Phylogeny of West African *Caryedon* (Coleoptera: Bruchidae): Congruence between Molecular and Morphological Data[1]

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Seed beetles belonging to the Old World genus *Caryedon* feed in the seeds of various Caesalpinioideae, Mimosoideae, and Combretaceae. In an attempt to resolve broad phylogenetic relationships within the genus, we obtained 332 base pair sequences of mitochondrial 12S ribosomal DNA and morphological data for the 16 West African *Caryedon* species. Morphological characters were analyzed under maximum parsimony and sequences were compared under maximum parsimony, maximum likelihood, and neighbor joining. Using a partition homogeneity test, we determined that morphological and molecular data sets were combinable. Combined data were analyzed under maximum parsimony. Morphological and molecular trees were congruent at the species group level and total evidence analyses yielded the same topologies as molecular data with each of the three outgroups used. Four main terminal clades are recognized, each corresponding with a group of species generally feeding on the same host plant family, subfamily, genus, or species. The monophyly of legume feeding *Caryedon* is supported by both data sets, and Combretaceae feeders split in two monophyletic assemblages. © 1998 Academic Press

INTRODUCTION

Bruchidae constitute a very homogeneous family of seed-feeding Coleoptera.

They show a strong tendency towards host specialization: although 31 plant families are used as hosts by Bruchidae, 84% of bruchid species feed on seeds of Leguminosae.[2] Bruchidae form with Cerambycidae and Chrysomelidae the Chrysomeloidea superfamily. According to Borowiec (1987), Cerambycidae separated first from the common branch and Bruchidae may have originated from a present day Chrysomelidae subfam-

ily, the Sagrinae. Among Bruchidae, the Rhaebinae, which retain many characters found in Sagrinae (Chrysomelidae), is considered as the most primitive subfamily. The Pachymerinae are also regarded as a primitive subfamily. As both subfamilies generally show preferences for plants other than Leguminosae, feeding on legumes is generally considered an apomorphic character in Bruchidae (Borowiec, 1987).

At the species level, bruchids are mostly oligophagous or monophagous (Borowiec, 1987). Such a host specificity has been correlated with the development of various seed defense mechanisms and particularly with the toxic secondary compounds found in many host seeds (Janzen, 1971; Janzen, 1980a; Gillon *et al.*, 1992). Several bruchids have developed an ability to feed on seeds rich in secondary compounds. They are able to detoxify and even use the toxic or some of the degraded products for their own metabolism (Rosenthal and Janzen, 1983, 1985).

Pachymerinae, a subfamily which includes the genus *Caryedon,* are more host specific than any of the two other large subfamilies, Bruchinae and Amblycerinae. They are primarily restricted to seeds of Palmae in the New World and to Combretaceae and Leguminosae in the Old World (Johnson, 1981).

Pachymerinae are classically subdivided in three tribes: (1) Pachymerini, whose species feed on Arecaceae (Palmae) (monocot); (2) Caryedonini, including *Caryedon* feed on Pandanaceae (monocots), Combretaceae, and Leguminosae (dicots) seeds; (3) Caryopemonini, the most specialized, with *Caryopemon* feeding on legumes.

The genus *Caryedon* comprises more than 30 Old World species. Most of them feed on legumes, but a few species develop in Combretaceae. One species, *C. serratus,* has been introduced in the neotropics. The host plants of *Caryedon* include three genera of Mimosoideae, 9 to 11 genera of Caesalpinioideae, and 3 genera of Papilionoideae. West African *Caryedon* feed mostly on Caesalpinioideae and Mimosoideae (Leguminosae) but also on Combretaceae (Delobel *et al.*, 1995; Delobel, 1997).

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[1] Sequence data reported in this article have been deposited in GenBank under Accession Nos. AF004114 through AF004132.

[2] As in "*Advances in Legume Systematics, Part 1,*" the legumes are treated here as a single family, the Leguminosae, with three subfamilies, the Caesalpinioideae, Mimosoideae, and Papilionoideae.

Janzen (1969) and followers suggested that bruchids and plants are engaged into a coevolutionary process in the sense of Ehrlich and Raven's (1964) hypothesis (for a review about coevolution in bruchids see Johnson, 1990). This hypothesis has been questioned, even by its first proponent (Janzen, 1980b) and challenged, principally because it was based on often inadequate correlative studies (Johnson, 1990).

With Johnson (1990), we considered that the study of seed-beetle systematics using cladistic methods in conjunction with ecological and biogeographical studies is the way to arrive at reasonable explanations of bruchid–seed interactions. This approach has been taken to study the historical relationships between different Chrysomelidae and their hosts (Farrell and Mitter, 1990; Funk et al., 1995; Becerra, 1997).

From a recent review of molecular data (Doyle, 1995) and a cladistic analysis (Chappill, 1995) the following statements can be made about Leguminosae phylogeny: (1) Leguminosae are monophyletic; (2) the subfamilies Mimosoideae and Papilionoideae are monophyletic; (3) Caesalpinioideae appear paraphyletic to the Mimosoideae and Papilionoideae (Doyle, 1995) or to Mimosoideae alone (Chappill, 1995); and (4) the Mimosoideae originate from a core group of Caesalpinioideae. These results are partially congruent with fossil records (Wheeler and Baas, 1992). The relationships of Leguminosae with other angiosperm families are not clear (Doyle, 1995). Although Combretaceae and Leguminosae were both placed under the Cronquist's system in the Rosidae subclass, now mostly considered unnatural (Doyle, 1995), there is no reason to consider them as closely related.

This limited knowledge of the phylogeny of Combretaceae and Leguminoseae prevents us to directly test congruence between Caryedon and host-plant phylogenies.

We have tested whether West African Caryedon species feeding on the same plant species are related. In order to investigate this hypothesis we used molecular and morphological characters to reconstruct a phylogeny of West African Caryedon species. We also used phylogenetic hypothesis to study more precisely the evolutionary history of a particular species, Caryedon serratus (Olivier). The latter, the so-called groundnut seed beetle, is a species of economic importance which feeds on wild Caesalpinioideae and recently became also adapted to groundnut, an introduced Papilionoideae (Delobel, 1995).

MATERIAL AND METHODS

Sixteen West African Caryedon and three outgroup species were studied (Table 1). All specimens were collected in the field in Senegal. Specimens used for molecular studies were preserved in pure ethanol.

Thirty-four morphological characters were used (Table 2). Nineteen were based on adult external

TABLE 1

West African *Caryedon* and Outgroup Taxa Studied

Taxon name	Main host plant genera
Caryedon acaciae (Gyllenhall)	Acacia (Mimosoideae)
Caryedon albonotatum (Pic)	Acacia (Mimosoideae)
Caryedon. excavatus auct.	Acacia (Mimosoideae)
Caryedon longispinosus auct.	Acacia (Mimosoideae)
Caryedon mauritanicus auct.	Acacia (Mimosoideae)
Caryedon sahelicus auct.	Acacia (Mimosoideae)
Caryedon crampeli (Pic)	Bauhinia, Cassia, Piliostigma, Prosopis, Tamarindus (Caesalpinioideae)
Caryedon dialii Decelle	Dialium (Caesalpinioideae)
Caryedon pallidus (Olivier)	Cassia (Caesalpinioideae)[a]
Caryedon serratus (Olivier)	Bauhinia, Cassia, Piliostigma, Prosopis, Tamarindus (Caesalpinioideae)
Caryedon fuliginosum Prevett	Combretum (Combretaceae)
Caryedon immaculatum Prevett	Combretum (Combretaceae)
Caryedon longipennis Prevett	Combretum (Combretaceae)
Caryedon lunatum Prevett	Combretum (Combretaceae)
Caryedon macropterae Delobel	Terminalia (Combretaceae)
Caryedon fathalae Delobel	Terminalia (Combretaceae)
Outgroup taxa	
Bruchidius senegalensis (Pic)	Acacia (Mimosoideae)
Callosobruchus maculatus (F.)	Vigna (Papilionoideae)
Sulcobruchus natalensis (Pic)	Acacia (Mimosoideae)

[a] Recorded also on *Acacia ataxacantha,* Mim.

morphology, 12 were based on male and female genitalia morphology, and 2 were based on first larval instar prothoracic plate. An ecological character was also used.

We sequenced the 3' region of the mitochondrial 12S ribosomal DNA (Fig. 1). Total DNA was extracted from single individuals according to Kocher et al. (1989) with slight modifications. DNA templates were produced through PCR. PCR primers were identical to SR-N-14588 and SR-J-14233 (Simon et al., 1994). PCR cycling conditions were initial denaturing step at 92°C (5 min), followed by 30 amplification cycles: 92°C (1 min), 50°C (1 min), 70°C (1 min), and final extension step at 70°C for 10 min. Partial 12S rDNA sequences were directly obtained by the dideoxy chain termination method (Sanger et al., 1977) using Sequenase V.2 DNA or Sequenase PCR product sequencing kits.

Sequence alignment was performed using ClustalW (Thompson et al., 1994). Aligned sequences were entered in McClade 3.06 (Maddison and Maddison, 1992) for subsequent treatments.

Maximum parsimony analyses (MP) were performed with PAUP 3.1.1 (Swofford, 1993) on morphological and molecular character matrices. Decay indices were calculated using AutoDecay (Eriksson, 1997). In addition, for molecular characters we performed maximum likelihood (ML) analyses using Puzzle 3 (Strimmer and Haeseler, 1997) and calculation of pairwise distance and neighbor-joining (NJ) analyses with MEGA (Ku-

TABLE 2

Morphological Characters of *Caryedon* and Outgroups, Used to Infer *Caryedon* Phylogeny

Male and female genitalia:
1. Spermatheca: with peripheral openings (0); with invaginated openings (1).
2. Spermatecal apex: thickened (0); slim (1).
3. Vaginal sclerites: absent or diffuse (0); well defined, strongly sclerotized (1).
4. Anterior part of the bursa copulatrix: with at most simple denticules (0); with a sclerotized flattened armature (1).
5. Ovipositor: long (0); short (1).
6. Apical patch of setae of the median lobe: absent (0); present (1).
7. Hooks on the median part of the median lobe: absent (0); present (1).
8. Apical hooks on the median lobe: absent (0); present (1).
9. Apical blades on the median lobe: absent (0); present (1).
10. Parameres (lateral lobes): short and fused (0); long and distinct (1).
11. Basal part of parameres: longer than wide (0); wider than long (1).
12. Basal part of parameres: without sclerotized arches (0); with sclerotized arches (1).

External morphology of adults:
13. Eye: ventrally separated by more than eye width (0); separated by less than eye width (1).
14. Frontal carina: complete (0); absent or partial (1).
15. Pronotal carina: equal to or longer than half the length of the pronotum (0); shorter or absent (1).
16. Scutellum: square or half-circular (0); triangular (1).
17. Humeral tubercle: absent (0); present (1).
18. Hind femur: widened and flattened (0); normal (1).
19. Ventral furrow on hind femur: absent (0); present (1).
20. Prepecten on hind femur: number of teeth much lower than pecten (0); number of teeth approximately equal to pecten (1).
21. Pecten on hind femur: present (0); absent or with less than three teeth (1).
22. Hind tibia: curved (0); straight (1).
23. Dorsal carinae on hind tibia: 2 (0); 1 (1); undetectable (3).
24. Mesosternal process: flat and wide (0); narrow (1).
25. Mesoepimeral plate: wide, reaching the coxa (0); narrow, does not reach the coxa (1).
26. Metasternum: normal (0); strongly convex (1).
27. Sexual dimorphism of abdominal sternites: none (0); moderate or pronounced (1).
28. Last abdominal sternite in female: straight (0); produced backward (1).
29. Sexual dimorphism of pygidium: none (0); moderate or pronounced (1).
30. Apex of pygidium, in female: straight (0); emarginate (1).
31. Tegument: uniformly brown (0); bi or multicolored (1).

First larval instar prothoracic plate:
32. With a single group of apical teeth (0); with at least two groups of apical teeth (1).
33. Apical transversal thickening of the prothoracic plate: straight (0); slightly curved (1); strongly curved (2).

Ecological character:
34. Pupation: outside the seed (0); inside the seed (1).

mar *et al.,* 1993). The morphological and molecular data sets were also combined into a single data matrix and the ILD test (Farris *et al.,* 1994) (partition homogeneity test in PAUP*, Swofford, 1997) used to analyze the congruence between morphological and molecular

partitions. Invariant characters were removed before applying the test (Cunningham, 1997) and default settings in PAUP* used (1000 randomizations). The combined data were analyzed under MP (total evidence approach, Kluge, 1989).

For all analyses, we used separately each of the three available outgroups, considering that congruence between trees obtained with different outgroups is an indication of the robustness of a topology.

RESULTS

Morphology

Strict consensus MP trees obtained with any of the three outgroups are mostly topologically congruent (Fig. 2A). The legume feeders, minus *C. albonotatum,* cluster together and the remaining species are part of a basal polytomy. The bootstrap values for the legume feeding species node (minus *C. albonotatum*) vary between 60 and 70 depending on which outgroup species or bootstrap options are used. It is also the sole node supported by a decay index different from zero. The majority rule consensus tree topologies indicate that several minor groups have to be considered even though bootstrap values or decay indices are low. With *Callosobruchus maculatus* and *Sulcobruchus natalensis* as outgroup taxa, the legume feeding *Caryedon* species can be divided into two sister groups: the mimosoid feeders (plus *C. serratus,* a caesalpinioid feeder) and the remaining caesalpinioid feeders. With *Bruchidius senegalensis* as outgroup, the caesalpinioid feeders cluster together but the mimosoid feeders are paraphyletic with respect to them. The *a posteriori* weighting reinforces the clustering tendencies observed with equally weighted characters and using *C. maculatus* and *S. natalensis* as outgroups. Regardless of the outgroup, the legume feeders split into the two previously described sister groups. In addition to that, the four species feeding on *Combretum* seeds (Combretaceae) cluster together even though bootstrap values are low. This clade is part of the basal polytomy in all trees. Branching positions of *C. albonotatum* and the two species feeding on seeds of *Terminalia* (Combretaceae) are more variable. With *C. maculatus* and *S. natalensis,* these three species group together and appear as the sister group of the legume feeders. With *B. senegalensis* as outgroup, *C. fathalae* appears as the sister species of the legume feeder clade, while *C. albonotatum* and *C. macropterae* successively merge with the *C. fathalae* plus legume feeders clade. The surprising placement of *C. albonotatum* in the tree is accounted for further in the discussion.

Molecules

Trees obtained under maximum parsimony with each of the three outgroups are topologically congruent.

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                  10        20        30        40        50        60        70        80        90        100       110       120       130       140
C.acaciae       ACTAAATGATTAATAGTTAAGTTCTTTAAAGTTAAAGAATTTGGCGGTATTTTAGTCTTTTCAGAGGAACCTGTTCTGTAATTGATAGTCCACGATTA-TAACTACTTTAGCTATAAGTTTGCATATCGTTGTAGTTT-G
C.albonotatum   ...............C..T.................A...............A...........................................AA..A........T.T...A..................-.
C.crampeli      G.................T............................................C...A.........A.A.-GT......AG.T....C.......................A-.
C.dialii        G.................T............................................C...A.........A.A.-GT......AG.T....C.......................A-.
C.excavatus     .T................T...........................................................T.......AT.T.................-.
C.fathalae      G....G......TA....T.........A..............T.......A....C..........GA.GA.......T.T..T...A..................-.
C.fuliginosum   .....................A.....A..............A...................TA..-........T.T.T.A...A...............-.
C.immaculatum   ..CT...........A..........AA...A..............A............AA..-......T.T.T..A...A...............A--.
C.longipennis   .....................A.....A..............A...................TA..-........T.T.T.A..................C.
C.longispinosus G.................T.......................................A.........-...T.......T.T...................-.
C.lunatum       ...............A..........A...............A...................A..........AAT.-......T.T.T.A..A.............-.
C.macropterae   G....G......TA....T.......A..T..A.............T...............C........GA.TA.......T.T..T...A...............C..-.
C.mauritanicus  .T................T........................................-...T.......AT.T.................-.
C.pallidus      G.................T..............................................C.........A.A..GA......AT.T.T.AC.............A-.
C.sahelicus     ..........C.............................................-...T.......T.T...................-.
C.serratus      G.................T.......................................A.........-...T.......T.T...................-.
Bru.senegalensis .......T.G..T......T......G...A....A..............T...C........-A.TT......TT...T.A..A.......C........-.
Cal.maculatus   G....T.G..T......T......G...A....A..............C...........-G..AA......TT..T.A..AT..............-.
Sul.natalensis  ...T...T.G..T......TT.....G...A....A..............C..........A.......-..TA......AT.T.T.....A.............A..-.
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                  150       160       170       180       190       200       210       220       230       240       250       260       270       280
C.acaciae       AATATTTTAAAAGAAA--AGAAATGTTCAATATATTTA--------------------ATTAAAATTTAAATCAAATCAAGGTGTAGTTTATGTTAAAGAAGAAATGGGTTACATTAATAGAAAAAATT-TAGGATTAT
C.albonotatum   .G.......T.....T--TAT...A.......GG..A.--------------------....................A.................G....TTT-T..T.A--.....A.A
C.crampeli      .........T.....G--GAT.......G..T..A.--------------------....................A...G.T........A.....A-T......-A.......
C.dialii        .........T.....G--GAT.......G..T..A.--------------------....................A...G.T........A.....A-T......-A.......
C.excavatus     .G.......G...T--GA....A.......T...--------------------....G..G........................A...C.AT....G..-.......
C.fathalae      .G.......GG.....-TT.G..A.......AT..CT--------------------....G.G..A......AA...........A......A...T..T.TT....-A....A.
C.fuliginosum   .........TG....G-GT.T..A.......G..AGA--------------------..........................A...T..............TA..T..T..A.....A.
C.immaculatum   .........T.....-AT.G.......G..A.TAATAAGAAAAATTATTAAAA.......................G..A..G..T........T..TA..T..T..--.....A.
C.longipennis   .G.......T.....T-TTAT..A.......G..A.T--------------------....................C.....A..T.......T..TA..T..T..--.....G..
C.longispinosus .G.......T....--.A...........G...--------------------....................A...G..........G.................-.......
C.lunatum       .G.......T....GTGT.........G..A.TT--------------------....................A...T.........TAG.T..T.A......A.
C.macropterae   .G.......GG.....-TT.-..A.......GT...T--------------------......A...........C....A.A........A......A...TA.T.T....-A....A.
C.mauritanicus  .G.......G....T--.AG...A.......G...--------------------....G..A.............A...T.............AT.......-.......
C.pallidus      .........T.....-GAT.......G..T..A.--------------------....................C..A.......T........A.....A-T......-A.......
C.sahelicus     .G.......T--TAG...A.......G...--------------------....................A...T.............A-......-.......
C.serratus      .G.......--.A...........G...--------------------....................A...G..........G.................-.......
Bru.senegalensis .........T-.TT....A...A..T..A..A.--------------------....AA.....G......AA........G....A....A..ATT---T.T..-.....A.
Cal.maculatus   .G....G--TT...A.....TG..T..A.--------------------....AA.......G.......A......AA....T..G.........A.A---T.T..-A.....A.
Sul.natalensis  ..........T....T--TTG...A.....G..T...T--------------------......AG.......G......A......C....A..T.......GG.............A.T---T.T..-......AT.
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                  290       300       310       320       330]
                                                              ]
C.acaciae       T---TTTGTAAAAAAAATATGAATTTGGATTTGAGAGTAATTTT-ATTTATA
C.albonotatum   .--T...T......T.T.....AC.................T.......
C.crampeli      .---...T.....G....T.....A.........A.........-.....T
C.dialii        .---...T.....G....T.....A.........A.........-.....T
C.excavatus     C---...A...G.G.......A.........T.........-......
C.fathalae      .---...T.T.TG.....T.............A.........-...A..T
C.fuliginosum   .---...T.....G..G.T.............T.........-.....T
C.immaculatum   .---...T......GG.TA.............T.........-.....T
C.longipennis   .---.....T...G....C.....G.........T.........-.....T
C.longispinosus .---.......................A...........-.......
C.lunatum       .AAT...T....GG.G.T.............T.........-.....T
C.macropterae   .---...T.T.T....G.T.............A.........-...A..T
C.mauritanicus  C---..........G........A...........-.......
C.pallidus      .---...T.T...GG...T.....A.........T.........-...A..T
C.sahelicus     C---.....G....G.................-.......
C.serratus      .---........................A...........-.......
Bru.senegalensis .---.A.T.....T....T....................-.AA...T
Cal.maculatus   .---...T.T....GG..T....G.........A......A.-.AA....
Sul.natalensis  .---...T.....G..AT.............T........A-..A...T
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FIG. 1. Alignment of mt. 12S rDNA sequences. Gaps inserted to improve alignment are indicated by a hyphen. The alignment was checked for conformity with mitochondrial small ribosomal subunit RNA secondary structure hypotheses (Kjer, 1995; Van de Peer *et al.*, 1997).
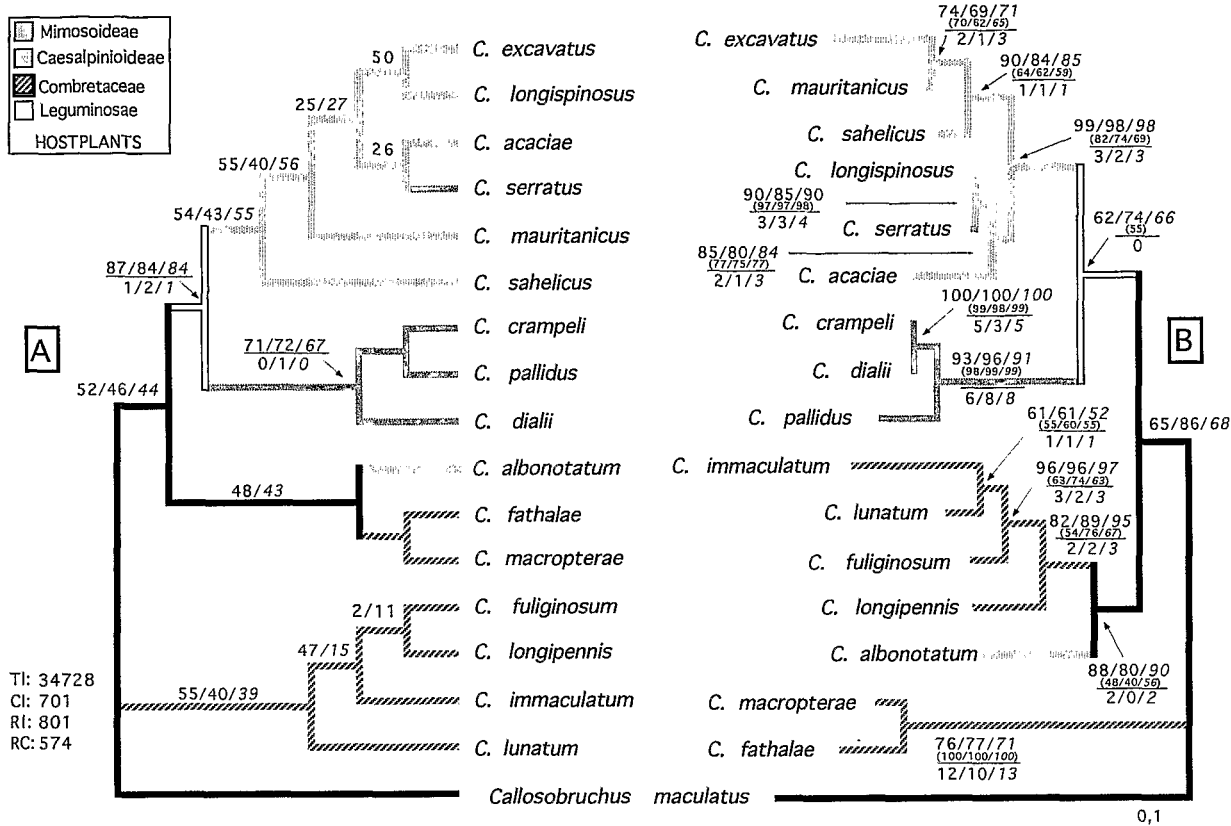
FIG. 2. *Caryedon* relationships inferred (A) from majority rule consensus tree (outgroup, *C. maculatus*) based on parsimony analysis of *a posteriori* reweighted morphological characters (heuristic search, gaps treated as missing) and (B) from maximum likelihood tree (same outgroup) based on 12S data set (quartet puzzling, Tamura-Nei substitution model). In A are figured for each node the bootstrap values (above lines) and the decay index values (beneath line) when decay index is different from 0 in at least one tree. In B top lines are figured the ML support values (top) and the MP bootstrap values (bottom, in small numbers in parentheses) and bottom lines are figured the decay index values. Bold, lightface, and italic numbers correspond to analyses that used *Calosobruchus maculatus*, *Bruchidius senegalensis*, and *Sulcobruchus natalensis* as outgroups, respectively. CI, RI, and RC: X1000.

Two clades, the *Terminalia* feeder clade (*C. fathalae* and *C. macropterae*) and the caesalpinioid feeder clade, are strongly supported by bootstrap values (≥98) and decay indices (≥10 and ≥6, respectively). Two other clades, the mimosoid feeder clade and the *Combretum* feeder clade are less strongly supported, with bootstrap values ranging from 69 to 82 (decay indices ≥2) for the former clade and from 54 to 76 (decay indices ≥2) for the latter. It is only with *B. senegalensis* as outgroup that the legume clade is recovered with a low bootstrap value (55) and no decay index support. Among the mimosoid feeders, *C. longispinosus* and *C. serratus* cluster together to form a clade well supported by both bootstrap (≥97) and decay (3) values. The same kind of relationship is observed between *C. crampeli* and *C. dialii* among the caesalpinioid feeders. With each of the three outgroups, *C. albonotatum* clusters with the *Combretum* feeders (decay index = 2 with *C. maculatus* and *S. natalensis* as outgroups). With *B. senegalensis* and *C. maculatus*, the *Terminalia* feeder clade separates first and is the sister group of a legume + *Combretum* feeder clade.

Topologies of trees obtained under maximum likelihood are strictly congruent and confirm the aforementioned relationships. With any outgroup, the legume feeder clade, minus *C. albonotatum*, is supported and splits into mimosoid and caesalpinioid feeder clades (including *C. serratus*), *C. albonotatum* clusters with the *Combretum* feeders, and the *Terminalia* feeder clade is part of the basal polytomy. We retain one of these ML tree as an example of molecular tree (Fig. 2B). With Tv + Ts data matrix, and complete or pairwise deletion, neighbor-joining analyses performed with any of the three outgroups give a consistent topology, identical to the ML topology.

Total Evidence Analysis

The ILD test found a *P* value of 0.400. Considering the incongruence threshold of 0.05 retained by various authors (Cunningham, 1997; Shaffer *et al.,* 1997) we concluded that the morphological and molecular data were combinable.

MP topologies (Fig. 3) obtained with any of the three outgroups are mostly congruent. The legume clade is

TABLE 3

Morphological Data Matrix for Calculation of *Caryedon* Phylogeny in Fig. 2

Character / Taxon name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
C. acaciae	1	0	0	0	1	1	1	1	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	2	1	0	0	1	0	0	1	1	1	2	0
C. albonotatum	1	1	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	1	0	1	1	1	1	0
C. crampeli	1	0	1	0	0	0	0	1	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	1	0	1	2	0
C. dialii	1	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	1	1	2	0
C. excavatus	1	0	1	0	0	1	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	2	1	0	0	1	0	0	0	1	1	2	0
C. fathalae	1	1	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	
C. fuliginosum	1	1	0	1	1	0	1	0	0	0	0	1	0	1	0	1	0	0	0	0	0	1	0	0	2	1	0	0	0	0	0	1	1	1	0	1
C. immaculatum	1	1	0	1	1	0	1	0	0	0	0	1	0	1	0	1	0	0	0	0	0	1	0	0	2	1	0	0	0	1	0	1	0	1	0	1
C. longipennis	1	1	1	1	1	0	1	0	0	0	0	1	0	1	0	1	0	0	0	0	0	1	0	0	2	1	0	0	0	0	0	1	1	1	0	1
C. longispinosus	1	0	1	0	0	1	1	1	0	0	1	0	1	0	1	1	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	1	1	2	0
C. macropterae	1	0	0	1	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	0	0	0	
C. mauritanicus	1	0	1	0	0	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1	2	0	
C. pallidus	1	0	1	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	0	1	2	0
C. sahelicus	1	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	1	1	2	0
C. serratus	1	0	1	0	0	1	1	1	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	2	1	0	0	0	0	0	0	1	1	2	0	
Su. natalensis	0	1	1	0	1	0	0	0	0	1	1	0	0	1	1	1	1	1	1	0	1	1	2	0	1	0	0	0	0	0	1	1	1	1	
Br. senegalensis	0	1	1	1	0	0	0	0	0	1	1	0	0	1	1	1	1	1	1	0	1	1	2	0	1	0	0	1	1	0	1	1	1	1	
Ca. maculatus	0	0	0	1	1	0	0	0	0	1	1	0	0	0	1	1	1	1	1	0	1	1	2	1	1	0	0	0	0	0	1	1	1	1	

Note. Characters and their coding correspond to Table 2.

recovered but poorly supported. The mimosoid (+ *C. serratus*) and the caesalpinioid feeder clades are also recovered with high bootstrap and decay index values for the latter. In all trees *C. albonotatum* clusters with a fairly well-supported *Combretum* feeder clade which is the sister group of the legume feeder clade. The strongly supported *Terminalia* feeder clade is part of the basal polytomy with *B. senegalensis* and *C. maculatus* as outgroups and clusters with the caesalpinioid feeders with *S. natalensis* as outgroup. Total evidence analyses confirm most of the results and tendencies observed with morphological data alone and all results obtained with molecular data. Decay values for the *Terminalia, Combretum, Combretum + C. albonotatum,* and caesal-
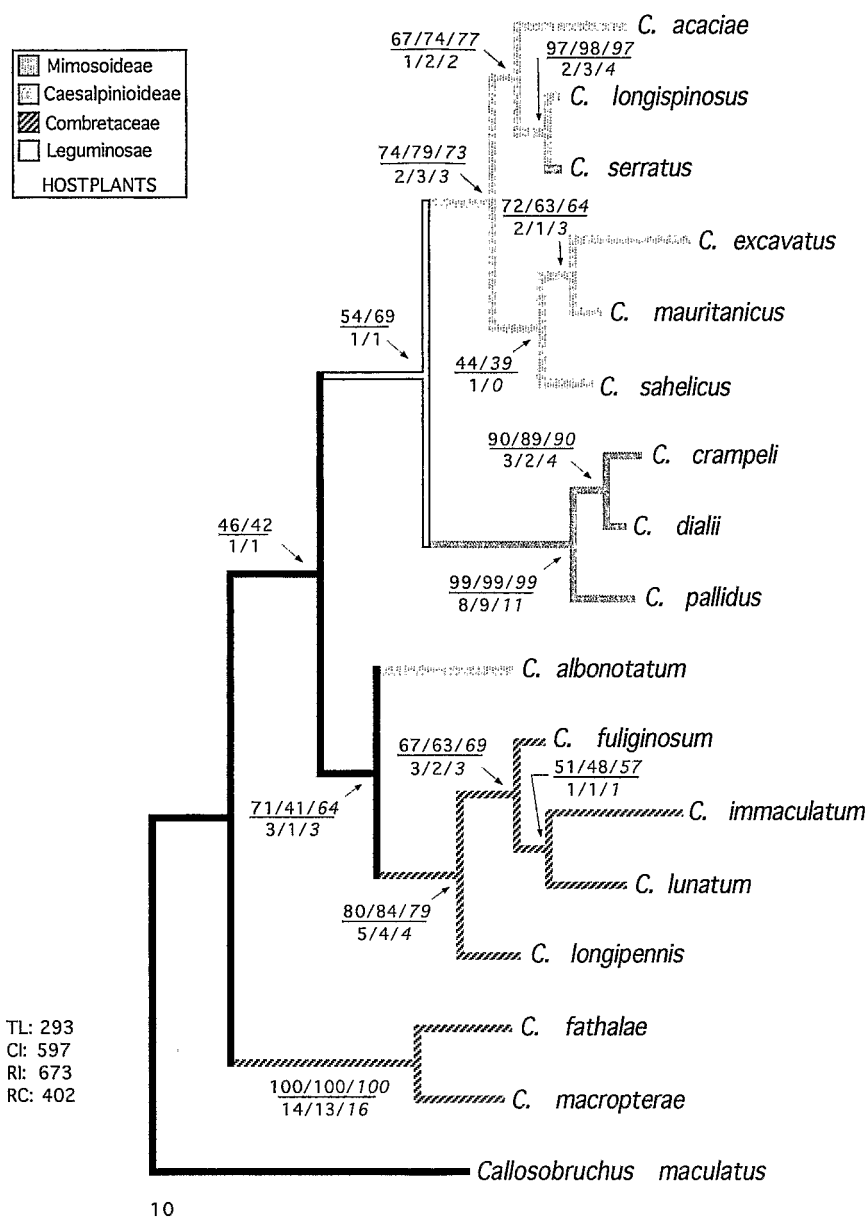
FIG. 3. *Caryedon* relationships inferred from MP tree (outgroup, *C. maculatus*) based on analysis of combined morphological and molecular data sets (total evidence). Bootstrap values (above lines) and decay index values (beneath lines) are figured for each node. Bold, lightface, and italic numbers have the same meaning as in Fig. 2.

pinioid feeder clades increase when morphological data are combined with molecular data.

DISCUSSION

The branching position of the *Terminalia* feeder clade in the analysis with *S. natalensis* as outgroup being kept apart, total evidence trees are in agreement with molecular trees and particularly with the ML single topology. This result may indicate that molecular data alone are able to recover the phylogenetic signal, an observation which contradicts some negative comments

made about the phylogenetic usefulness of the mitochondrial 12S rDNA gene (Simon *et al.*, 1994). Morphological data alone are useful because their analysis suggests the same species groupings as molecular data. Clearly, at present we have too few morphological characters to resolve the group and especially the sister species relationships between terminal taxa, a situation likely due to the great morphological homogeneity of the genus.

In the particular case of West African *Caryedon* phylogeny, total evidence analysis appears as a useful way to combine a fairly strong molecular phylogenetic signal suggesting possible terminal taxa relationships

within each of the main assemblages and a lower morphological phylogenetic signal that will nevertheless reinforce the robustness of several main nodes. Using the total evidence topology we will be able to look for new morphological characters useful at different systematic levels.

According to the total evidence topology, the following conclusions can be put forward:

(1) Among *Caryedon* species, there is a close relationship between cladogenesis and host plant association. Consequently, closely related *Caryedon* species can be expected to share the same kind of plant secondary compound detoxification mechanisms. Such a result was expected according to the taxonomic and ecological literature (Johnson, 1981; Borowiec, 1987) but we provide here the first systematic evidence confirming such statements. This first statement is however contradicted in two cases. The first case concerns *C. albonotatum,* a Mimosoideae feeder which does not cluster with species having the same feeding preference according to both morphological and molecular data sets. Biologically, *C. albonotatum* differs from all other West African *Caryedon* feeding on Leguminosae. Consequently, the hypothesis of an independent host shift of *C. albonotatum* from *Combretum* to Mimosoideae cannot be ruled out. The second case emphasizes the need for a phylogenetic hypothesis to understand the evolution of ecological traits. *C. serratus,* an oligophagous caesalpinioid feeder which recently added to its diet an introduced Papilionoideae (*Arachis hypogaea*) (Delobel, 1995), is clearly a member of the mimosoid seed user clade and is therefore assumed to have directly arisen from a mimosoid feeding ancestor. According to our phylogenetic hypothesis, *C. serratus* acquired a higher seed secondary compound detoxification ability than its close relatives, and this ability gave it a potential first to shift from Mimosoideae to Caesalpinioideae and secondly to expand its host plant spectrum to a cultivated Papilionoideae. An interesting parallel can be done with *C. crampeli,* another oligophagous Caesalpinioideae feeder which is sympatric with *serratus* and has a very similar host range but has not extended it to Papilionoideae. It may be hypothesized that *C. serratus* has an ecological advantage over *crampeli* in the use of papilionoid seeds because it is a member of a clade already adapted to Mimosoideae, a legume subfamily possibly originating from a core group of Caesalpinioideae (Doyle, 1995).

(2) The present data sets do not allow us to answer the question of whether or not the relationships between *Caryedon* and their host plants are driven by a coevolutionary process (Janzen, 1969), but the tree topologies obtained do not contradict such an hypothesis. Although character optimization does not allow us to assert that feeding on nonlegume host plant is the ancestral character, as hypothesized by Borowiec (1987), two observations nonetheless support such an assumption: (i) the basal position of the morphologically primi-

tive *Terminalia* feeders on molecular and total evidence trees (Figs. 2 and 3) and (ii) the branching position of the *Combretum* feeders plus *C. albonotatum* clade in the same trees. Considering the behavior of extant species, the most parsimonious feeding preference hypothesis for the common ancestor of that clade would either be the legume or the *Combretum* feeding pattern. However, if we retain the hypothesis of a *C. albonotatum* host shift from Combretaceae to Mimosoideae, the ancestral feeding behavior for the clade would more likely be the *Combretum* feeding pattern. Accordingly, the common ancestor of both "*Combretum*" and legume clades would then have been a Combretaceae feeder. Similarly, we cannot state that among legume feeders the feeding behavior on Caesalpinioideae is ancestral to feeding on Mimosoideae. Such an hypothesis would agree with recent phylogenetic data about Leguminosae (Doyle, 1995) and would have brought new arguments to the discussion about the possibility of coevolutionary relationships. But ancestral feeding on Caesalpinioideae is nonetheless one of the two most parsimonious hypotheses for the common ancestor of the legume clade.

These remarks underline the need for a taxonomically and geographically extended phylogenetic study of the genus *Caryedon*. Including representatives of New World Pachymerini would also clarify the phylogenetic relationships with *Terminalia* feeding *Caryedon*.

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