HM770680
1987_02	*Ficomila*	sp.	*variegata*	Malaysia	GQ367946	GQ368043	HM770520	GQ367656
2028_05w01a	*Apocryptophagus*	*fusca*	*racemosa*	Indonesia	HM770619	HM770575	HM770521	HM770681
2085_02w01a	*Conidarnes*	sp.	*sumatrana*	Indonesia	HM770620	HM770576	HM770522	HM770682
2136_05w01b	*Idarnes*	sp.	*citrifolia*	Brazil	HM770621	HM770577	HM770523	HM770683
2171_02w01a	*Idarnes*	sp.	*trachelosyce*	Costa Rica	HM770622	HM770578	HM770524	HM770684
2171_03w01b	*Idarnes*	sp.	*trachelosyce*	Costa Rica	HM770623	–	HM770525	HM770685
2177_02w01b	*Anidarnes*	sp.	*perforata*	Costa Rica	HM770624	–	HM770526	HM770686
2177_03w01a	*Idarnes*	sp.	*perforata*	Costa Rica	HM770625	HM770579	HM770527	HM770687
2182_02w01a	*Idarnes*	sp.	*goldmanii*	Costa Rica	HM770626	HM770580	HM770528	HM770688
2195_02w01a	*Apocryptophagus*	sp.	*subcuneata*	Indonesia	HM770627	HM770581	HM770529	HM770689
2196_01	*Ceratosolen*	sp.	*comitis*	Indonesia	GQ367958	GQ368053	HM770530	GQ367670
2237_01w01a	*Sycophaga*	*cyclostigma*	*sur*	Gabon	HM770628	HM770582	HM770531	HM770690
2293_02w01a	*Apocryptophagus*	sp.	*congesta*	Indonesia	HM770629	HM770583	HM770532	HM770691
2301_02w01a	*Apocryptophagus*	sp.	*variegata*	Indonesia	HM770630	HM770584	HM770533	HM770692
2315_02w01a	*Apocryptophagus*	sp.	*mollior*	Indonesia	HM770631	HM770585	HM770534	HM770693
2331_01w01a	*Apocryptophagus*	sp.	*variegata*	Indonesia	HM770632	HM770586	HM770535	HM770694
2355_03b	*Odontofroggattia*	sp.	*microcarpa*	Indonesia	HM770633	HM770587	HM770536	HM770695
2355_05a	*Odontofroggattia*	*ishii*	*microcarpa*	Indonesia	HM770634	HM770588	HM770537	HM770696
2356_04w013	*Apocryptophagus*	sp.	*nodosa*	Indonesia	HM770635	HM770589	HM770538	HM770697
2448_04w01a	*Apocryptophagus*	*gigas*	*sycomorus*	Senegal	HM770636	HM770590	HM770539	HM770698
2451_03w01a	*Apocryptophagus*	sp.	*sur*	Senegal	HM770637	HM770591	HM770540	HM770699
2459_02w01a	*Apocryptophagus*	sp.	*tiliifolia*	Madagascar	HM770638	HM770592	HM770541	HM770700
2510_02w01a	*Apocryptophagus*	sp.	*dissipata*	Solomon Islands	HM770639	HM770593	HM770542	HM770701
2523_02w011	*Pseudidarnes*	sp.	*baola*	Solomon Islands	HM770640	HM770594	HM770543	HM770702
2529_01w013	*Eukoebelea*	sp.	*glandifera*	Solomon Islands	HM770641	HM770595	HM770544	HM770703
2558_01w01a	*Pseudidarnes*	sp.	*obliqua*	Australia	HM770642	HM770596	HM770545	HM770704
2562_02w011	*Idarnes*	sp. 7	*citrifolia*	Brazil	HM770643	–	HM770546	HM770705
2565_02w013	*Idarnes*	sp. 3	*eximia*	Brazil	HM770644	HM770597	HM770547	HM770706
2566_02w013	*Idarnes*	sp. 6	*eximia*	Brazil	HM770645	HM770598	HM770548	HM770707
2569_02w013	*Idarnes*	sp. 17	*eximia*	Brazil	HM770646	HM770599	HM770549	HM770708
2574_02w01a	*Idarnes*	sp. 6	*crocata*	Brazil	HM770647	HM770600	HM770550	HM770709
2575_02w013	*Idarnes*	sp. 23	*crocata*	Brazil	HM770648	HM770601	HM770551	HM770710
2578_02w01a	*Anidarnes*	sp. 2	*crocata*	Brazil	HM770649	HM770602	HM770552	HM770711

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Table 2 Continued

Voucher	Genus	Species	Host *Ficus* species	Countries	COI	Cyt *b*	EF1α	rRNA 28S
2580_02w013	*Idarnes*	sp. 9	*obtusifolia*	Brazil	HM770650	HM770603	HM770553	HM770712
2581_02w013	*Idarnes*	sp. 12	*obtusifolia*	Brazil	HM770651	HM770604	HM770554	HM770713
2584_02w013	*Idarnes*	sp. 26	*obtusifolia*	Brazil	HM770652	HM770605	HM770555	HM770714
2586_02w01a	*Anidarnes*	sp. 1	*obtusifolia*	Brazil	HM770653	HM770606	–	HM770715

–, No sequence was available.

Linux Cluster at CBGP. The most appropriate model of gene evolution for each data subset most likely to have experienced similar evolutionary processes (mitochondrial genes, EF1α, rRNA stems and loops) was identified using the Akaike information criterion implemented in MrAIC.pl 1.4.3 (Nylander, 2004).

We performed maximum likelihood analyses (ML) and associated bootstrapping using the MPI-parallelized RAxML 7.0.4 (Stamatakis, 2006b). GTRCAT approximation of models was used for ML bootstrapping (Stamatakis, 2006a) (1000 replicates). A bootstrap percentage (BP) > 95% was considered as strong support and BP < 70% as weak.

Bayesian analyses were conducted using a parallel version of MrBayes v. 3.1.1. (Huelsenbeck & Ronquist, 2001). We assumed across-partition heterogeneity in model parameters by considering the parameter *m*. Parameter values for the model were initiated with default uniform priors and branch lengths were estimated using default exponential priors. To improve mixing of the cold chain and avoid it converging on local optima, we used Metropolis-coupled Markov chain Monte Carlo (MCMC), with each run including a cold chain and three incrementally heated chains. The heating parameter was set to 0.02 in order to allow swap frequencies from 20% to 70%. We ran two independent runs of 10 million generations. All values were sampled every 1000 generations. For the initial determination of burn-in, we examined the plot of overall model likelihood against generation number to find the point where the likelihood started to fluctuate around a constant value. The points sampled prior to convergence of the chains were then discarded. We used a range of MCMC convergence and good mixing diagnostics following Cruaud *et al.* (2010). The results were based on the pooled samples from the stationary phases of the two independent runs. Posterior probabilities (PP) > 0.95 were considered as strong support.

Molecular dating

A number of molecular dating methods are currently available. Many of them take into account uncertainty in the topology, branch lengths and calibration points. Most of these methods are based on the assumption that evolutionary rates among branches in a phylogenetic tree are inherited and are correlated with physiology and life history. Consequently, these methods assume a degree of autocorrelation between molecular rates in adjacent branches of the tree. On the contrary, other methods use uncorrelated clock models based on the hypothesis that evolutionary rates among branches are random variables drawn from a specified probability distribution (Ho, 2009).

Here, we used two relaxed clock methods with or without rate autocorrelation implemented in PhyloBayes 3.2c (lognormal autocorrelated, uniform prior on divergence times) (Lartillot *et al.*, 2004) and in beast 1.5.3 (uncorrelated lognormal; Drummond & Rambaut, 2007), respectively:

1. PhyloBayes. The default combination of independent Dirichlet processes was used to model site-specific features of sequence evolution. Two chains were run in parallel and convergence was assessed using the tracecomp program. Chains were stopped when maximum discrepancy between summary variables of the trace files was < 0.1 and the minimum effective size of these summary variables was > 100 (Lartillot *et al.*, 2004).

2. beast. The same modelling strategies as for MrBayes and RAxML were used. We assumed a Yule tree prior. Node constraints were assigned a normal prior distribution with the standard deviation encompassing the minimum and maximum age of each calibration. We used default priors for all other parameters. Two runs of 30,000,000 generations were performed with sampling every 3000 generations. The two separate runs were then combined using LogCombiner 1.4.8. We ensured convergence for each parameter using both tracer 1.4 (Drummond & Rambaut, 2007) and AWTY (Nylander *et al.*, 2008). Following the removal of 10% burn-in, the sampled posterior trees were summarized using TreeAnnotator 1.4.8 to generate a maximum clade credibility tree and calculate the mean ages, 95% highest posterior density intervals and PP.

We used the following calibration constraints.

1. *Idarnes* crown. A species of *Idarnes* has been recently discovered by S.G. Compton (University of Leeds, UK) in Dominican amber. Given uncertainties over the age of Dominican amber (Iturralde-Vinent & MacPhee, 1999), beast analyses were performed using a normal prior with a mean of 22.5 Ma and a standard deviation (SD) of 4.5 Ma. PhyloBayes analyses were conducted specifying an upper and a lower constraint of 30 and 15 Ma, respectively.

2. Mascarene Archipelago. Mauritius, the oldest island, is *c.* 8 Myr old based on K–Ar dating (McDougall & Chamalaun, 1969; McDougall, 1971). *Apocryptophagus explorator* is endemic to La Réunion. Consequently the maximum age constraints on the node grouping *A. explorator*, *Apocrypto-*

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phagus sp. ex *Ficus trichoclada* and *Apocryptophagus* sp. ex *Ficus tiliifolia* was modelled with a normal distribution with a mean of 8 Ma and SD of 0.05 Myr. This constraint assumes rapid colonization after island emergence.

3. Solomon Islands. The Solomon Islands have a complex geological history. The uplift of the South Solomon block dates back to 11–12 Ma and the north-east-directed South Solomon arc of volcanism to 6 Ma (Petterson *et al.*, 1999). *Apocryptophagus* sp. (ex *Ficus dissipata*) is endemic to the Solomon Islands. Consequently the maximum age constraint on the node grouping *Apocryptophagus* sp. (ex *F. dissipata*) and *Apocryptophagus* sp. (ex *Ficus congesta*) was modelled with a normal distribution with a mean of 9.5 Ma and SD of 1.0 Myr. PhyloBayes analyses were conducted specifying an upper and a lower constraint of 11 and 7.9 Ma, respectively. beast and PhyloBayes chronograms were visualized with FigTree v. 1.2 (Rambaut, 2006).

Reconstruction of ancestral areas

We chose a ML approach to infer where the different groups of Sycophaginae originated. Ancestral area was inferred on the ML tree using the stochastic Markov model of evolution implemented in Mesquite 2.72 (Maddison & Maddison, 2008). Following Lopez-Vaamonde *et al.* (2009), current species distributions were categorized into five character states: Afrotropical (Africa, Madagascar and the Mascarene Archipelago), Australasia (east of Wallace's Line), Neotropical (southern and central Americas), Asia (continental and Sundaland) and Australasia + Asia to encode taxa occurring from continental Asia to Australasia. Encoding took into account all published geographic localities for Sycophaginae, museum specimens and *c.* 3000 samples of fig wasp communities we had collected over the last 15 years.

RESULTS

Sequence data

The final matrix contained 55 ingroup and 5 outgroup species for a total length of 4267 bp (COI + cyt b = 2231 bp, EF1α = 516 bp, 28S core and stems = 933 bp, 28S loops and Clustal-aligned parts = 587 bp). Of these, 2158 bp were variable and 1615 bp parsimony informative. Alignment of exons revealed no indels. For all partitions the best-fitting model was GTR+I+G.

Phylogenetic analysis

All reconstructions produced similar topologies. We arbitrarily chose to map node support values on the beast topology (Fig. 1). We give node support as follows: (1) bootstrap proportions (BP) of ML, (2) PP given by MrBayes, and (3) PP given by beast. The topology is well resolved and provides strong support for most notable relationships within Sycophaginae. In all analyses, Sycophaginae is recovered as monophyletic with strong support (BP 100, PP 1.0) with the exception of the beast reconstruction (PP 0.81). All Sycophaginae genera – with the exception of *Apocryptophagus* – are monophyletic with strong support. Sycophaginae is subdivided into three main clades.

1. *Eukoebelea*, recovered as the sister lineage to all other Sycophaginae (BP 99, PP 1.0, PP 1.0).

2. A strongly supported clade of three genera, namely *Pseudidarnes*, *Anidarnes* and *Conidarnes* (BP 100, PP 1.0, PP 0.99). *Pseudidarnes* always appears basal to *Anidarnes* + *Conidarnes*.

3. A well-supported clade (BP 74, PP 1.0, PP 0.98) composed of two groups: *Apocryptophagus*/*Sycophaga* (BP 100, PP 1.0, PP 0.99) and *Idarnes* (BP 100, PP 1.0, PP 1.0) (Fig. 1).

Within the *Apocryptophagus*/*Sycophaga* group, the internodes are short (Fig. 1), making the recovery of unambiguous phylogenetic information difficult. *Apocryptophagus* associated with *Ficus* section *Sycocarpus* group in a strongly supported clade (Fig. 1, i). This group and two continental Asiatic species respectively associated with *Ficus oligodon* (subsection *Neomorphe*) and *Ficus orthoneura*, an atypical *Ficus* from subsection *Urostigma* (Fig. 1, ii), root basally to the remaining *Apocryptophagus*/*Sycophaga*. The remaining *Apocryptophagus*/*Sycophaga* species are distributed in five well-supported clades with no firmly established order of branching.

1. An East Wallacean clade (BP 100, PP 1.0, PP 1.0) that comprises *Apocryptophagus* species associated with *Adenosperma* fig trees (Fig. 1, iii).

2. One *Apocryptophagus* species associated with *Ficus prostata*, an atypical *Ficus* of the ambiguous section *Hemicardia*, from continental Asia (Fig. 1, iv).

3. A clade (BP 100, PP 1.0, PP 1.0) including *Apocryptophagus agraensis* and *Apocryptophagus spinitarsus*, cleptoparasites associated respectively with *Ficus racemosa* and *Ficus variegata* (section *Sycomorus*) (Fig. 1, v).

4. The two *Apocryptophagus* species associated with *F. variegata* and *Ficus nodosa* (section *Sycomorus*) in New Guinea (PP 1.0, BP 100) (Fig. 1, vi).

5. A large and well-supported clade (BP 99, PP 1.0, PP 0.99) of *Apocryptophagus* and *Sycophaga* species exclusively associated with monoecious species of section *Sycomorus* (Fig. 1, vii). These species are mostly Afrotropical but three of them are associated with the Oriental *F. racemosa*.

The *Apocryptophagus*/*Sycophaga* clade associated with section *Sycomorus* is subdivided into five groups.

1. *Apocryptophagus gigas* (*Ficus sycomorus* and *Ficus mucuso*) plus *Apocryptophagus stratheni* (*F. racemosa*), two species of early gallers (Fig. 1, viii).

2. All *Sycophaga* species that consequently render genus *Apocryptophagus* paraphyletic (Fig. 1, ix).

3. All *Apocryptophagus* species from Madagascar and the Mascarene Archipelago (Fig. 1, x).

4. *Apocryptophagus testacea* and *Apocryptophagus fusca* associated with *F. racemosa* (Fig. 1, xi).

5. The Afrotropical *Apocryptophagus* with long ovipositors associated with *Ficus sur* and *F. sycomorus* (Fig. 1, xii).

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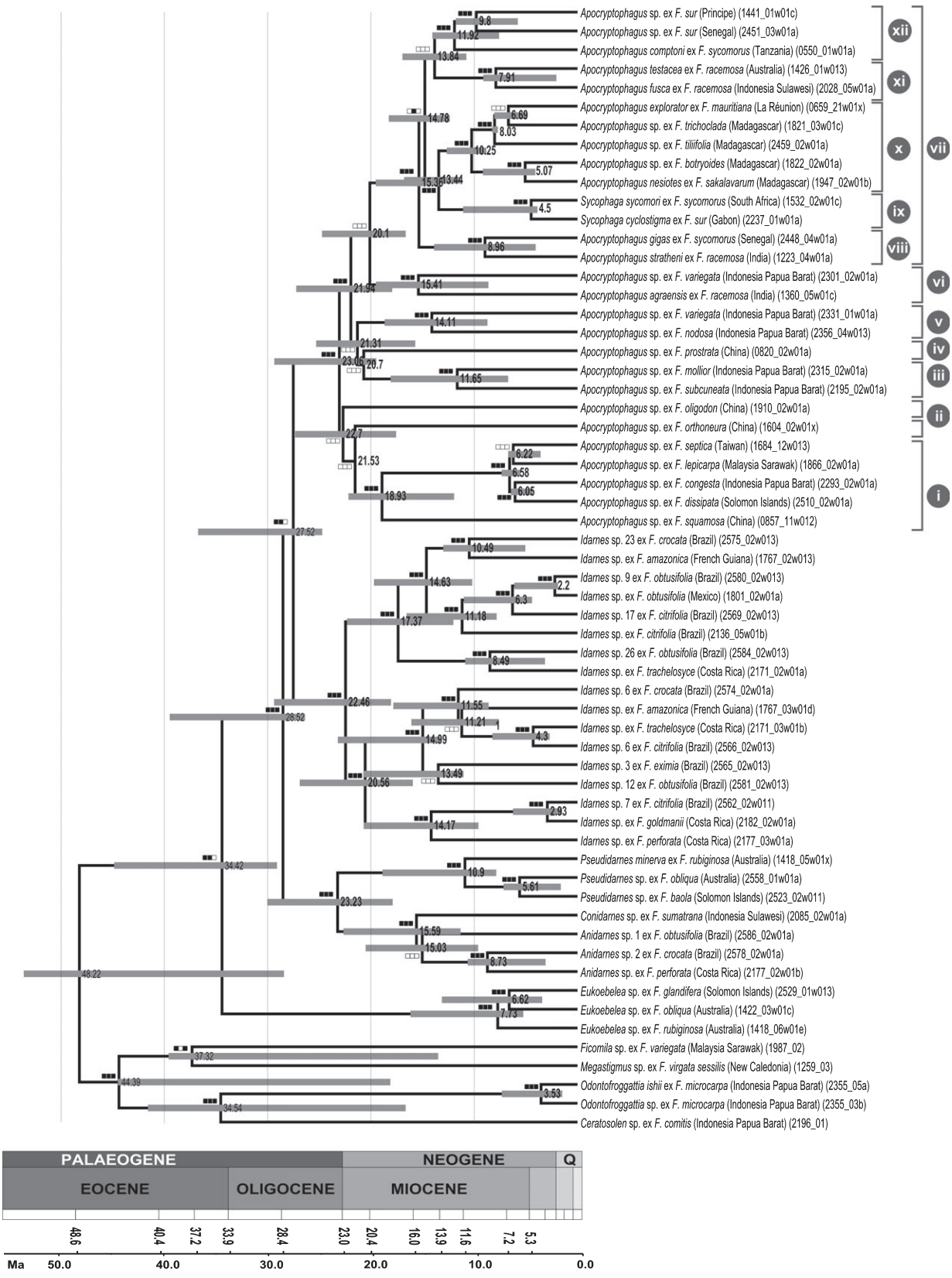

Figure 1 BEAST chronogram showing the timing of evolution of the Sycophaginae. Grey bars around node ages (Ma) indicate the 95% highest posterior density (HPD) intervals. The geological time-scale is shown at the bottom. Squares correspond to node supports and are respectively given for RAxML, MrBayes and BEAST analyses. Black squares highlight bootstrap values > 70 or posterior probability > 0.95. *Apocryptophagus* groups (i–xii) are detailed in the text.

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Table 3 BEAST and PHYLOBAYES mean age estimates (Ma) for selected nodes in the phylogeny of Sycophaginae with 95% lower and upper highest posterior distribution.

Nodes	Estimated date Ma (95% confidence interval)	
	BEAST	PHYLOBAYES
Stem group Sycophaginae	48.2 (53.4–28.2)	41.9 (51.0–35.5)
Crown group Sycophaginae	34.4 (44.7–29.3)	41.2 (50.5–34.9)
Pseudidarnes + *Idarnes*/*Apocryptophagus*	28.5 (39.3–26.6)	38.8 (47.0–32.8)
Idarnes + *Apocryptophagu Sycophaga*	27.5 (36.6–24.9)	37.8 (45.5–32.0)
Apocryptophagus/*Sycophaga*	23.1 (29.2–19.7)	34.0 (43.0–28.8)
Idarnes	22.5 (29.2–18.2)	28.2 (29.9–24.1)
Pseudidarnes + (*Conidarnes* + *Anidarnes*)	23.2 (29.8–18.1)	26.1 (32.3–20.3)
Idarnes incerta group + *I. flavicollis* group	20.6 (26.7–16.1)	25.8 (28.4–21.1)
Apocryptophagus associated with sect. *Sycocarpus*	18.9 (22.0–12.1)	30.7 (38.7–26.1)
Conidarnes + *Anidarnes*	15.6 (22.5–11.5)	16.5 (24.6–9.5)
Afrotropical *Apocryptophagus*	15.4 (19.4–13.5)	19.9 (24.1–16.4)
Idarnes carme group	17.4 (22.1–12.2)	22.2 (25.9–18.0)
Anidarnes	15.0 (20.3–9.8)	13.7 (20.2–7.1)
Sycophaga + Malagasy *Apocryptophagus*	13.4 (16.6–11.5)	16.6 (19.4–13.2)
Eukoebelea	7.7 (16.0–5.4)	18.2 (27.1–10.6)
Sycophaga	4.5 (10.9–4.1)	10.9 (14.2–7.9)

Molecular dating

The mean ages of major nodes with 95% confidence intervals are indicated in the chronogram (Fig. 1) and in Table 3. The estimates of divergence times suggest that Sycophaginae is a post-Gondwanan group that appeared between 34.4 and 41.2 Ma (BEAST and PHYLOBAYES estimates, respectively). Most clades and all genera within Sycophaginae result from Palaeogene diversification.

Biogeographic analysis

The Markov-ML reconstruction of ancestral areas indicated that the most recent common ancestor of all extant Sycophaginae lived in Australasia. The proportions of the maximum likelihood (PML) attributed to each biogeographic regions were: Australasian region = 0.63, Neotropical = 0.20, continental Asia = 0.13, remaining areas = 0.04 (Fig. 2). From Australasia, Sycophaginae migrated to Southeast Asia and continental Asia at least twice independently (ancestral *Apocryptophagus/Idarnes* and *Conidarnes*).

From Australasia or continental Asia, two lineages reached South America independently. For *Idarnes*, colonization of the New World was followed by rapid cladogenesis and diversification.

Our ML analysis favoured an origin of *Apocryptophagus*/*Sycophaga* either in Asia (PML = 0.64) or in Australasia (PML = 0.32; Fig. 2). However, because the Australasian and Afrotropical realms are disjunct areas, a direct colonization of Africa from Australasia is biogeographically unlikely for these genera. We therefore favour an origin in continental Asia. Furthermore, the nested position of Afrotropical and Malagasy *Apocryptophagus*/*Sycophaga* within Asian taxa (Fig. 1) suggests a continental Asian origin of Afrotropical *Apocryptophagus* and a subsequent migration into Africa. The ancestors of Afrotropical *Apocryptophagus* apparently reached the African continent and from there Madagascar and the Mascarene Archipelago during the Miocene. The nested position of three of the taxa associated with *F. racemosa* (a fig tree distributed from India to Australia) within the Afrotropical clade indicates further dispersal events from Africa back to Asia. The ancestor of the clade of *Apocryptophagus* associated with section *Sycocarpus* may have dispersed back from continental Asia to New Guinea and the Pacific islands.

DISCUSSION

Out of Australia, but how?

Sycophaginae are widely distributed, spanning all tropical regions (*Idarnes* and *Anidarnes* in South America, *Pseudidarnes* and *Eukoebelea* in Australasia, *Conidarnes* and *Apocryptophagus* in the Oriental region, and *Apocryptophagus* and *Sycophaga* in the Afrotropics; Table 1). These tropical disjunctions between extant lineages could suggest vicariance resulting from the break-up of Gondwanaland. Our data provide a new perspective on the historical biogeography of Sycophaginae and suggest instead a post-Gondwanan origin for Sycophaginae and out-of-Australia dispersal during the Middle Palaeogene (Fig. 3a–c).

Eukoebelea is sister to all other Sycophaginae and only occurs east of Wallace's Line on *Malvanthera* fig trees, a group of figs supposed to be of Australian origin (Rønsted *et al.*, 2008). Furthermore, *Pseudidarnes*, the basal group of the clade (*Pseudidarnes* (*Conidarnes* + *Anidarnes*)) is also associated with *Malvanthera* in Australia and also includes at least six Papuan species associated with series *Hesperidiiformes* of *Malvanthera*. Consequently Sycophaginae that constitute species-rich

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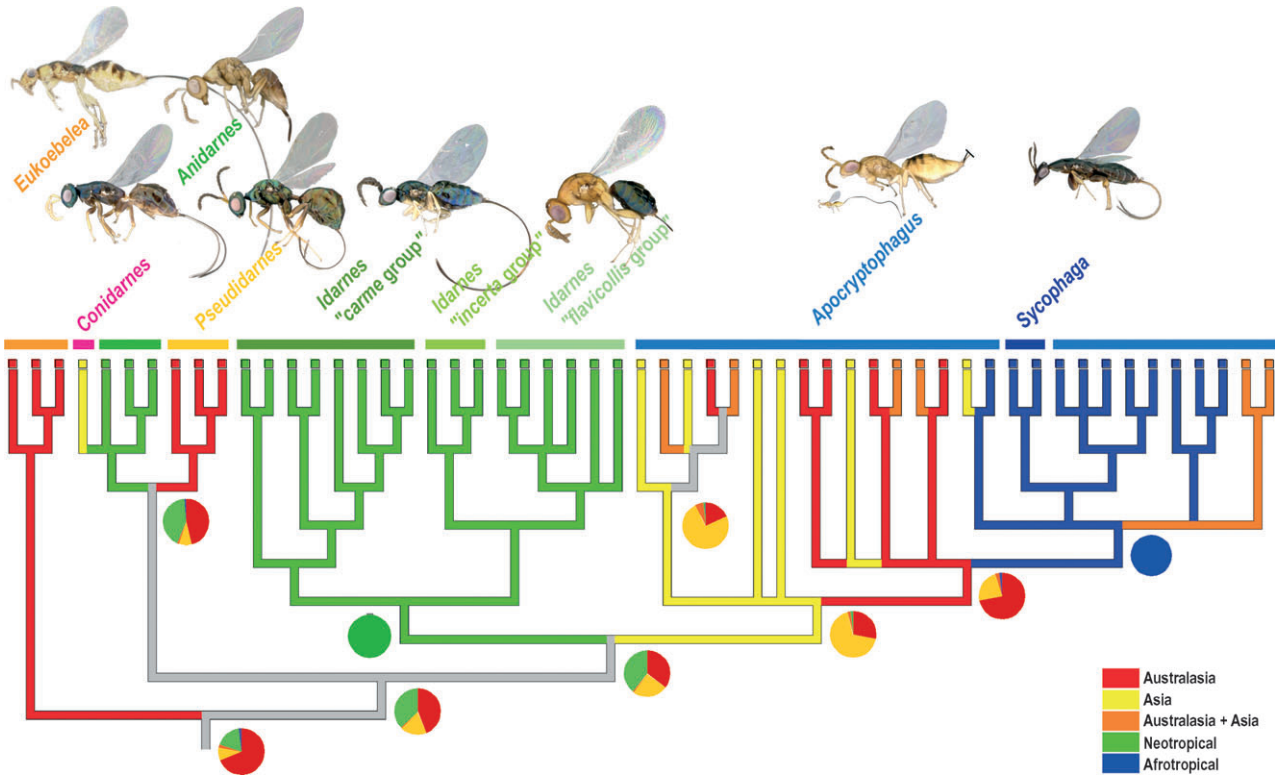

Figure 2 Reconstruction of the ancestral area of major clades of Sycophaginae. The proportion of the total likelihood received by each biogeographic region as the ancestral area of the major clades (calculated with Mesquite) is represented by pie charts at nodes.

communities in Australasia, Asia and the Neotropics originated from two independent out-of-Australia dispersal events during the Late Eocene and Early Miocene, respectively.

Our analyses provide an age of 41.9–48.2 Ma (mean age, respectively PhyloBayes and beast) for the origin of Sycophaginae and of 41.2–34.4 Ma (respectively PhyloBayes and beast) for the crown group of Sycophaginae (Table 3). Therefore, Sycophaginae probably invaded the fig microcosm in Australia 50 to 40 Ma after its origin (100–60 Ma). A similar result was found for cynipid gallwasps that colonized oaks long after their diversification (Stone *et al.*, 2009). The estimated age of Sycophaginae is similar to the independently estimated age of *Malvanthera*, which are probably their ancestral host plants. According to Rønsted *et al.* (2005), section *Malvanthera* originated at least 41 Ma and radiated gradually from *c.* 35 Ma. Our age estimate for the basal lineages of Sycophaginae associated with *Malvanthera* is also congruent with the age of 40 Ma independently estimated for the *Pleistodontes* crown group, using different molecular dating methods (Lopez-Vaamonde *et al.*, 2009) (Table 1).

These congruent cross-estimates of the respective ages of interacting groups (plants, pollinators and gall-makers in the figs) using independent datasets and dating methods support our out-of-Australia scenario for the Sycophaginae. It furthermore highlights the power of phylogenetic multiple dating of interacting groups (plants/gall-makers) to reconstruct the historical biogeography of these associations. Divergence within an ancestral plant group and its specialized associates may have been synchronous over geological time even if co-speciation did not play a major role in the evolutionary process.

Sycophaginae expanded out of Australia at least twice. The first dispersal event concerned the ancestor of the clade (*Apocryptophagus*/*Sycophaga*) + *Idarnes* (Fig. 3a), the second one concerned the ancestor of the clade *Anidarnes* + *Conidarnes* (Fig. 3a,b). The first dispersal of Sycophaginae is estimated between 28.5 Ma and 27.5 Ma by beast and between 37.8 Ma and 38.8 Ma by PhyloBayes, so roughly between 40 and 30 Ma. At that time Australia was isolated from Asian and African landmasses and there is no trivial explanation of how this lineage expanded out of Australia. Three hypotheses may explain the observed pattern of Sycophaginae distribution, as follows.

Hypothesis 1. Through Antarctica and via America

This hypothesis assumes that the ancestor of *Apocryptophagus*/*Idarnes* dispersed through Antarctica into South America, and subsequently colonized the Old World via the North Atlantic land bridges or Beringia. Until 45–30 Ma Antarctica was connected to Australia (Convey *et al.*, 2008). However, although Antarctica remained largely ice free during the Eocene (Thorn & DeConto, 2006), it was occupied by a cold-climate flora dominated by Araucariaceae, Podocarpaceae and *Nothofagus* (Truswell & Macphail, 2009). This flora was subjected during the Middle Eocene (*c.* 43 Ma) to up to

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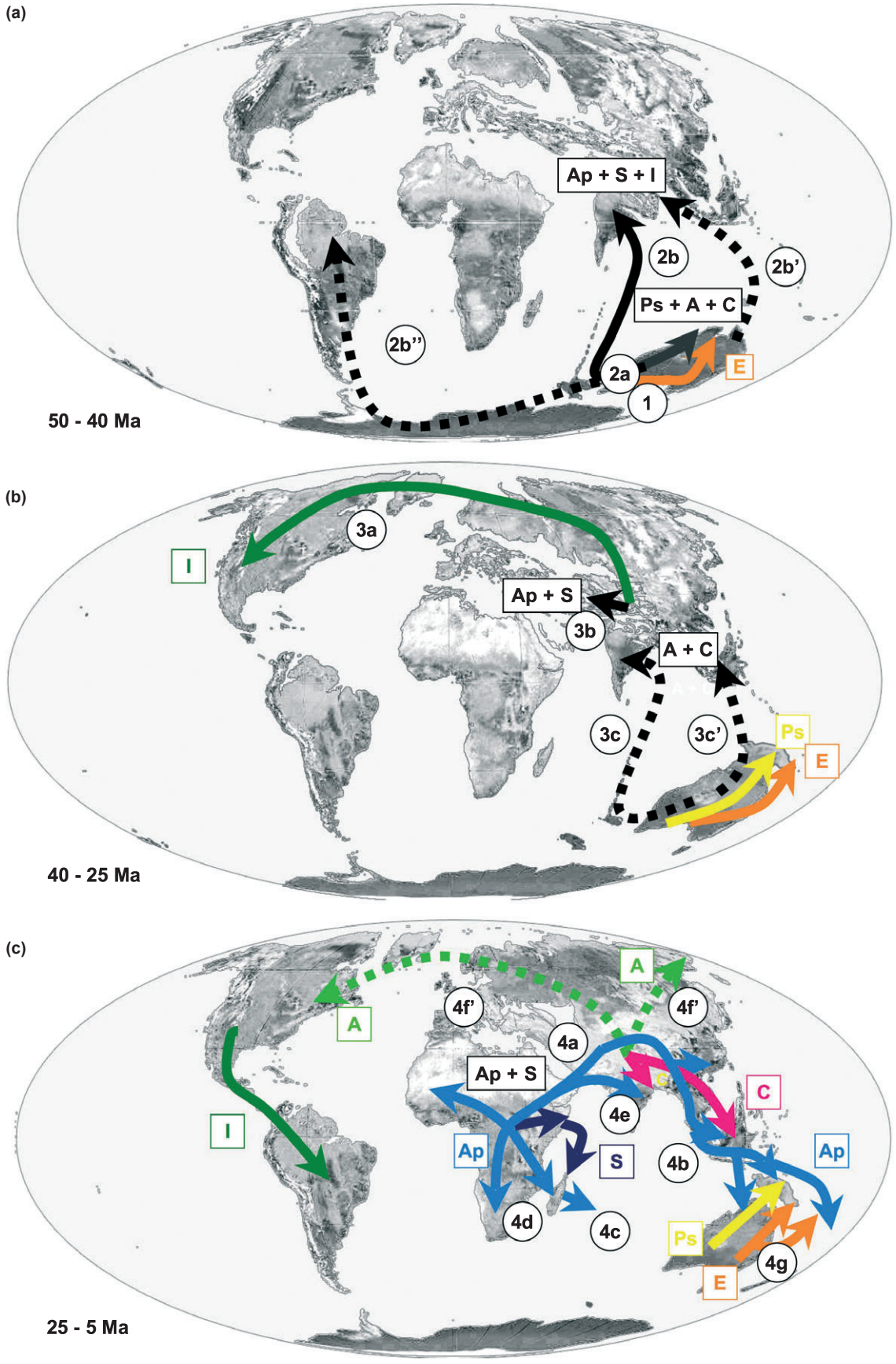

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6 months of total darkness and to mean annual temperatures of *c.* 10–15 °C (Francis & Poole, 2002). Hence, after the Early Eocene, the local climate was unlikely to support *Ficus* and their associates which thrive only in tropical/subtropical climates (Zerega *et al.*, 2005). Hence, current knowledge of Antarctic palaeoclimates is not compatible with this scenario. Nevertheless, a lack of old fossils may have biased our estimates of the migration periods. If dispersal of the *Apocryptophagus*/*Idarnes* ancestor happened earlier than our estimates, then colonization of continental Asia may have occurred through the temperate connection that still linked Australia, Antarctica and South America during the Early Eocene.

Hypothesis 2. Long-distance dispersal events from Australia to Sundaland across Wallacea

During the Eocene or Early Oligocene, Australia continued to move northwards. Before 30 Ma, dispersal from this continent to Southeast Asia was not possible. By 25–30 Ma some organisms could disperse via the fragments broken off from the Australian Plate (terranes) and the emergent land in Wallacea (East Sulawesi, Vogelkop; Hall, 1998). However, the suggested low dispersal ability of NPFW compared with pollinators (Harrison & Rasplus, 2006) makes transoceanic dispersal of Sycophaginae from Australia to Sundaland, or to Greater India, Africa and even South America, unlikely. Indeed, the longest colonization of isolated islands inferred for a Sycophagine species is *c.* 700 km between Madagascar and La Réunion, which contradicts this hypothesis. However, our dating estimates could be inaccurate and emigration of the ancestor of *Apocryptophagus*/*Idarnes* may have occurred later. Alternatively, exchanges of flora and fauna between Australia and Southeast Asia may have occurred earlier than currently accepted. However, because we did not record any basal *Apocryptophagus* species from Borneo, this scenario is unlikely.

The ancestor of *Anidarnes* and *Conidarnes* may, however, have followed this dispersal route. Indeed, we estimated that this second out-of-Australia dispersal event occurred later, between 23.2 Ma and 15.6 Ma (BEAST estimate) and between 26.1 Ma and 16.5 Ma (PHYLOBAYES), a timing compatible with a passage along that route given their dispersal abilities. Moreover, the species richness of *Conidarnes* is higher in Borneo (seven of the nine known species) than in continental Asia.

Hypothesis 3. Through the Ninetyeast Ridge

This dispersal route was proposed to explain the disjunct distributions of some oscine birds (Passerida; Fuchs *et al.*, 2006; Jønsson *et al.*, 2008). The authors proposed that the birds dispersed from Australia to Africa across stepping-stones in the southern Indian Ocean during the warm Eocene period (Kennett, 1995). At that time, this part of the Indian Ocean may have been connected to India and Africa via stepping-stone dispersal through the Réunion hotspot trace islands and the Ninetyeast Ridge (Ali & Aitchison, 2008). Concerning Sycophaginae, our analyses support Australasia and continental Asia as the most likely ancestral areas of *Apocryptophagus*/*Sycophaga* + *Idarnes* clade (Fig. 2). A key argument for such a scenario is the presence of two basal species of *Apocryptophagus* on section *Urostigma* (*F. orthoneura*) in continental Asia (Fig. 1). This is the first and only report of *Apocryptophagus* species associated with a non-*Sycomorus* fig tree. Furthermore, these species have a metallic tinge, a character exhibited by most *Idarnes* species but by no other *Apocryptophagus* species. *Ficus orthoneura* is considered as a basal *Ficus* species within subsection *Urostigma*. Indeed, this Sino-Himalayan species exhibits a bifid stigma, a character that is also reported from some *Malvanthera* species from Australia but not elsewhere within subgenus *Urostigma* (Corner, 1978). These observations suggest that basal *Ficus* of section *Conosycea* inhabiting

Figure 3 Maps illustrating key dispersal events relevant to Sycophaginae biogeography. The shading represents the relief of the continents at that time. Abbreviations: E, *Eukoebelea*; Ps, *Pseudidarnes*; A, *Anidarnes*; C, *Conidarnes*; I, *Idarnes*; Ap, *Apocryptophagus*; S, *Sycophaga*. (a) Map 50–40 Ma. (1) Origin of the Sycophaginae in Australia *c.* 50–40 Ma. Divergence between *Eukoebelea* and other Sycophaginae. (2a) Divergence between *Pseudidarnes* + (*Anidarnes* + *Conidarnes*) and *Idarnes* + *Apocryptophagus*/*Sycophaga*. About 40–30 Ma, long-distance dispersal of the ancestor of *Idarnes* + *Apocryptophagus*/*Sycophaga* to continental Asia via: (2b) the Kerguelen Plate, the Ninetyeast Ridge and Greater India (most likely hypothesis), (2b′) directly to Sundaland, and (2b″) Antarctica and South America. This dispersal event could be concomitant with the dispersal of the ancestor of *Anidarnes* + *Conidarnes* and was followed by the split between *Idarnes* and *Apocryptophagus*/*Sycophaga*. (b) Map 40–25 Ma. (3a) Between 35 and 20 Ma, colonization of the Neotropics by the ancestor of *Idarnes*. This dispersal event probably occurred via the North Atlantic land bridges during the Late Oligocene (26–23 Ma) (this is supported by an Oligocene Agaonidae fossil from Isle of Wight). Dispersal of *Idarnes* and *Anidarnes* may have been concomitant. (3b) From 25 to 20 Ma, diversification of *Apocryptophagus* and shift to *Sycomorus*. Between 26 and 16 Ma, dispersal of the ancestor of (*Anidarnes* + *Conidarnes*) to continental Asia, via two potential routes (3c) the Ninetyeast Ridge and Greater India, (3c′) directly through Sundaland, following the collision between the Australian and the Asian plates. (c) Map 25–5 Ma. (4a) Around 20 Ma *Apocryptophagus* colonized Africa via the Arabic corridors. (4b) Simultaneously, the genus dispersed to Sundaland, Australasia and the Pacific islands. Some species reached Taiwan to the north and the Solomon Islands to the south by 10–5 Ma. (4c) 10 Ma, colonization of Madagascar and later the Mascarene Archipelago by Afrotropical *Apocryptophagus*. (4d) 15–10 Ma, one lineage of *Apocryptophagus* adapted to enter the fig through the ostiole and subsequently diversified (*Sycophaga*). (4e) 20–10 Ma, a few Afrotropical *Apocryptophagus* returned to continental Asia with their fig tree (*Ficus racemosa*), they subsequently reach Australia. (4f or 4f′) Between 23 and 15 Ma dispersal of *Anidarnes* to New World through the North Atlantic land bridges or Beringia. (4g) From 10 Ma, *Eukoebelea* and *Pseudidarnes* colonize Pacific islands and extend to Wallace's Line.

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continental Asia and India (*Ficus arnottiana*, *Ficus beddomei*, *Ficus dalhousiae*, *Ficus costata* and *Ficus hookeriana*) may be pivotal for our understanding of the evolution of Sycophaginae. Another strong argument is the presence in India of basal *Apocryptophagus* species associated with *F. oligodon* and *Ficus prostrata*, two *Sycomorus* species endemic to continental Asia that may also represent out-of-India dispersers (Fig. 1). Consequently, the host shift from subsection *Urostigma* to subgenus *Sycomorus* may have occurred in continental Asia during the Oligocene.

One or two voyages to the New World?

The timing of New World colonization seems to differ between the ancestors of *Idarnes* and *Anidarnes*, although the estimates slightly overlap (around 20 Ma). These results lead us to propose two alternative hypotheses.

Hypothesis 1. Independent colonization of the Neotropics

The ancestor of *Idarnes* may have colonized South America between 27.5 Ma and 22.5 Ma (BEAST) or between 37.8 Ma and 28.2 Ma (PHYLOBAYES) (Table 3, Fig. 3b), so roughly between the Late Eocene and Early Miocene. Ancestors of *Anidarnes* may have colonized the Neotropics more recently, between 15.6 Ma and 15 Ma (BEAST) or between 16.5 Ma and 13.7 Ma (PHYLOBAYES) (Fig. 3c).

Hypothesis 2. Concomitant colonization of the Neotropics

While less likely, we cannot totally rule out a simultaneous colonization during the Late Oligocene or Early Miocene (around 20 Ma).

From the beginning of the Tertiary period, Eurasia and the Americas were connected by the trans-Beringian and the North Atlantic (deGeer and Thulean) land bridges. During the Eocene to Miocene warm periods (Raven & Axelrod, 1974; Wolfe, 1975; Zachos *et al.*, 2001), both of these connections facilitated inter-continental migrations of thermophilic evergreen flowering plants and their associated insects that today inhabit the Neotropical region (Sanmartín *et al.*, 2001; Hines, 2008). However, these connections acted differently at different periods, and for different taxa. While the latitude of the Beringian land bridge was globally unfavourable to dispersal of tropical taxa it nevertheless acted as a dispersal corridor for these taxa in pre-Cretaceous and Quaternary times. However, in the Late Oligocene to Early Miocene (25–15 Ma), Beringia was covered by a continuous mixed hardwood and deciduous forest. This forest belt possibly enabled taxa adapted to warm climates to migrate (Stebbins & Day, 1967), but it is an unlikely dispersal route for *Ficus* species as they require a tropical climate. The North Atlantic land bridges enabled several groups of tropical plants to migrate: (1) from or into North America, when climates supported tropical forests during the Eocene (Tiffney, 1985; Tiffney & Manchester, 2001; Davis *et al.*, 2002), and (2) between Africa and America (Xiang *et al.*, 2005). They ceased to act as a migration corridor at the Eocene/Oligocene boundary but opened again during the 'Late Oligocene Warming Event' offering new opportunities for tropical plant migrations (Zachos *et al.*, 2001). By the Middle Miocene (15 Ma), they were no longer viable for tropical taxa (Tiffney & Manchester, 2001).

The dispersal of ancestors of *Idarnes* from continental Asia to the New World took place some time between 35 Ma and 20 Ma. This dispersal event coincided with the 'Late Oligocene Warming Event' (26–23 Ma) that enabled subtropical and, possibly, also tropical taxa to migrate to or from America (Milne, 2006). Consequently the North Atlantic land bridges were the most favourable connection for these wasps to disperse to the New World (Fig. 3). This hypothesis is corroborated by the recent discovery of an agaonid fossil collected on the Isle of Wight from Eocene/Oligocene limestone dated to 32 Ma (Compton *et al.*, 2010; Antropov *et al.*, in press). Dispersal of *Anidarnes* may have occurred contemporaneously and by the same route. However, if New World colonization occurred later (between 23 Ma and 15 Ma), then it probably took place via Beringia as the North Atlantic land bridges were closed.

The colonization of South America by ancestors of *Idarnes* could coincide with its colonization by their host plants. However, the age of the stem and crown lineage of *Americana* fig trees have been estimated to 42.2–36.8 Ma and 33.65–27.69 Ma, respectively, by Rønsted *et al.* (2005) (Table 1), and pre-date by at least 7 Myr our estimated ages of the stem lineages of *Idarnes* (29.2–18.2 Ma for BEAST 95% confidence interval and 29.9–24.1 Ma for PHYLOBAYES).

Interestingly, the dispersal of Sycophaginae to America occurred simultaneously with a shift of host plants, as they are now associated with section *Americana*. However, analyses of the phylogeny of *Ficus* failed to recover a sister group relationship between the Neotropical and Oriental *Urostigma*. Instead an Afrotropical–Neotropical connection for *Ficus* was proposed (Renoult *et al.*, 2009). One possible explanation for both events, dispersal to the New World and shift of host plants, is that the shift occurred somewhere in western Laurasia when the Afrotropical ancestor of *Americana* migrated via the North Atlantic land bridges. Rønsted *et al.* (2005) suggested an age of 40 Ma for the crown group of *Galoglychia*, 30 Ma for the crown diversification of *Americana* and 40 Ma for their split with *Galoglychia*. These estimates are consistent with our scenario.

Old World exploration long before Livingstone and Cook

While *Idarnes* subsequently spread and diversified into South America, via continental or volcanic islands that existed at various times through the Tertiary, ancestors of *Apocryptophagus* remained in the Old World but shifted to subgenus *Sycomorus* (Fig. 3c). The explosive radiation of *Sycomorus* was paralleled by the probably contemporaneous diversification of

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Apocryptophagus. From continental Asia, *Apocryptophagus* associated with *Sycocarpus* and *Adenospermae* figs spread to Africa and back to Australia at least twice independently (Figs 1 & 3).

Until the Early Miocene, Asia and Africa were isolated by the Tethys Sea. The sea and the climatic differences between northern and southern shores acted as an effective barrier to dispersal between these continents, with few exchanges during the early Palaeogene. During the Early Miocene (20–17 Ma) new land bridges connected Asia and Africa through the Arabian Peninsula and led to intensive faunal and floristic exchanges (Bernor *et al.*, 1987; Krijgsman, 2002). The estimated age of the dispersal of *Apocryptophagus* from continental Asia to Africa is dated to 32.0–16.4 Ma (PhyloBayes) or 24.5–13.5 Ma (beast) (Fig. 1). This genus subsequently diversified in the African continent during the Late Oligocene and the Middle Miocene (beast), or later in the Miocene (PhyloBayes). These dates correspond to the opening of the Arabic corridors. Between 19.4 and 7.9 Ma (PhyloBayes) and between 16.1 and 4.1 Ma (beast), one Afrotropical lineage (*Sycophaga*) evolved to enter figs through the ostiole and subsequently diversified (Fig. 3c). *Apocryptophagus* reached Madagascar and the Mascarene Archipelago where it underwent local radiation on the endemic *Sycomorus*.

Our results strongly support a reverse southward movement of *Apocryptophagus* associated with *Sycocarpus* fig trees, from continental Asia back to New Guinea, Australia and all major archipelagos in the Pacific, following the expansion of tropical forests in Sundaland (Fig. 3c). This radiation is dated to 30.6–14.2 Ma (PhyloBayes) or 18.9–6.6 Ma (beast) and occurred over a short period of time. Two species groups of Afrotropical *Apocryptophagus* returned independently to Australia via Asia through association with their widely distributed host plant (*F. racemosa*).

CONCLUSIONS

We provide the first phylogeny and divergence time estimates for Sycophaginae genera. We propose dispersal scenarios and interpret them in the light of geological history and palaeoclimatology. Despite a distribution pattern that mimics a Gondwanan origin, our results invalidate a vicariant history of sycophagine divergence. On the contrary, they strongly suggest that the current disjunct distribution pattern of Sycophaginae is a result of trans-oceanic dispersal.

With reasonable certainty, the group emerged somewhere in Australia during the Eocene. This result fits the observation that Australia hosts many lineages of phytophagous chalcidoids (e.g. Melasomellini, Megastigminae) representing a large diversity of species associated with numerous host plants (*Eucalyptus*, *Acacia*, *Ficus*; Bouček, 1988; LaSalle, 2005).

The Sycophaginae probably invaded the fig microcosm in Australia *c.* 50–40 Ma after the origin of the mutualism. Once associated with *Ficus* the subfamily underwent regional diversification following diversification of their host plants and associated pollinators.

Globally, our results revealed similar ages and synchronous colonization events between *Ficus*, pollinators and Sycophaginae. Additional support for this idea will be possible by exploring the biogeography of other groups of non-pollinating fig wasps that are also specialized on *Ficus*.

Tracking the routes followed by Sycophaginae is difficult. Nevertheless, Greater India appears to play a key role in their evolution and biogeography. This area is also strongly suggested as playing a key role in the origin of a number of different groups of pollinating fig wasps (*Platyscapa*, *Eupristina*, *Dolichoris*) and fig trees (*Urostigma*, *Conosycea*). It is tentatively proposed that Australian Sycophaginae may have colonized Greater India after it had rifted away from the African continent. The islands of the Ninetyeast Ridge may have acted as stepping-stones for dispersal throughout the Indian Ocean. This hypothesis is mostly retained because no other satisfying hypothesis can explain the disjunction and the timing observed. However, such a route has been suggested to explain similar disjunction patterns observed between Australasian and African/Indian taxa. Notably, the fruit pigeons that disperse the Lauraceae fruits (genera *Ducula* and *Ptilinopus*) originated in the Australasian region *c.* 57 Ma (Pereira *et al.*, 2007b) and probably spread through the Indian Ocean via the Ninetyeast Ridge *c.* 38 Ma (Carpenter *et al.*, 2010). This observation strongly corroborates our out-of-Australia dispersal of fig wasps via the Ninetyeast Ridge as these pigeons are the main fig dispersers (Innis, 1989) and may exhibit some degree of fig specialism (Shanahan *et al.*, 2001).

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BIOSKETCH

Astrid Cruaud is a post-doctoral research associate at CBGP at Montferrier-sur-Lez, France. This paper is from her PhD research on fig wasp community phylogenetics and biogeography. The members of the research teams who co-authored the paper have worked together for the past decade on fig/fig wasp ecology and evolution (http://www.figweb.org).

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