



Extraordinary haplotype diversity in haplodiploid inbreeders: phylogenetics and evolution of the bark beetle genus *Coccotrypes*

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Abstract

Regular inbreeding by sib-mating is one of the most successful ecological strategies in the bark beetle family Scolytinae. Within this family, the many species (119) in *Coccotrypes* are found breeding in an exceptional variety of untraditional woody tissues different from bark and phloem. Species delineation by morphological criteria is extremely difficult, however, as in most other inbreeding groups of beetles, perhaps due to the unusual evolutionary dynamics characterizing sib-mating organisms. Hence, we here performed a phylogenetic analysis using molecular data in conjunction with morphological data to better understand morphological and ecological evolution in this sib-mating group. We used partial DNA sequences from the nuclear gene EF- α and the mitochondrial genes 12S and CO1 to elucidate patterns of morphological evolution, haplotype variation, and evolutionary pathways in resource use. Sequence variation was high among species and far above that expected at the species level (e.g., 19% for CO1 within *Coccotrypes advena*). The tendency for exhaustive sequence variation at deeper nodes resulted in ambiguous reconstructions of the deepest splits. However, all results suggested that species with the broadest diets were clustered in a single derived position—another piece of evidence against specialization as a derived evolutionary feature. © 2002 Elsevier Science (USA). All rights reserved.

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1. Introduction

Our empirical understanding of the origin and evolution of haplodiploid clades is poor. Most extant haplodiploid clades are very ancient and divergent from their closest relatives, and our understanding of their origins is hampered by uncertainty about phylogenetic topology (e.g., pinworms, iceryine scale insects), by uncertainty about reconstruction of ancestral ecological characters (e.g., Hymenoptera, monogonont rotifers), or by both sources of uncertainty at once (e.g., mite clades, Thysanoptera, whiteflies) (Normark et al., 1999). An exception to this generalization is the haplodiploid, inbreeding clade of scolytine beetles within the tribe

Dryocoetini. We now have a precise understanding of its basal relationships (Jordal et al., 2000, 2002; Normark et al., 1999), across which many important ecological characters remain constant, yielding an exceptionally clear picture of the ecological context in which haplodiploidy arose in this case. However, important sources of uncertainty remain in our understanding of the evolution of the clade.

We now have clear evidence that the basal lineage in the clade is the genus *Ozopemon* Hagedorn (Jordal et al., 2002), and the preponderance of the evidence suggests that the remainder of the clade consists of two monophyletic sister taxa: (a) the tribe Xyleborini and (b) the genus *Coccotrypes* Eichhoff (including *Dryocoetiops*; see Jordal et al., 2000, 2002). Two of these taxa are fairly easy to characterize ecologically: all *Ozopemon* are phloem-feeders and all Xyleborini feed on ambrosia fungi cultured on the walls of tunnels bored into xylem.

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However, *Coccotrypes* is not so easily characterized. Though it has barely more than 10% of the number of species found in Xyleborini, the range of their feeding habits is vastly greater. Some of them, like *Ozopemon*, have retained the ancestral character of feeding in phloem, but almost all of them have dwarf males like Xyleborini. Clearly, *Coccotrypes* is a critical group for understanding the evolution of this haplodiploid clade.

Coccotrypes is a fairly large genus of sib-mating beetles, with currently 119 species assigned to it (Bright and Skidmore, 1997; Wood and Bright, 1992). Most species are distributed in humid tropical forests of Asia and Africa, and recent introductions of several species into the Neotropics has made the genus circumtropical (Bright and Peck, 1998; Wood, 1982). Among the most widespread species, we find a few species that also are well adapted to drier conditions in warm temperate areas, where they feed and breed in small seeds, in particular palm seeds. The best known species, the “ivory button” beetle *Coccotrypes dactyliperda* (Fabr.) is well known as a pest of palm seeds (Blumberg and Kehat, 1982; Hamilton, 1993; Herfs, 1950).

Overall, the species of *Coccotrypes* have adopted a wide range of unusual resources for feeding and breeding. They have been collected from many different fruits and seeds (Browne, 1961), leafstalks (Beaver, 1979a; Jordal and Kirkendall, 1998), phloem, and the pith of twigs (Browne, 1959). *Coccotrypes* is the only bark beetle genus known to breed in ferns (Browne, 1973; Gray, 1970, 1972) and in mangrove radicles (Browne, 1961; Woodruff, 1970). In contrast to the narrow specialization of most scolytines on a particular host tissue—usually phloem, as the common name “bark beetle” implies—fewer than 10 species of *Coccotrypes* use phloem exclusively (Browne, 1959). In contrast, many species of *Coccotrypes* are host-tissue (and host-taxon) generalists, capable of breeding in two or more widely different tissues, usually in a wide range of tree species.

The reproductive biology of the entire haplodiploid clade (1400 spp) is characterized by strongly female-biased offspring sex ratios, in which a single dwarfed male develops in close association with his many sisters (Kirkendall, 1993). In all cases where experimental or cytological studies have been reported, the males are produced by arrhenotokous parthenogenesis (Entwistle, 1964; Herfs, 1950; Ueda, 1997) and are haploid (Takeuchi and Takagi, 1967; B.B.N. and B.H.J., unpublished flow cytometry data for *C. dactyliperda*). In conjunction with regular sib-mating, haplodiploidy contributes an advantage to colonizing females, which can either mate with a brother before dispersal, or produce haploid sons parthenogenetically, one of which she can eventually mate with (Kirkendall, 1993). Sib-mating species of Scolytinae contribute a disproportionately high fraction of the bark and timber beetle

faunas on tropical islands, and the wide distribution and ecological success for sib-mating species might be explained by their extraordinary reproductive biology (Jordal et al., 2001).

Inbreeding species complexes often tend to be taxonomically difficult to resolve, and inbreeding groups of Scolytinae are no exception (Wood, 1982). Until recently, *Coccotrypes* and *Poecilips* Schaufuss were considered two genera difficult to distinguish (Browne, 1961) and the latter was synonymized when complete character intergradation was found (Wood, 1973). Long lists of generic and specific synonymies (Wood and Bright, 1992) reflect the problematic classification of this genus, whose species are regarded particularly difficult to identify (Wood, 1986). A similar pattern of ambiguous morphology and taxonomic synonymies is also observed in the inbreeding Cryphalini and Xyleborini (Wood and Bright, 1992), demonstrating a general taxonomic problem with sib-mating species.

The genus *Coccotrypes* is of great interest due to its remarkable sib-mating habits, its extremely great lability of ecological characters, both within and between species, compared with other scolytine beetles, and its pivotal position in the evolutionary history of a haplodiploid clade. Here we present the first phylogenetic study of the genus, using characters from mitochondrial and nuclear DNA, morphology, and behavior.

2. Material and methods

2.1. Specimens

The species analyzed, the GenBank accession numbers, and their geographical sources and main food resources are listed in Table 1. Samples were collected into 100% ethanol or acetone by the senior author or persons credited in the acknowledgements. Vouchers are deposited at the Museum of Comparative Zoology, Harvard University or at the Department of Zoology, University of Bergen. We included 26 specimens from 19 species of *Coccotrypes* which were selected to represent the overall variation in morphological and ecological characters. Two species of *Dryocoetiops*, which are known to be part of a paraphyletic *Coccotrypes* (Jordal et al., 2000, 2002, and the outgroups *Ozopemon* and *Dryocoetes* were also included.

2.2. Morphological and behavioral characters

Adults of each species were extensively examined and compared, and 28 morphological characters were coded for phylogenetic analysis. Because larval characters are only marginally informative for scolytine genera (Lekander, 1968), larvae were not examined. In

Table 1

Collecting area for the species sequenced in this study, along with their main food resource (monophagous resources in boldface), and GenBank accession numbers

Species	Alias	Collection	Food source	Acc. EF-1 α	Acc. 12S	Acc. CO1
<i>Coccotrypes aciculatus</i> Schedl	dry16	Ecuador	Seed	—	AF444047	AF444059
<i>Coccotrypes advena</i> Blandford	scd24	Uganda	seed, fruit, petiole, phloem	AF186664	AF259818	AF187116
<i>Coccotrypes advena</i> Blandford	dry11	Uganda	seed, fruit, petiole, phloem	AF444072	AF444044	AF444056
<i>Coccotrypes advena</i> Blandford	scd32	Japan	seed, fruit, petiole, phloem	AF186668	AF259821	AF187120
<i>Coccotrypes advena</i> Blandford	scd36	Borneo	seed, fruit, petiole, phloem	AF259868	AF259824	AF444068
<i>Coccotrypes advena</i> Blandford	dry20	Costa Rica	seed, fruit, petiole, phloem	AF444076	AF444050	AF444062
<i>Coccotrypes cardamomi</i> Schaufuss	scd38	Japan	seed, fruit, petiole, fern, phloem	AF259869	AF259826	AF375308
<i>Coccotrypes carpophagus</i> (Hornung)	scd43	USA, Florida	palm seed	AF259872	AF259829	AF444069
<i>Coccotrypes</i> cf. <i>cardamomi</i>	dry12	PNG	petiole, palm seed, more?	AF444073	AF444045	AF444057
<i>Coccotrypes cyperi</i> (Beeson)	scd11	Costa Rica	seed, fruit, petiole, phloem, pith	AF259863	AF259813	AF375309
<i>Coccotrypes cyperi</i> (Beeson)	dry15	Queensland	seed, fruit, petiole, phloem, pith	AF444074	AF444046	AF444058
<i>Coccotrypes dactyliperda</i> (Fabricius)	scd14	Argentina	palm seed	AF186659	AF259815	AF187111
<i>Coccotrypes dactyliperda</i> (Fabricius)	dry22	USA, Florida	palm seed	AF444078	AF444052	AF444064
<i>Coccotrypes</i> cf. <i>distinctus</i> (Motschulsky)	dry17	Costa Rica	palm seed	AF444075	AF444048	AF444060
<i>Coccotrypes</i> sp. near <i>rhizophorae</i>	dry09	PNG	petiole, more?	AF444071	AF444043	AF444055
<i>Coccotrypes fallax</i> (Eggers)	scd13	Queensland	mangrove radicle	—	AF444053	AF444066
<i>Coccotrypes fallax</i> (Eggers)	dry18	Bangladesh	mangrove radicle	—	AF444049	AF444061
<i>Coccotrypes gedeanus</i> (Eggers)	scd35	Borneo	seed, fruit, petiole, pith	AF259867	AF259823	AF375310
<i>Coccotrypes graniceps</i> (Eichhoff)	scd31	Japan	small seed	AF259866	AF259820	AF438513
<i>Coccotrypes impressus</i> Eggers	scd54	Thailand	small seed	AF259874	AF259831	AF444070
<i>Coccotrypes litoralis</i> (Beeson)	scd12	Bangladesh	mangrove radicle	AF259864	AF259814	AF444065
<i>Coccotrypes longior</i> (Eggers)	scd40	Borneo	phloem (also under bark of petioles)	AF259871	AF259828	AF438515
<i>Coccotrypes marginatus</i> (Browne)	scd34	Singapore	petiole	AF186669	AF259822	AF187121
<i>Coccotrypes medius</i> (Eggers)	scd55	Singapore	phloem, petiole, fruit	AF259875	AF259832	AF438518
<i>Coccotrypes petioli</i> (Browne)	dry21	Borneo	petiole	AF444077	AF444051	AF444063
<i>Coccotrypes variabilis</i> (Beeson)	scd30	Japan	fruit, seed, petiole, phloem	AF259865	AF259819	AF444067
<i>Dryocoetes affaber</i> (Mannerheim)	scd20	USA, NH	phloem	AF259817	AF259817	AF187113
<i>Dryocoetes autographus</i> (Ratzeburg)	scd53	Japan	phloem	AF259873	AF259830	AF444054
<i>Dryocoetiops</i> cf. <i>eugeniae</i> (Schedl)	dry19	Malaysia	pith	AF439741	AF438492	AF438507
<i>Dryocoetiops coffeae</i> (Eggers)	scd37	Borneo	pith (also pith of petiole)	AF186670	AF259825	AF187122
<i>Ozopemon brownei</i> Schedl	scd39	Borneo	phloem	AF259870	AF259827	AF438514
<i>Ozopemon uniseriatus</i> Eggers	dry04	PNG	phloem	AF439740	AF438491	AF438506

Note. Alias refers to voucher specimens (see Section 2).

addition, 1 developmental character and 2 behavioral characters were compiled from the literature and checked against the location, structure, and occupancy of the gallery systems from which the beetles were collected (Appendix A). These characters were included in the “morphology” data partition. The evolution of each character was traced on the total-evidence trees, and consistency indices for characters from different

body regions were tabulated and compared. Particular attention was paid to the evolution of character 30, resource use.

2.3. DNA sequencing and alignments

DNA was extracted and amplified using primers and protocols described elsewhere (Jordal et al., 2000;

Normark et al., 1999). Sequences were assembled and edited in Sequencher 3.1 (Gene Codes Corp., Ann Arbor, MI). Of the two different copies of EF-1 α , we used the copy that has only one intron, between coding positions 753 and 754 in scolytine beetles (Danforth and Ji, 1998; Normark et al., 1999). The intron was aligned and used in the analyses. For COI and the EF-1 α coding region, alignments were unambiguous due to the lack of any insertions or deletions in the coding region. Alignments of 12S and the EF-1 α intron were done in ClustalX (Thompson et al., 1997) under four different gap cost ratios (2, 4, 8, and 16) with transitions and transversions weighted equally and with opening and extension gaps equally costly. Maximum-parsimony (MP) trees were inferred by heuristic search (see below) for each alignment. For the EF-1 α intron, the strict consensus tree for each alignment was compared by inspection with the strict consensus tree for the EF-1 α coding region, and the alignment yielding the most congruent consensus tree (gap cost 4) was chosen for further analysis. Eight base pairs consisting of AT-repeats unique to *C. litoralis* were deleted from the EF-1 α intron matrix. For 12S, an elision matrix was constructed (Wheeler et al., 1995) and the strict consensus trees for the individual alignments were compared with the strict consensus tree for the elision matrix. The alignment yielding the most congruent consensus tree (gap cost 8) was chosen for further analysis. Two inserted sites unique to *C. longior* were deleted; the matrix was re-analyzed (again with gap cost 8), resulting in a 12S matrix with no gaps occurring in that region. After alignment, the combined matrix was 2239 bp in length, including 324 aligned sites of 12S and 65 aligned sites of EF-1 α intron. Due to the high degree of consistency between different alignments, the few remaining gaps were each treated as a fifth character state.

2.4. Optimal outgroup analysis

A preponderance of molecular and morphological evidence suggests that *Coccotrypes* + *Dryocoetiops* constitutes a monophyletic group whose sister group is the tribe Xyleborini (Jordal, 2001). However, the precise relationship between Xyleborini and *Coccotrypes* + *Dryocoetiops* is unclear; one may be paraphyletic with respect to the other (Jordal et al., 2000). We are sufficiently persuaded of the monophyly of *Coccotrypes* + *Dryocoetiops* to treat them as monophyletic in this paper, but for rooting their phylogeny we have conservatively chosen the unequivocal outgroup *Ozopemon* (Jordal et al., 2000, 2002; Farrell et al., 2001). As a further outgroup to test for sensitivity to outgroup composition in a functional ingroup/outgroup analysis (Watrous and Wheeler, 1981), we have selected the sister group of the

haplodiploid clade, the genus *Dryocoetes* (Normark et al., 1999).

The program RASA (Lyons-Weiler, 1998) was used to explore putative plesiomorphy contents of the different outgroup genera (Lyons-Weiler et al., 1998), separate and in combination, using all data unweighted. The outgroup composition that results in the highest increase in phylogenetic signal for the ingroup in a rooted analyses is supposed to provide the best estimate of the ingroup root.

2.5. Phylogenetic analysis

MacClade was used for editing matrices, translating to amino acids, translating to standard format, and calculating substitution frequencies. Paup* 4.0 (Swofford, 1999) was used for calculating pairwise sequence transitions and transversions, base composition, sequence divergences, and tree statistics and for all phylogenetic analyses. Under the maximum-parsimony criterion, we performed 100 random-addition replicates of heuristic searches for each of the data partitions and the combined matrix. Bootstrap support for individual nodes was assessed by 100 bootstrap replicates (Felsenstein, 1985) of 10 random-addition heuristic searches each. For the least variable data partition, the morphology matrix, we set maxtrees to 1000 during bootstrapping.

Maximum-likelihood (ML) was used on the molecular data only, with parameters estimated on the topology resulting from the weighted parsimony analysis. We used a general time reversible model in which the proportion of invariable sites and a gamma distribution of rates of substitution were estimated. Several iterations were done to optimize these parameters, until the topology did not change.

We used the incongruence difference length (ILD) test (Farris et al., 1995a,b) to measure level of incongruence between data sets. Although we would ultimately combine all data in a total-evidence approach (Kluge, 1989, 1998), we used the ILD test to evaluate eventual lack of resolution or topological conflicts. TreeRot (Sorenson, 1999) was used to calculate partitioned Bremer support indices (Baker and DeSalle, 1997; Baker et al., 1998) showing the relative contribution from each data partition to the combined analysis. We used heuristic searches with 20 random-addition sequences for each node in each of the partitions.

We further explored the relative performance of each data partition using RASA to measure phylogenetic signal (Lyons-Weiler et al., 1996) in the ingroup for each of the data partitions and compared these measures to the overall bootstrap support and saturation plots for the same partitions. We used this information to inform differential weighting between partitions.

3. Results

3.1. Sequence structure and divergence

There was a slight (EF-1 α coding region) to strong (mitochondrial and EF-1 α intron) AT bias for all gene fragments (Table 2). This bias was also associated with generally high substitution rates, in particular in the mitochondrial 12S and CO1 third codon positions and in the nuclear noncoding EF-1 α intron (Fig. 1). While there was no significant heterogeneity in base composition between taxa, among-site rate variation was pronounced within the protein-encoding EF-1 α and CO1 (Table 3), reflecting different rates of substitution in the three codon positions.

A direct comparison between the EF-1 α coding region and the CO1 highlights the threefold higher divergence in the latter, for the ingroup (Table 3). The maximum intrageneric CO1 divergence was 20.4% (uncorrected), and most pairwise comparisons had more than 15% divergence, with slightly lower levels for 12S. Even between individuals of the morphologically nearly invariable *C. advena* complex, we observed a remarkable 19.0% divergence in the CO1 sequences. High variation was also observed between individuals of the mangrove beetle, *C. fallax* (9.5%). In contrast, the morphologically distinguishable species *C. cyperi* and another mangrove beetle *C. litoralis* were only 2.6% divergent, indicating more rapid morphological and ecological evolution in this clade.

The high rates of substitution in CO1 third positions resulted in high levels of homoplasy (Fig. 1). More than half of the informative characters for these sites were changing eight times or more over the total-evidence tree. This same subpartition also revealed evident saturation from the plot of transitions vs transversions, as did 12S (Fig. 1). On the contrary, we observed a complete absence of saturation of transitions in the least variable molecular data partition, EF-1 α , for the ingroup. More than 80% of the variable characters from the latter partition changed fewer than four times through the total-evidence tree. Despite the high sequence divergence found in the much more rapidly evolving EF-1 α intron, this subpartition mimicked the saturation plot for the coding region (Fig. 1), albeit with a much lower relative rate of transitional substitutions (Table 3).

Saturation in both of the mtDNA gene partitions was also evident from exhaustive sequence divergence observed at deeper nodes (Table 3). This trend was not observed in the EF-1 α intron which instead revealed considerable increase in sequence divergence also from the ingroup to the primary outgroup *Ozopemon*. The exhaustive sequence variation and marked saturation in transitional substitutions in the two mtDNA gene partitions also coincided with the lowest phylogenetic signals measured by the RASA regression method (Table 4).

Table 2
Base composition for partial gene sequences

Locus	Position	A	C	G	T	AT-bias
<i>EF-1α</i>	1	0.29	0.17	0.34	0.20	0.49
	2	0.30	0.22	0.15	0.32	0.62
	3	0.23	0.26	0.13	0.40	0.63
	1 + 2 + 3	0.27	0.22	0.21	0.30	0.57
	Intron	0.28	0.13	0.08	0.52	0.80
<i>CO1</i>	1	0.30	0.18	0.26	0.25	0.55
	2	0.19	0.24	0.16	0.41	0.60
	3	0.48	0.16	0.03	0.33	0.81
	1 + 2 + 3	0.33	0.19	0.15	0.33	0.66
<i>12S</i>	All	0.39	0.19	0.09	0.33	0.72

3.2. Separate analyses

As a means to assess the relative contribution from each data partition to the total-evidence hypothesis, we analyzed each partition separately (Fig. 2). Clearly, the EF-1 α data, which showed the lowest substitution frequency (Fig. 1) and the second highest RASA signal (Table 4), had also the highest overall bootstrap support and the highest proportion (52%) of partial Bremer support values (Table 5). Adding the EF-1 α intron to the analysis of the coding region resulted in higher resolution with increased bootstrap support. Although CO1 also contributed much to the total Bremer support, half of this support was confined to two nodes involving conspecifics (nodes 10 and 23 in Fig. 3).

EF-1 α resulted in a consensus topology most similar to the consensus resulting from morphological data (Fig. 2). With the inclusion of the mtDNA gene fragments, which had the lowest RASA signal and the fewest bootstrap-supported nodes, the overall support did not improve. If *Ozopemon* was used as the sole outgroup, the number of supported nodes actually decreased with the addition of mtDNA data.

Separate analyses of amino-acid-translated CO1 sequences did not perform much better than CO1 nucleotides (cf., Pruess et al., 2000; Simmons, 2000). However, when CO1 amino acids were substituted for nucleotides in the combined analysis, the number of ingroup nodes with more than 50% bootstrap support increased from 12 to 15.

3.3. Combined analyses: optimal outgroup analysis

The ingroup alone contained significant and high phylogenetic signal (tRASA = 11.82) for all data combined (Table 4). The outgroup designation with the highest increase in signal for the ingroup was the phylogenetic sister group *Ozopemon* alone (tRASA = 19.48), followed by all outgroups simultaneously (tRASA = 17.54). However, because both of the *Ozopemon* species had significantly long branches as

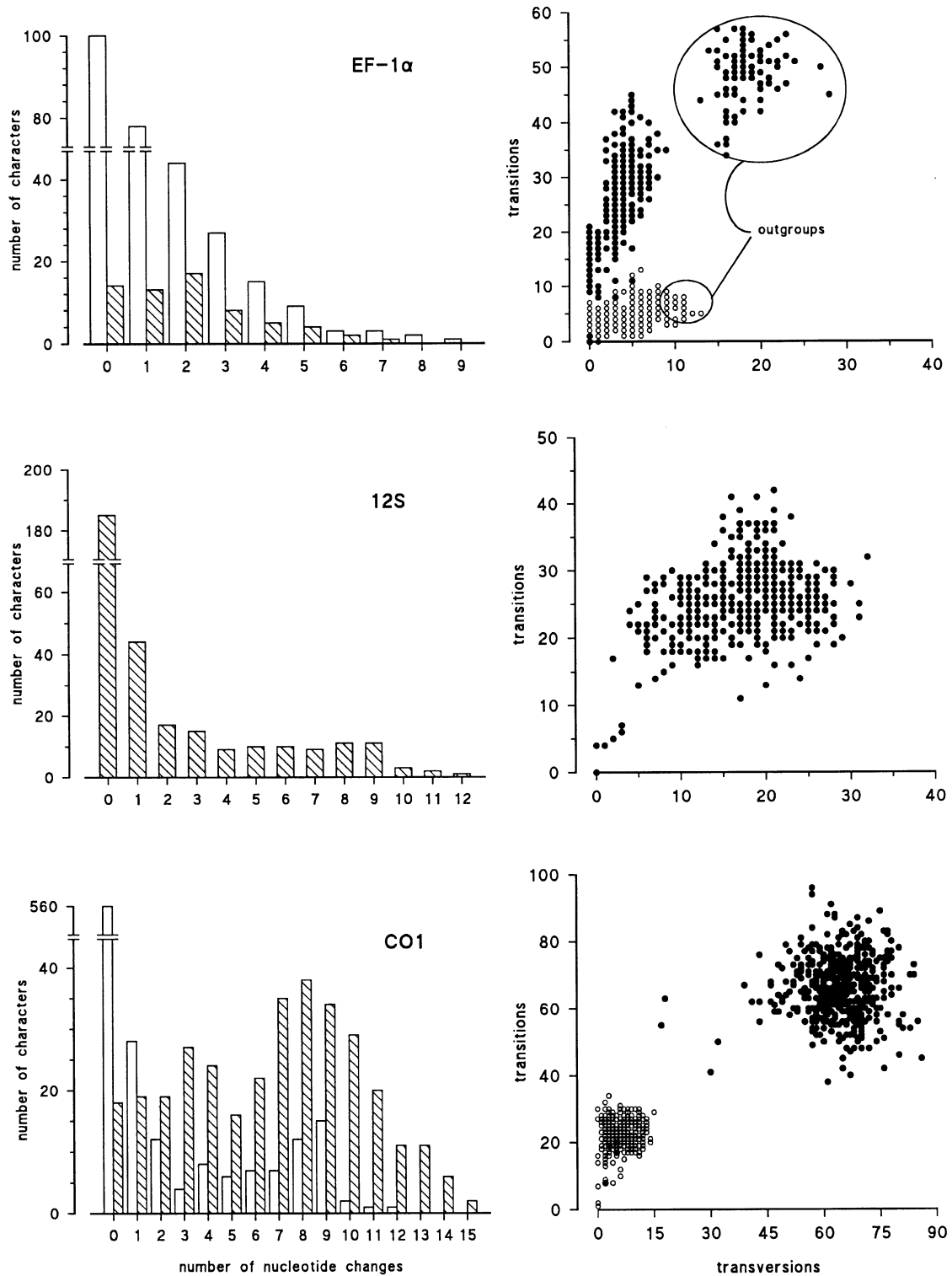


Fig. 1. DNA substitution patterns of the three gene fragments analyzed. Numbers of nucleotide changes were estimated over the tree topology depicted in Fig. 3. Plots of transitions vs. transversions reflect pairwise divergences. For EF-1 α , open bars and filled dots indicate third positions for the exon and hatched bars and open dots indicate the intron. For CO1, open bars and dots indicate first + second codon positions and hatched bars and filled dots indicate third positions only.

indicated by the taxon variance ratio plot (Lyons-Weiler and Hoelzer, 1997), we should be cautious with interpreting topologies where only *Ozopemon* are used as

outgroups, and hence we continue here with exploring the effect of outgroup composition on the ingroup rooting, with preferentially all outgroups included.

Table 3
Sequence variation for different subsets of the genes used in this study

Locus	Position	Total characters	Informative characters	ti/tv ratios ^a	Max divergence <i>C. advena</i>	Max divergence <i>Coccotrypes</i>	Max divergence <i>Cocc + Ozop</i>	Max divergence <i>Cocc + Dryo</i>
EF-1α	1	285	9	14.3	1.1	2.4	2.1	3.3
	2	285	6	0.6	1.4	1.4	1.4	2.2
	3	284	139	4.3	10.6	17.7	27.9	27.3
	All coding	854	154	–	4.3	6.2	10.5	10.6
	Intron	65	44	1.1 ^b	15.2	34.8	41.3	41.3
COI	1	332	71	3.5	9.3	12.3	11.1	13.3
	2	332	14	1.3	1.8	2.7	2.4	2.4
	3	332	295	1.0	48.0	49.6	48.2	45.0
	All coding	996	380	–	19.0	20.4	20.5	19.5
	aa	332	38	–	3.6	6.6	6.3	7.5
12S	All	324	112	1.5	11.1	18.7	19.9	19.5

Note. *Cocc*, *Coccotrypes*; *Dryo*, *Dryocoetes*; *Ozop*, *Ozopemon*; *aa*, amino acids

^a ti/tv signifies the relative proportions of transitional to transversal substitutions.

^b ti/tv = 1.8 when averaged over MP tree. For other subsets, tree-based ratios differed marginally from the average pairwise estimates in the table.

Table 4
Phylogenetic signal for 25 ingroup taxa and eventual outgroups as measured by RASA and the number of ingroup nodes supported by 50 and 90% bootstrap support

Partition	b_{obs}	b_0	tRASA	df	> 50 BP	> 90 BP
EF-1 α , ingroup	7.46	3.41	13.26	272	16	7
12S, ingroup	7.77	4.82	9.68	272	8	2
COI nuc, ingroup	5.73	3.70	7.36	272	6	3
COI aa, ingroup	9.26	6.58	8.91	272	8	1
Morphology, ingroup	11.04	6.83	14.55	272	7	3
All data, ingroup	8.95	5.29	11.82	272	–	–
–rooted all outgroups	15.32	10.48	17.54	272	14	10
–rooted <i>Dryocoetes</i>	13.04	8.70	14.16	272	16	10
–rooted <i>Ozopemon</i>	13.99	8.39	19.48 ^a	272	15	9

^a Outgroup with the highest putative plesiomorphy content.

3.4. Combined analyses: ingroup rooting

Choice of outgroup affected the placement of the ingroup root. Each of the three outgroup compositions resulted in a different root. In the unweighted MP analysis of 29 taxa the ingroup root consisted of one large basal polytomy (indicated by Bremer support of zero in Fig. 3). The topological conflict between the two most parsimonious trees may be a result of conflict among outgroups. When using only *Dryocoetes* species as outgroups, *C. longior* was the basal taxon supported by 71% bootstrap support. When only *Ozopemon* species were used as outgroups, a clade of seed feeders (*C. cf. distinctus*, *C. impressus*, *C. carpophagus*, and *C. dactyliperda*) was basal, supported by 58% bootstrap support, a result similar to the ML analysis with both outgroup genera present (Fig. 4). When the rapidly evolving mtDNA gene fragments were downweighted by a factor 0.5, *C. longior*, *C. petioli*, and *C. marginatus* made up the basal clade (all outgroups). If weighted 0.3 or less, *C. longior* again

recovered as the sole basal taxon—a result also consistent with amino acid translation of COI (in combination with remaining data). Using *C. longior* as a functional outgroup resulted in functional ingroup rooting by the seed-feeding clade.

3.5. Combined analyses: ingroup topology

Some relationships were consistent across the various outgroup configurations, including the following. The four species feeding on small seeds (the seed-feeding clade) made up the best-supported larger clade, and a fifth seed-feeding species, *C. aciculatus*, grouped with these in the mtDNA and morphology analyses (Fig. 3). The two petiole-feeding specialists *C. petioli* and *C. marginatus* clustered as expected. The two pith-feeding *Dryocoetiops* species clustered together with high support and clustered further with two, primarily petiole-feeding, species of *Coccotrypes*. However, a Kishino–Hasegawa (parsimony) variance test (Kishino and Hasegawa, 1989) could not reject monophyly of *Coccot-*

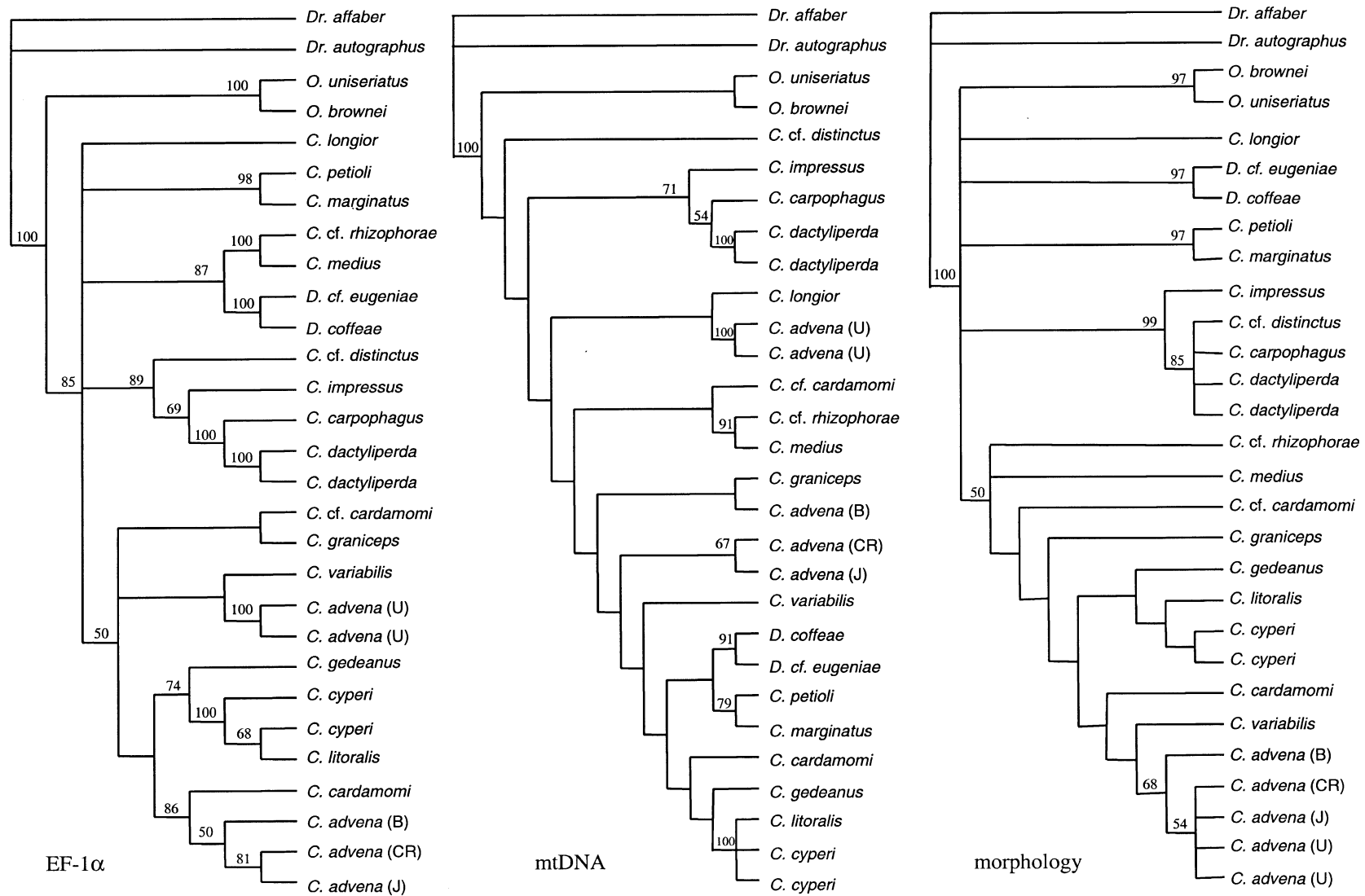


Fig. 2. Separate unweighted MP analyses of the 25 ingroup taxa and 4 outgroup taxa having no missing data partitions. Bootstrap support values are shown on nodes with more than 50% support. Collecting areas are indicated by their initials for the nonmonophyletic *Coccotrypes advena* (see Table 1). Morphology: strict consensus of 3 trees of length 87 steps each, CI = 0.57, RI = 0.80. mtDNA: 1 tree of length 3040 steps, CI = 0.28, RI = 0.37. EF-1 α : strict consensus of 17 trees of length 628 steps each, CI = 0.56, RI = 0.62.

Table 5
Partitioned bremer support indices for each of the four data partitions

Node	EF-1 α	12S	CO1	Morphology	All combined
1	34.5	14.5	7	7	63
2	2.5	-2.5	9	-1	8
3	11.2	1.3	8.7	3.8	25
4	0.5	1.5	-1	-1	0
5	15.5	1.5	9	7	33
6	0.5	1.5	-1	-1	0
7	6.6	0.4	2.6	3.4	13
8	7.5	0	2.2	-3.7	6
9	8.2	3.8	4.1	-2.1	14
10	15.2	13.5	48.3	-3	74
11	0	0	0	0	0
12	7.5	-0.5	-4	1	4
13	7.8	-1.5	6.3	1.4	14
14	15	-2.5	6.5	5	24
15	-0.5	-1.5	1	1	0
16	-0.5	-1.5	1	1	0
17	0	0	0	0	0
18	6.5	-2.5	1.5	0.5	6
19	13.5	-2.5	30	2	43
20	-0.5	-1.5	1	1	0
21	2.5	-2	-0.5	1	1
22	4.5	-1.8	-1.4	-0.3	1
23	21.5	1	27	3.5	53
24	8.5	-1.5	-3	-3	1
25	12.5	2.5	-7	-4	4
26	1.5	-1.5	5	-1	4
Total	202	18.2	152.3	18.5	391
Inf. ch.	198	112	380	29	708
Tot/Inf. ch.	1.02	0.16	0.40	0.64	0.55

Note. Values are estimated from a topology based on combined data for 25 ingroup taxa and 4 outgroups, with no missing partitions (see Fig. 3).

rypes, to the exclusion of *Dryocoetiops*. *C. gedeanus* grouped with *C. cyperi* and *C. litoralis*. The latter species had haplotypes very similar to those of *C. cyperi* (EF-1 α coding region was identical, but intron differed by one 11-bp insertion) and was nested within the latter in many analyses, including some analyses of partial gene fragments, and in the ML analysis. On the contrary, and perhaps the most remarkable result of the analyses, was the consistent nonmonophyly and long branches of *C. advena*, a widespread resource generalist. The latter result holds for all gene partitions when analyzed separately, but not morphology.

The general absence of well-supported deep nodes was not associated with incongruence among data partitions. Only when each of the mtDNA data partitions was tested separately against EF-1 α was incongruence significant ($P = 0.03$ [12S], 0.01 [CO1]). However, because the mtDNA gene partitions are linked in the mitochondrial genome, they must ultimately be combined, and as such, the combined mtDNA partition was not significantly different from any other partition ($P = 0.20$ [EF-1 α], 0.74 [morphology]).

3.6. Morphology and behavior

Table 6 shows the relative performance of morphological and behavioral characters on the topology depicted in Fig. 3. Among those characters with maximum CI values, only characters 1, 16, 23, and 27 could potentially vary within the ingroup. The phylogenetic utility of proventricular characters (Nobuchi, 1969) is little known, but we note here that the shape of the anterior plate and the placement of marginal bristles define groupings also suggested by our current best estimate of the phylogeny (Fig. 3: node 7, char. 5; node 15, char. 1). We also note that the characters with the worst performance were all describing cuticular granules and ridges (char. 12, 14, 15, and 17). Resource use (char. 30) performed equally to the average for all characters combined.

4. Discussion

4.1. Unusual evolutionary dynamics under sib-mating

Perhaps the most striking result from our study is the unusually high sequence divergence and morphological stasis in many lineages. Variation in the genus *Coccotrypes* in fact equals that reported for the entire Prodoxidae (Brown et al., 1994) or Silphidae (Dobler and Müller, 2000) for mtDNA or for the Noctuidae (Mitchell et al., 1997) for EF-1 α . Not only is the observed CO1 divergence among the highest reported for a genus, but the 19% uncorrected divergence among the five *C. advena* haplotypes (22.3% Kimura two-parameter (k2) corrected) is the highest intraspecific CO1 variation known to us for any insect or most organisms (but see Hedin, 2001; Rocha-Olivares et al., 2001). Such high intra specific variation is not unique to *C. advena*, however. Although lower variation was found between the two *C. fallax* haplotypes (9.5%), considerable variation was found between ecologically and morphologically near-identical species, such as *C. petioli* and *C. marginatus* (18.1%) and *C. dactyliperda* and *C. carpophagus* (16.1%). Some other sib-mating scolytine genera contain similar genetic structure as revealed by unpublished CO1 haplotypes of *Xylosandrus morigerus* (Blandford) (16.0%) and *Hypothenemus eruditus* Westwood (16.7%) (B.H.J. and L.R.K., unpublished).

One might argue that such high levels must reflect morphologically cryptic species, in particular because outbreeding scolytine beetle species—and even species complexes—typically encompass less than 5% sequence divergence at COI (Cognato and Sperling, 2000; Kelley and Farrell, 1998). The consistent nonmonophyly of *C. advena* further supports this view, suggesting that the Ugandan populations constitute a separate species. On

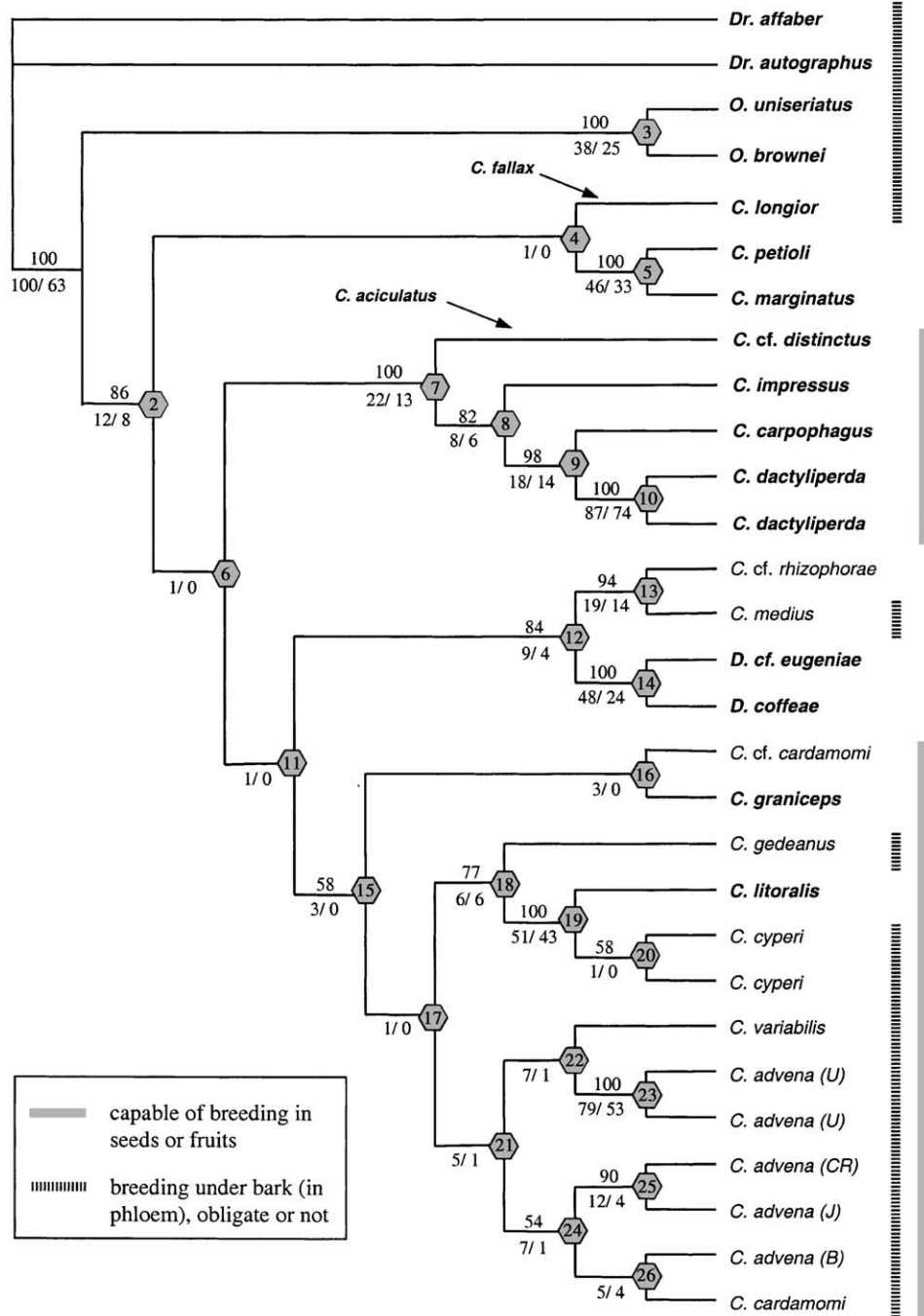


Fig. 3. Most parsimonious tree for 25 ingroup taxa and 4 outgroup species, with EF-1 α and morphology weighted 2 and mtDNA weighted 1. Length 4540 steps, CI = 0.34, RI = 0.46. Numbers in gray refer to node numbers (see Table 5). Values above nodes are bootstrap support values and below are Bremer support values for weighted/unweighted analyses. Bremer support of zero indicates an unresolved or a slightly conflicting node. Arrows point to the placement of taxa for which only mtDNA sequences were available, if included in the equally weighted analysis. Vertical bars show resource preferences.

the other hand, none of the markers used were congruent with respect to the topological placement of the different *C. advena* specimens, pointing toward possible events of introgression and incomplete lineage sorting. Only further sampling of other populations and additional nuclear markers can resolve this ambiguity. However, there are reasons to expect sib-mating lineages

to show more incongruence and higher levels of intra-specific DNA sequence divergence than outbreeders.

Sexual selection is thought to be one of the major components driving species diversification in a variety of outbreeding organisms (Schluter, 2000), and the loss of sexual selection in sib-mating scolytines may reduce divergence rates between species. This also involves the

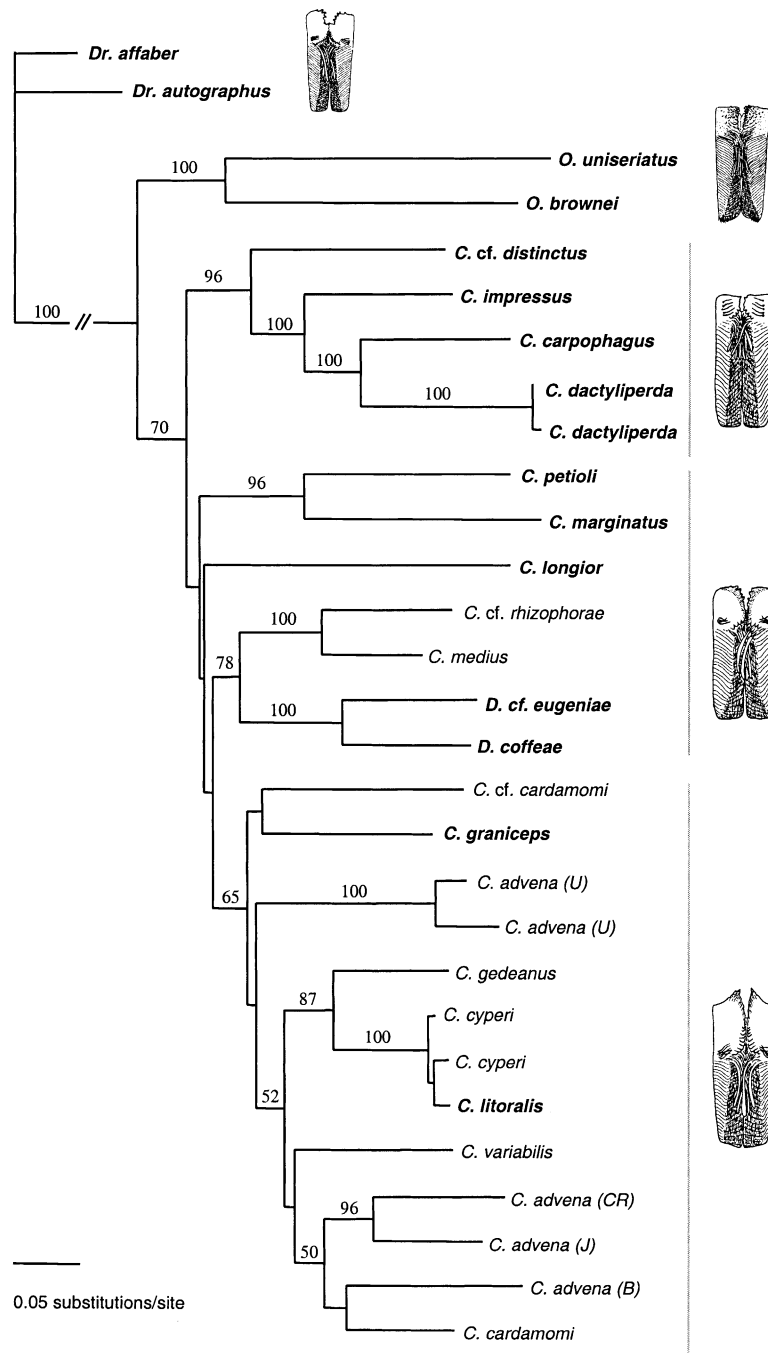


Fig. 4. Most likely tree (score -17489.89) found in the ML analysis of all molecular data partitions combined for 29 taxa (and no missing data partitions). Parameter settings used with the GTR + Γ + I model were empirical base frequencies, I = 0.496, and Γ = 0.487 with four rate categories and substitution frequencies A \leftrightarrow G 11.46, A \leftrightarrow C 4.85, A \leftrightarrow T 8.92, T \leftrightarrow G 1.00, C \leftrightarrow T 66.90, and C \leftrightarrow G 1.93. Parameters were approached through iterations of parameter estimates, starting with the topology depicted in Fig. 3. To the right are drawings from the internal side of one of the eight proventricular plates from the following species, top to bottom: *Dryocoetes affaber*, *Ozopemon uniseriatus*, *Coccotrypes cf. distinctus*, *Dryocoetops coffeae*, and *Coccotrypes gedeanus*. Vertical bars indicate similarity in proventricular characters 1 and 5 (see Table 6, Appendix A).

loss of specific mate recognition characters which define most outbreeding species. Indeed, the characters most commonly used to distinguish outbreeding bark beetle species are secondary sexual characters (Jordal, 1998) which are presumably important for exchange of tactile cues during mate selection (Page and Willis, 1982). The

slowdown in morphological evolution in species of *Coccotrypes* and other sib-mating beetles is therefore largely expected. Still, morphological differences could develop between shallowly diverged sib-mated lineages because, under zero outcrossing, the fixation of mutations in any lineage is virtually instantaneous. This may

Table 6

The relative fit of morphological and behavioral characters as measured by the consensus index (CI) over tree topology in Fig. 3

Proventriculus					Head									
1	2	3	4	5	6	7	8	9	10	11	12	13	14	
1.00	0.5	0.33	0.33	0.75	1.0	1.0	1.0	1.0	0.67	0.5	0.17	0.4	0.25	
0.58					0.67									
Pronotum					Elytra					Legs				
15	16	17	18	19	20	21	22	23	24	25	26	27	28	
0.29	1.0	0.25	1.0	0.5	0.4	1.0	0.5	1.0	1.0	0.6	0.5	1.0	0.5	
0.61					0.73					0.72				
Behaviour and development														
							29	30	31					
							1.0	0.67	1.0					
Average morphology: 0.63					0.89					Average all: 0.66				

Note. Indices vary marginally between alternative tree topologies. Numbers in boldface are average CI for each body section and for behavior. See Appendix A for description of characters 1–31.

explain the distinct morphological differences between the genetically close *C. cyperi* and *C. litoralis*. Another well-studied ecological mate recognition character is pheromone production, where outbreeding species find conspecific mates by sorting diverse pheromone plumes during dispersal. Pheromones are not known from sib-mating species, as expected, because females mate with their brother before dispersal. Consequently, the huge genetic divergences that we observe in some sib-mating lineages rarely represent ecologically segregated cryptic species.

While close relatives in *Coccotrypes* sometimes demonstrate distinct ecological segregation (see below), the overwhelming impression is one of ecological niche overlap in sympatry. It is not unusual to find closely related species breeding in the same unit, for instance the petiole specialists *C. petioli* and *C. marginatus* or the seed specialists *C. dactyliperda* and *C. carpophagus* (B.H.J. and L.R.K., pers. obs.). We also expect to find the same pattern in species of *Dryocoetiops* where all use pith of tiny twigs or of petioles and in species of *Ozopemon* where all breed under bark of large logs. The observation of a marked increase in niche overlap for many sib-mating sister species contrasts with the sometimes clear evidence for allopatric distribution or sympatric resource division between closely related species in general (Barraclough and Vogler, 2000) and for outbreeding bark beetles in particular (Cognato and Sperling, 2000; Jordal, 1998; Kelley and Farrell, 1998).

If the overall outcrossing rate for a population is low, and if hybridization is physically possible, the relaxed selection on mate recognition characters may also permit more frequent matings between these ecologically similar and sympatric “species.” Judged by field observations (B.H.J., L.R.K.), broods from multiple colonizations sometimes coalesce, providing opportunities for outcrossing. The genetic outcome of this process could

sometimes mimic the principles of artificial breeding programs in agriculture, in which a cross between inbred “pure strains” creates hybrids, and then the new hybrid strain is inbred until it “breeds true,” i.e., until it is homozygous. A long history of such low-frequency recombination events may be the cause of the extreme haplotype diversity and incongruence between morphological characters and gene trees for some *Coccotrypes* lineages. We also note that hybridization in nature of genetically highly diverged individuals seems only possible for subclonally reproducing lineages. For instance hybridizing *Daphnia* species in the *galeata* complex are as much as 19% diverged (K2 corrected) in their CO1 sequences (Schwenk et al., 2000), consistent with our view that hybridization may also be possible between the different lineages of *C. advena* and related species.

We are currently working on assessing more closely the intrapopulation genetic variation within and between sites, but it is clear that variation is considerable for most sib-mating lineages studied (B.H.J. and L.R.K., unpublished). Whatever is causing the extreme haplotype variation in *Coccotrypes*, is also resulting in certain drawbacks of phylogeny reconstruction as discussed below.

4.2. Gene utility and phylogeny reconstruction

The most rapidly evolving mitochondrial genes showed high levels of substitutional saturation and homoplasy between the most divergent lineages. Although high substitution rates rarely contribute mere noise in a data matrix (Wenzel and Siddall, 1999; Yang, 1998) and often provide the majority of phylogenetic signal in terms of node support (Allard et al., 1999; Baker et al., 2001; Björklund, 1999; Källersjö et al., 1999), the negative effect of noise from mtDNA was in

this study demonstrated by the reduced number of bootstrap-supported (> 50%) nodes (16 vs 12) when mtDNA nucleotides were added to the combined data matrix. By comparison, using amino-acid-translated COI data, or downweighting mtDNA by a factor of two or more, we increased the number of supported ingroup nodes to 15 or more. Taken together with the many bizarre relationships found in the mtDNA phylogeny, the lack of support and negative contribution to the total-evidence tree suggest that the mitochondrial genes are far too rapidly evolving to be useful for phylogeny reconstruction above species-group level in *Coccotrypes*.

A commonly used method to accommodate poor resolution is downweighting rapidly evolving sites—third positions or transitions—to elevate signal from more conservative sites (Swofford et al., 1996). However, combined analysis of first and second positions yielded only slightly better results than third positions alone for our COI data, and transversions performed no better than transitions for all mtDNA data. Despite the appeal of this type of differential weighting, studies showing that such character weighting does not increase node support or decrease incongruence (Baker and DeSalle, 1997; Baker et al., 2001; Broughton et al., 2000; Durando et al., 2000; Fu, 2000; Garin et al., 1999), and that it performs even worse when second positions are especially favoured (Xia, 1998), are now accumulating.

The sometimes limited utility of mitochondrial genes is in contrast to the usefulness of certain rapidly evolving nuclear genes (e.g., Campbell et al., 2000). Nuclear genes usually outperform mtDNA genes in combined analyses (Baker and DeSalle, 1997; Baker et al., 2001; Durando et al., 2000; Remsen and DeSalle, 1998), especially at deeper splits (Springer et al., 1999), and the value of nuclear protein-coding genes to the resolution of deeper nodes of insect genera cannot be overstated (Durando et al., 2000). Also in this study, the nuclear gene contributed most of the phylogenetic signal within a combined data matrix which otherwise consisted of highly diverged mitochondrial sequences. Thus, higher weights could be given to the best-performing gene to also recover the deepest splits (see Reed and Sperling, 1999). With higher weights applied to EF-1 α and morphology, topologies converged toward the one in Fig. 3 or the one with *C. longior* as a single basal taxon when mtDNA data were excluded altogether.

As noted previously (Jordal et al., 2000, 2002), *Dryocoetiops* appear to be nested within *Coccotrypes*. Morphological differences between the two genera are small and not consistent, in particular the separation of the procoxae and shape of the pronotum (Wood, 1986; but see Appendices A and B). Although more nuclear markers and species of *Dryocoetiops* must be

sampled before reaching a final conclusion, synonymy of this genus seems likely when such data become available.

The two preferred topologies (Figs. 3 and 4) are also similar to our previous study where *C. longior*, *C. marginatus*, and the seed-feeding clade were among the most basal taxa (Jordal et al., 2000). However, we could not confidently place the basal node of the ingroup due to a presumably strong conflict among the outgroups. Because there is no consistent rule on how to root phylogenetic trees, and because general recommendations differ among authors (Lyons-Weiler et al., 1998; Smith, 1994; Watrous and Wheeler, 1981), it is unclear which rooting should be preferred. Hence we prefer to err on the side of inclusion by using both *Dryocoetes* and *Ozopemon* as outgroups. The two ingroup roots found in the MP and ML analyses using both outgroup genera (Figs. 3 and 4) are not very different, and both alternatives place the seed-feeding clade close to the base in each topology. Furthermore, the topology depicted in Fig. 3 is identical to the *Coccotrypes* topology reconstructed as a part of an analysis of 80 dryocoetine and xyleborine EF-1 α sequences (B.H. Jordal, unpublished), demonstrating topological stability with increased sampling. On the other hand, we note in the ML analysis that the resulting basal position of the seed-feeding clade is similar to the result obtained with *Ozopemon* as the sole outgroup in the MP analysis. Despite these ambiguities, the combined data still provided evidence for certain evolutionary patterns in resource use that we discuss below.

4.3. Implications for resource-use evolution

The expansion of niches and extreme resource generalism in sib-mated lineages is a paradox, given the presumed loss of genetic variation due to inbreeding. However, we have demonstrated huge amounts of haplotype diversity in populations, hinting at a broad array of genotypic variation conducive to ecological adaptability and evolutionary change. The resolution of the genetic variation paradox may lie in the ability of sib-mating *Coccotrypes* to regain “lost” genetic variation through occasional outcrossing/hybridization events. During the long periods of inbreeding that separate such events, any beneficial mutations enabling use of a particular resource will be immediately fixed in the inbred lineages on that resource. If that resource is locally abundant such lineages may proliferate, and when outcrossing/hybridization events do occur, such alleles may be captured by other inbred lineages in the same or different species. Selection for ecological adaptability in opportunistic generalists such as *Coccotrypes* may act to promote distant outcrossing and to eliminate any vestiges of a specific mate recognition system.

The clear preference for small resources set some sibling groups apart from most outbreeding bark and timber beetles (Kirkendall, 1993). Smaller resources such as seeds and petioles are scattered in the forest and even more ephemeral than the logs and branches used by other scolytines (Beaver, 1979b). Because larger, longer-lived resources attract more beetle individuals over a longer time period than do smaller resource units, the probability of finding a mate for an outbreeding individual decreases with decreasing resource size. Small resources such as petioles (Beaver, 1979b; Jordal and Kirkendall, 1998) and palm seeds (Kirkendall, 1993) have usually one or a few conspecific colonizing females (inbreeders) or colonizing pairs (outbreeders) per resource unit. Because colonizing females of inbreeders carry with them sperm from their natal nest, these breed independently of colonization densities. This is the most obvious reason that outbreeders are generally less frequent in small resources. Another important factor is the near twofold advantage of sib-maters' female-biased offspring sex ratios, allowing demographic viability in less productive resource units (Beaver, 1979a; Jordal and Kirkendall, 1998).

Some of the highly unusual resources used by *Coccotrypes* are seemingly evolutionary novelties with little or no subsequent diversification. Perhaps the most unusual such novelty is bracken fern (Gray, 1970, 1972), with three associated species of *Coccotrypes*. We were not able to obtain DNA from the single monophagous species in ferns, *C. pteridophytæ* (Schedl), but judged by morphological characters, this species seems closely related to *C. cardamomi*, a generalist species with preference for seeds (Browne, 1961) and occasionally found in bracken fern (Gray, 1972). A third species found in ferns (*Asplenium*), *C. confusus* (Eggers), is most probably the closest relative of two other generalists, *C. medius* and *C. cylindricus* (Eggers) (see Browne, 1973). Based on the widely separated phylogenetic placement of *C. medius* and *C. cardamomi*, it seems likely then that fern feeding evolved at least twice in *Coccotrypes*.

The seeds and radicles of viviparous mangroves also have a limited number of associated beetles (Beeson, 1961; Browne, 1961; Woodruff, 1970) and three species of *Coccotrypes* breed exclusively in such tissues. In at least two of these (*C. litoralis* and *C. rhizophoræ* [Hopkins]), the mangrove association has evolved from the use of fruits and seeds as part of their more generalized diet. With the lack of EF-1 α data, we were not able to assess the placement of *C. fallax* in this study. However, judged by proventricular and other morphological characters in conjunction with mitochondrial data, *C. fallax* is not associated with the "generalist" clade (node 15 in Fig. 3). Hence, two or three independent origins of mangrove seed feeding occurred. Because there is no example of speciation within each mangrove-

feeding lineage, and because close relatives of *C. litoralis* and *C. fallax* have diverged little in genome and morphology, the association with mangroves appear to be recent in each case.

Seeds of various size and form are clearly the most productive resources in terms of brood size (Kirkendall, 1993) and the number of species capable of breeding in this type of resource (Browne, 1961). Despite its productivity, seed feeding has only evolved (in Scolytinae) in two inbreeding clades (*Coccotrypes* and *Hypothenemus*) and in a few outbreeding species. Nonetheless, more than half of the species in *Coccotrypes* show slight or strong preferences for seeds, although only a minor fraction are specialists on this resource. These specialists feed on small seeds (primarily palm seeds) and have followed the recent expansion of palms throughout the tropics. Although we have sampled only a minor fraction of putative seed specialists, a majority of the species seems to constitute a monophyletic group (cf. Figs. 3 and 4) and as such demonstrates evolutionary consistency of this habitat.

Given the high-energy endosperm provided by large seeds (e.g., dipterocarp seeds) and the frequent but facultative use of larger seeds by species of *Coccotrypes*, why is there apparently so little specialization upon this resource? The most important sources of large seeds are those of dipterocarp trees growing in southeast Asia, the area where *Coccotrypes* originated during the Miocene (Jordal et al., 2000). Since dipterocarps exhibit multi-species gregarious flowering and mast fruiting (Ashton et al., 1988), dipterocarp seeds are not available every year, and it is not possible for multivoltine insects such as *Coccotrypes* to specialize on them. They can be utilized only opportunistically, by resource generalists. To specialize, a species is constrained to use predictably available resources—for instance, in the case of *Coccotrypes*, palm seeds, mangrove radicles, Moraceae petioles, or bracken ferns. When dipterocarp seeds are artificially made permanently available, as in ilipe nut (oil-seed) plantations, many different *Coccotrypes* species converge to destroy much of the crop (Browne, 1973), suggesting preference for this type of resource when available.

Regardless of the ambiguity associated with our phylogenetic hypothesis, all reconstructions indicate an evolutionary trend from specialization upon one kind of host tissue toward a more opportunistic behavior using many different tissue types (Figs. 3 and 4). And other generalist species, not sampled for DNA—*C. cinnamomi* (Eggers), *C. nitidus* (Eggers), and *C. papuanus* (Eggers) (Browne, 1961)—have morphological features indicating a clear relationship to the generalist-dominated sub clade (node 15). Our study thus adds to the increasing bulk of evidence that specialization does not necessarily evolve from more generalized lifestyles (Thompson, 1994).

Acknowledgments

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Appendix A. Morphological and behavioral characters coded for *Coccotrypes*, *Dryocoetiops*, and outgroups

Proventriculus

1. Anterior margin of anterior plate: (0) straight or slightly curved; (1) angular.
2. Anterior plate with tubercles: (0) absent; (1) present.
3. Median suture: (0) oval; (1) linear or diverging.
4. Median denticles: (0) pointed, sharp; (1) small, rounded; (2) absent.
5. Marginal bristles: (0) tuft of few; (1) longitudinal rows of > 10; (2) longitudinal rows of widely spaced, soft bristles; (3) absent.

Head

6. Labial palpus segment 1: (0) shorter than 2 + 3 combined; (1) longer than 2 + 3 combined, barrel shaped.
7. Labial palpus, segment 3: (0) longer than segment 2, narrow; (1) subequal to segment 2, broad.
8. Maxillary fringe with setae: (0) few and coarse; (1) many, medium coarse; (2) numerous fine and soft.
9. Maxillary palpus segment 3: (0) shorter than 1 + 2 combined; (1) longer than 1 + 2 combined.
10. Antennal club: (0) more than basal 1/3 corneous; (1) less than basal 1/4 corneous; (2) hairy to base.
11. Setose area of anterior face of club with transverse suture: (0) visible (1) not visible.
12. Epistoma with granules: (0) present; (1) absent.
13. Aciculation in frons: (0) absent; (1) narrowly, from below level of eyes; (2) broadly, from vertex.
14. Median, longitudinal carina in frons: (0) absent; (1) present.

Pronotum

15. Pronotal asperities on anterior half: (0) absent; (1) low or granulate; (2) sharply elevated.
16. Row of asperities along apical margin: (0) absent; (1) present.
17. Asperities on basal disc: (0) present; (1) laterally only; (2) absent.
18. Pronotal disc: (0) shining; (1) reticulate.
19. Dorsum: (0) flat; (1) lightly curved; (2) dome-shaped in middle; (3) dome-shaped posteriorly.

Elytra

20. Interstitial setae: (0) thin; (1) stout; (2) spatulate.
21. Strial punctures (0) in rows; (1) confused at least on declivity.
22. Strial setae: (0) absent; (1) tiny, recumbent; (2) erect, at least half length of interstitial setae.

Legs

24. Probibiae with: (0) 4 socketed teeth, (1) 5 teeth; (2) 7 teeth.
25. Mesotibiae with: (0) 4 socketed teeth; (1) 5 teeth; (2) 6 teeth; (3) 7 teeth; (4) 8 teeth.
26. Metatibiae with: (0) 4 socketed teeth; (1) 5 teeth; (2) 6 teeth; (3) 7 teeth; (4) 9 teeth.
27. Procoxae: (0) contiguous, prosternal process pointed; (1) separated, prosternal process broad/blunt.
28. Mesocoxae separated: (0) approximately width of scapus; (1) twice or more the width of scapus.

Males

29. Male development: (0) similar to females: (1) smaller, with adult characters (2) larviform.

Behavior

30. Feeding on: (0) phloem; (1) pith; (2) leafstalks; (3) small, hard seeds; (4) fruits; (5) mangrove radicles.
 31. Mating system: (0) outbreeding; (1) inbreeding.
-

Appendix B. Data matrix of the 31 morphological characters described in Appendix A

<i>Dryocoetes affaber</i>	0	0	0	0	0	00000	100	0	2	0100	0	01001	2010	0	0
<i>Dr. autographus</i>	0	0	0	0	0	00000	100	0	1	0100	0	01024	5010	0	0
<i>Ozopemon brownei</i>	0	0	0	0	3	10111	010	1	2	0003	0	12001	1002	0	1
<i>O. uniseriatus</i>	0	1	0	0	3	10111	010	1	2	0003	0	12002	2002	0	1
<i>Coccotrypes aciculatus</i>	0	0	1	1	2	11210	112	0	2	1002	1	02000	0011	3	1
<i>C. advena</i> (B)	1	0	1	1	1	11211	110	0	0	0200	2	00001	1011	{024}	1
<i>C. advena</i> (CR)	1	0	1	1	1	11211	111	0	0	0201	2	00001	1011	{024}	1
<i>C. advena</i> (J)	1	0	1	1	1	11211	111	0	0	0201	2	00001	1011	{024}	1
<i>C. advena</i> (U)	1	0	1	1	1	11211	111	0	0	0201	2	00001	1011	{024}	1
<i>C. advena</i> (U)	1	0	1	1	1	11211	111	0	0	0201	2	00001	1011	{024}	1
<i>C. cardamomi</i>	1	0	1	1	1	11211	101	{01}	1	0100	0	00001	1011	{34}	1
<i>C. carpophagus</i>	0	0	1	1	2	11210	112	0	2	1002	0	02000	0011	3	1
<i>C. cf. carpophagus</i>	1	0	1	1	0	11211	111	0	1	0101	1	01001	101?	3	1
<i>C. cf. rhizophorae</i>	0	0	0	0	0	11211	112	0	1	0100	0	01001	111?	2	1
<i>C. cyperi</i> (CR)	1	0	1	1	0	11211	111	0	2	0101	1	00001	1011	{01234}	1
<i>C. cyperi</i> (Qld)	1	0	1	1	0	11211	111	0	2	0101	1	00001	1011	{01234}	1
<i>C. dactyliperda</i> (Arg.)	0	0	1	1	2	11210	112	0	2	1002	0	02000	0011	3	1
<i>C. dactyliperda</i> (FL)	0	0	1	1	2	11210	112	0	2	1002	0	02000	0011	3	1
<i>C. cf. distinctus</i>	0	0	1	1	2	11210	112	0	2	1002	0	02000	0011	3	1
<i>C. falax</i> (Bangl)	0	1	0	0	0	11211	100	0	2	0011	0	00001	2111	5	1
<i>C. fallax</i> (Qld)	0	1	0	0	0	11211	100	0	2	0011	0	00001	2111	5	1
<i>C. gedeanus</i>	1	{01}	1	{01}	0	11211	101	1	2	0101	0	00001	1011	{0124}	1
<i>C. graniceps</i>	1	0	1	1	0	11211	101	1	1	0001	0	01001	1001	3	1
<i>C. impressus</i>	0	0	1	1	2	11210	112	0	2	1002	0	02001	101?	3	1
<i>C. litroralis</i>	1	0	1	1	0	11211	111	0	2	0101	0	00001	1011	5	1
<i>C. longior</i>	0	0	0	0	0	11211	010	0	2	0110	{12}	00001	1011	{02}	1
<i>C. marginatus</i>	0	0	1	1	3	11212	010	0	0	0000	0	02101	1011	2	1
<i>C. medius</i>	0	0	1	0	0	11211	111	0	1	0000	0	01011	111?	{012}	1
<i>C. petioli</i>	0	0	1	1	3	11212	010	0	0	0000	0	02101	1011	2	1
<i>C. variabilis</i>	1	0	1	1	1	11211	101	0	1	0000	2	00001	1011	{034}	1
<i>Dryocoetiops coffeae</i>	0	1	1	0	0	11211	100	0	2	0002	0	01002	211?	1	1
<i>D. cf. eugeniae</i>	0	1	1	0	0	11211	100	1	2	0002	0	01002	211?	1	1

References

- Allard, M.W., Farris, J.S., Carpenter, J.M., 1999. Congruence among mammalian mitochondrial genes. *Cladistics* 15, 75–84.
- Ashton, P.S., Givnish, T.J., Appanah, S., 1988. Staggered flowering in the Dipterocarpaceae: New insights into floral induction and the evolution of mast fruiting in the aseasonal tropics. *Am. Nat.* 132, 44–66.
- Baker, R.H., DeSalle, R., 1997. Multiple sources of character information and the phylogeny of Hawaiian drosophilids. *Syst. Biol.* 46, 645–673.
- Baker, R.H., Wilkinson, G.S., DeSalle, R., 2001. Phylogenetic utility of different types of molecular data used to infer evolutionary relationships among stalk-eyed flies (Diopsidae). *Syst. Biol.* 50, 87–105.
- Baker, R.H., Yu, X., DeSalle, R., 1998. Assessing the relative contribution of molecular and morphological characters in simultaneous analysis trees. *Mol. Phylogent. Evol.* 9, 427–436.
- Barraclough, T.G., Vogler, A.P., 2000. Detecting the geographical pattern of speciation from species-level phylogenies. *Am. Nat.* 155, 419–434.
- Beaver, R.A., 1979a. Leafstalks as a habitat for bark beetles (Col.: Scolytidae). *Z. Angew. Entomol.* 88, 296–306.
- Beaver, R.A., 1979b. Non-equilibrium ‘island’ communities: A guild of tropical bark beetles. *J. Anim. Ecol.* 48, 987–1002.
- Beeson, C.F.C., 1961. *The Ecology and Control of the Forest Insects of India and the Neighbouring Countries*, 2nd ed. Government of India, New Delhi.
- Björklund, M., 1999. Are third positions really that bad? A test using vertebrate Cytochrome b. *Cladistics* 15, 191–197.
- Blumberg, D., Kehat, M., 1982. Biological studies of the date stone beetle, *Coccotrypes dactyliperda*. *Phytoparasitica* 10, 73–78.
- Bright, D.E., Peck, S.B., 1998. Scolytidae from the Galapagos Islands, Ecuador, with descriptions of four new species, new distribution records, and a key to species. *Koleopt. Rund.* 68, 233–252.
- Bright, D.E., Skidmore, R.E., 1997. *A Catalog of Scolytidae and Platypodidae* (Coleoptera), Supplement 1 (1990–1994). NRC Research Press, Ottawa.
- Broughton, R.E., Stanley, S.E., Durrett, R.T., 2000. Quantification of homoplasy for nucleotide transitions and transversions and a reexamination of assumptions in weighted phylogenetic analysis. *Syst. Biol.* 49, 617–627.
- Brown, J.M., Pellmyr, O., Thompson, J.M., Harrison, R.G., 1994. Mitochondrial DNA phylogeny of the Prodoxidae (Lepidoptera: Incurvarioidea) indicates rapid ecological diversification of yucca moths. *Ann. Entomol. Soc. Am.* 87, 795–802.

- Browne, F.G., 1959. Notes on two Malayan scolytid bark-beetles. Malay. For. 22, 292–300.
- Browne, F.G., 1961. The biology of Malayan Scolytidae and Platypodidae. Malay. For. Rec. 22, 1–255.
- Browne, F.G., 1973. The African species of *Poecilips* Schaufuss. Rev. Zool. Bot. Afr. 87, 679–696.
- Campbell, D.L., Brower, A.V.Z., Pierce, N.E., 2000. Molecular evolution of the *wingless* gene and its implications for the phylogenetic placement of the butterfly family Riodinidae (Lepidoptera: Papilionoidea). Mol. Biol. Evol. 17, 684–696.
- Cognato, A.I., Sperling, F.A.H., 2000. Phylogeny of *Ips* species (Coleoptera: Scolytidae) inferred from mitochondrial cytochrome oxidase I sequence. Mol. Phylogenet. Evol. 14, 445–460.
- Danforth, B.N., Ji, S., 1998. Elongation Factor-1 α occurs as two copies in bees: Implications for phylogenetic analysis of EF-1 α sequences in insects. Mol. Biol. Evol. 15, 225–235.
- Dobler, S., Müller, J.K., 2000. Resolving phylogeny at the family level by mitochondrial Cytochrome Oxidase sequences: Phylogeny of carrion beetles (Coleoptera, Silphidae). Mol. Phylogenet. Evol. 15, 390–402.
- Durando, C.M., Baker, R.H., Etges, W.J., Heed, W.B., Wasserman, M., DeSalle, R., 2000. Phylogenetic analysis of the *repleta* group of the genus *Drosophila* using multiple sources of characters. Mol. Phylogenet. Evol. 16, 296–307.
- Entwistle, P.F., 1964. Inbreeding and arrhenotoky in the ambrosia beetle *Xyleborus compactus* (Eich.) (Coleoptera: Scolytidae). Proc. R. Soc. Lond. A 39, 83–88.
- Farrell, B.D., Sequeira, A., O'Meara, B., Normark, B.B., Chung, J., Jordal, B., 2001. The evolution of agriculture in beetles (Curculionidae: Scolytinae and Platypodinae). Evolution 10, 2011–2027.
- Farris, J.S., Källersjö, M., Kluge, A.G., Bult, C., 1995a. Constructing a significance test for incongruence. Syst. Biol. 44, 570–572.
- Farris, J.S., Källersjö, M., Kluge, A.G., Bult, C., 1995b. Testing significance of incongruence. Cladistics 10, 315–319.
- Felsenstein, J., 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39, 783–791.
- Fu, J., 2000. Towards the phylogeny of the family Lacertidae—Why 4708 base pairs of mtDNA sequences cannot draw the picture. Biol. J. Linn. Soc. 71, 203–217.
- Garin, C.F., Juan, C., Petitpierre, E., 1999. Mitochondrial DNA phylogeny and the evolution of host-plant use in Palearctic *Chrysolina* (Coleoptera, Chrysomelidae) leaf beetles. J. Mol. Evol. 48, 435–444.
- Gray, B., 1970. Observations on the first scolytid, *Poecilips pteridophytæ* (Coleoptera: Scolytidae), to be found in association with a fern (Pteridophyta). Can. Entomol. 102, 578–585.
- Gray, B., 1972. Observations on *Poecilips cardamomi* (Schaufuss), the second species of Scolytidae to be found in bracken fern (Col.). Entomol. Tidskr. 93, 229–237.
- Hamilton, W.D., 1993. Inbreeding in Egypt and in this book: A childish perspective. In: Thornhill, N.W. (Ed.), The Natural History of Inbreeding and Outbreeding: Theoretical and Empirical Perspectives. University of Chicago Press, Chicago, pp. 429–450.
- Hedin, M.C., 2001. Molecular insights into species phylogeny, biogeography, and morphological stasis in the ancient spider genus *Hypochilus* (Araneae: Hypochilidae). Mol. Phylogenet. Evol. 18, 238–251.
- Herfs, A., 1950. Studien an dem steinussborkenkäfer, *Coccotrypes tanguanus* Eggers. Hofsch. Wiss. Praxis. 3, 3–31.
- Jordal, B.H., 1998. A review of *Scolytodes* Ferrari (Coleoptera: Scolytidae) associated with *Cecropia* (Cecropiaceae) in the northern Neotropics. J. Nat. Hist. 32, 31–84.
- Jordal, B.H., 2001. The origin and radiation of sib-mating haplodiploid beetles (Coleoptera, Curculionidae, Scolytinae). Ph.D. Thesis, Department of Zoology, University of Bergen, Bergen.
- Jordal, B.H., Beaver, R.A., Kirkendall, L.R., 2001. Breaking taboos in the tropics: Inbreeding promotes colonization by wood-boring beetles. Glob. Ecol. Biogeogr. 10, 345–358.
- Jordal, B.H., Beaver, R.A., Normark, B.B., Farrell, B.D., 2002. Extraordinary sex ratios and the evolution of male neoteny in sib-mating *Ozopemon* beetles. Biol. J. Linn. Soc. 75, 353–360.
- Jordal, B.H., Kirkendall, L.R., 1998. Ecological relationships of a guild of tropical beetles breeding in *Cecropia* leafstalks in Costa Rica. J. Trop. Ecol. 14, 153–176.
- Jordal, B.H., Normark, B.B., Farrell, B.D., 2000. Evolutionary radiation of an inbreeding haplodiploid beetle lineage (Curculionidae, Scolytinae). Biol. J. Linn. Soc. 71, 483–499.
- Källersjö, M., Albert, V.A., Farris, J.S., 1999. Homoplasy increases phylogenetic structure. Cladistics 15, 91–93.
- Kelley, S.T., Farrell, B.D., 1998. Is specialization a dead end? The phylogeny of host use in *Dendroctonus* bark beetles (Scolytidae). Evolution 52, 1731–1743.
- Kirkendall, L.R., 1993. Ecology and evolution of biased sex ratios in bark and ambrosia beetles. In: Wrensch, D.L., Ebbert, M.A. (Eds.), Evolution and Diversity of Sex Ratio: Insects and Mites. Chapman & Hall, New York, pp. 235–345.
- Kishino, H., Hasegawa, M., 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. J. Mol. Evol. 29, 170–179.
- Kluge, A.G., 1989. A concern for evidence and a phylogenetic hypothesis of relationships among *Epicrates* (Boidae, Serpentes). Syst. Zool. 38, 7–25.
- Kluge, A., 1998. Total evidence or taxonomic congruence: Cladistics or consensus classification. Cladistics 14, 151–158.
- Lekander, B., 1968. Scandinavian bark beetle larvae; descriptions and classification. Rep. No. 4, Department of Forest Zoology, Royal College of Forestry, Stockholm.
- Lyons-Weiler, J., 1998. RASA for the Mac. Available from <http://loco.biology.unr.edu/archives/rasa/rasa.html>.
- Lyons-Weiler, J., Hoelzer, G.A., 1997. Escaping from the Felsenstein zone by detecting long branches in phylogenetic data. Mol. Phylogenet. Evol. 8, 375–384.
- Lyons-Weiler, J., Hoelzer, G.A., Tausch, R.J., 1996. Relative Apparent Synapomorphy Analysis (RASA) I: The statistical measurement of phylogenetic signal. Mol. Biol. Evol. 13, 749–757.
- Lyons-Weiler, J., Hoelzer, G.A., Tausch, R.J., 1998. Optimal outgroup analysis. Biol. J. Linn. Soc. 64, 493–511.
- Mitchell, A., Cho, S., Regier, J.C., Mitter, C., Poole, R.W., Matthews, M., 1997. Phylogenetic utility of Elongation Factor-1 α in Noctuidae (Insecta: Lepidoptera): The limits of synonymous substitution. Mol. Biol. Evol. 14, 381–390.
- Nobuchi, A., 1969. A comparative morphological study of the proventriculus in the adult of the subfamily Scolytoidea (Coleoptera). Bull. Government For. Exp. Sta. 224, 39–110.
- Normark, B.B., Jordal, B.H., Farrell, B.D., 1999. Origin of a haplodiploid beetle lineage. Proc. R. Soc. Lond. B 266, 2253–2259.
- Page, R.E., Willis, M.A., 1982. Sexual dimorphism in ventral abdominal setae in *Scolytus multistriatus* (Coleoptera: Scolytidae): Possible role in courtship behaviour. Ann. Entomol. Soc. Am. 76, 78–82.
- Pruess, K.P., Adams, B.J., Parsons, T.J., Zhu, X., Powers, T.O., 2000. Utility of the mitochondrial Cytochrome Oxidase II for resolving relationships among black flies (Diptera: Simuliidae). Mol. Phylogenet. Evol. 16, 286–295.
- Reed, R.D., Sperling, F.A.H., 1999. Interaction of process partitions in phylogenetic analysis: An example from the Swallowtail butterfly genus *Papilio*. Mol. Biol. Evol. 16, 286–297.
- Remsen, J., DeSalle, R., 1998. Character congruence of multiple data partitions and the origin of the Hawaiian *Drosophilidae*. Mol. Phylogenet. Evol. 9, 225–235.

- Rocha-Olivares, A., Fleeger, J.W., Foltz, D.W., 2001. Decoupling of molecular and morphological evolution in deep lineages of a meiobenthic harpacticoid copepod. *Mol. Biol. Evol.* 16, 1088–1102.
- Schluter, D., 2000. *The Ecology of Adaptive Radiation*. Oxford University Press, Oxford.
- Schwenk, K., Posada, D., Hebert, P.D.N., 2000. Molecular systematics of European *Hyalodapnia*: The role of contemporary hybridization in ancient species. *Proc. R. Soc. Lond. B* 267, 1833–1842.
- Simmons, M.P., 2000. A fundamental problem with amino-acid-sequence characters for phylogenetic analyses. *Cladistics* 16, 274–282.
- Smith, A.B., 1994. Rooting molecular trees: Problems and strategies. *Biol. J. Linn. Soc.* 51, 279–292.
- Sorenson, M.D., 1999. *TreeRot.v2*. Boston University, Boston.
- Springer, M.S., Amrine, H.M., Burk, A., Stanhope, M.J., 1999. Additional support for Afrotheria and Paenungulata, the performance of mitochondrial versus nuclear genes, and the impact of data partitions with heterogeneous base composition. *Syst. Biol.* 48, 65–75.
- Swofford, D., 1999. *PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods) version 4*. Sinauer, Sunderland, MA.
- Swofford, D.L., Olsen, G.J., Waddell, P.J., Hillis, D.M., 1996. Phylogenetic inference. In: Hillis, D.M., Moritz, C., Mable, B.K. (Eds.), *Molecular Systematics*. Sinauer, Sunderland, MA.
- Takenouchi, Y., Takagi, K., 1967. A chromosome study of two parthenogenetic scolytid beetles. *Ann. Zool. Jpn.* 40, 105–110.
- Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., Higgins, D.G., 1997. The ClustalX windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res.* 24, 4876–4882.
- Thompson, J.N., 1994. *The Coevolutionary Process*. Chicago University Press, Chicago.
- Ueda, A., 1997. Brood development of an inbreeding spermatophagous scolytid beetle *Coccotrypes graniceps* (Eichhoff) (Coleoptera: Scolytidae). *Jpn. J. Entomol.* 65, 677–687.
- Watrous, L.E., Wheeler, Q.D., 1981. The outgroup comparison method of character analyses. *Syst. Zool.* 30, 1–11.
- Wenzel, J.W., Siddall, M.E., 1999. Noise. *Cladistics* 15, 51–64.
- Wheeler, W.C., Gatesy, J., DeSalle, R., 1995. Elision: A method for accommodating multiple molecular sequence alignments with alignment-ambiguous sites. *Mol. Phylogenet. Evol.* 4, 1–9.
- Wood, S.L., 1973. New synonymy in American bark beetles (Scolytidae: Coleoptera). *Gt. Basin Nat.* 33, 169–188.
- Wood, S.L., 1982. The bark and ambrosia beetles of North and Central America (Coleoptera: Scolytidae), a taxonomic monograph. *Gt. Basin Nat. Mem.* 6, 1–1359.
- Wood, S.L., 1986. A reclassification of the genera of Scolytidae (Coleoptera). *Gt. Basin Nat. Mem.* 10, 1–126.
- Wood, S.L., Bright, D., 1992. A catalog of Scolytidae and Platypodidae (Coleoptera) Part 2: Taxonomic index. *Gt. Basin Nat. Mem.* 13, 1–1553.
- Woodruff, R.E., 1970. A mangrove borer, *Poecilips rhizophorae* (Hopkins) (Coleoptera: Scolytidae). *Florida Dept. Agric. Entomol. Circ.* 98, 1–2.
- Xia, X., 1998. The rate heterogeneity of non-synonymous substitutions in mammalian mitochondrial genes. *Mol. Biol. Evol.* 15, 336–344.
- Yang, Z., 1998. On the best evolutionary rates for phylogenetic analysis. *Syst. Biol.* 47, 125–133.