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Systematics and character evolution in Durio s. lat. (Malvaceae/Helicteroideae/Durioneae or Bombacaceae-Durioneae)

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Abstract

A molecular phylogenetic study of *Durio* s.lat. was conducted based on sequences of the internal transcribed spacer (ITS) of nuclear ribosomal DNA for 30 ingroup exemplars, representing 16 species, and two taxa of *Cullenia* as outgroups. The phylogeny suggests the existence of two well-circumscribed clades composed, respectively, of species with poricidal pollen locules (*Boschia*) and species with pollen locules that open with longitudinal slits (*Durio* s.str.). The latter clade is subdivided into two strongly supported clades: /Tubulidurio, with highly fused filaments and free calyx lobes, and /Palatadurio, with largely free filaments and connate calyx lobes. We provide phylogenetic definitions for the names of these well-supported clades. Reconstruction of floral evolution is consistent with the hypothesis that vertebrate pollination is ancestral for D*urio* s.lat. and *Cullenia*. However, there have been further shifts in pollination system within *Durio* s.lat., which may account for some of the current diversity of floral characters. The correlation of fruit and aril characteristics suggests that there are two major dispersal syndromes, involving either birds (fruits opening on trees; aril red/yellow and odorless) or terrestrial mammals (fruits opening only after falling to the ground; aril pale colored and pungent). The distribution of extant taxa implies a Malesian origin and radiation of the study group, with a single dispersal of *Cullenia* to India and Sri Lanka. However, the fossil pollen record raises the possibility that *Durio* and relatives may have migrated to Southeast Asia from the Indian subcontinent after it collided with Asia. A molecular clock analysis suggests that the earliest divergence within the study group occurred about 20 to 32 mya and, hence, does not favor either biogeographic scenario.

Key words: *Durio, Boschia, Cullenia,* molecular systematics, phylogenetic nomenclature, flower evolution, pollination biology, fruit evolution, dispersal biology, historical biogeography

Introduction

The genus *Durio* Adans. is native to Southeast Asia and, as traditionally circumscribed (e.g., Kostermans, 1958; Brown, 1997), comprises about 30 species of rather large, often buttressed trees which predominantly grow in primary lowland dipterocarp forests (Ashton, 1988). *Durio* is characterized by (1) a densely lepidote or stellate indumentum on the lower side of the leaves, on young branches, and various parts of the flowers, (2) a cup-shaped calyx, (3) rather large, showy flowers with generally whitish or reddish petals, (4) anthers consisting of clusters of globular or elongate pollen locules which open either via an apical pore or a longitudinal slit, and (5) big spiny fruits with large seeds that are covered with a fleshy or, in some species, rather leathery aril. A number of different *Durio* species are esteemed locally in Malesia for their fruits with edible arils (Soegeng Reksodihardjo, 1962). In particular, numerous cultivars of *Durio zibethinus* L. are grown commercially throughout Southeast Asia (Nakasone & Paull, 1998). In the western world, durians have a mixed reputation for their offensively smelling and odd-tasting fruits (e.g., König, 1804; Fairchild, 1938; Lee, 1980).

The first systematic treatment of *Durio* was compiled by Masters (1874). Soon thereafter, Beccari (1889) provided a detailed account of this genus based on several years of fieldwork in Southeast Asia. Major taxonomic investigations of *Durio* in the 20th century were conducted by Bakhuizen van den Brink (1924), Wyatt-Smith (1953), and Kostermans (1958). However, while these studies helped settle a number of older confusions, there is still uncertainty about the status of some names and taxa (Brown, 1997). In part this lack of solid information reflects the limited number of studies of the wild species. Despite the extensive body of applied literature related to

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cultivation and harvesting of durians (see Brown, 1997 for a detailed bibliography), interspecific comparative studies are more or less limited to just a few investigations, such as on floral morphology by van Heel (1966), on leaf morphology by Brown (1994), on leaf indumentum by Salma (1999), and on pollination biology by Yumoto (2000).

The genus *Cullenia* Wight from Sri Lanka and Southeast India (Robyns, 1970) is thought to be the closest relative to *Durio* (Aubréville, 1975; Nyffeler & Baum, 2000). In particular, the highly modified anther architecture and the aril covering the entire seed, and data from the chloroplast marker *ndh*F support this sistergroup relationship (Nyffeler & Baum, 2000). Bakhuizen van den Brink (1924) and Mabberley (1997) went so far as to subsume *Cullenia* under *Durio*. However, *Cullenia* differs from *Durio* in having (1) a narrowly tubular calyx, (2) flowers that lack petals, and (3) anthers consisting of club-shaped connectives covered with numerous small pollen locules which open via circumscissile slits.

Based on androecium morphology, *Durio* sensu Kostermans (= *Durio* s.lat.) consists of two well-characterized subgroups. The pollen locules of *Durio* s.str. are elongate, wedge-shaped, often curved, and open with a longitudinal slit, whereas those of *Boschia* Korth. are globular and open with an apical pore (van Heel, 1966). In addition, the complex peltate scales (i.e., scales where the cells of the multicellular head are delimited by primary and secondary radial walls) of *Durio* s.str. are 350–1200 µm in diameter, while in *Boschia* they are generally 95–240 µm (Salma, 1999). The only exception to this pattern was *B. acutifolia* Mast. (as *D. griffithii* [Mast.] Bakh. var. *acutifolius* [Mast.] Bakh.), with scales of 316–473 µm in diameter (Salma, 1999). The close relationship between *Boschia* and *Durio* s.str. has never been questioned and was supported by a molecular analysis of chloroplast and nuclear markers (Nyffeler & Baum, 2000). Kostermans (1958) distinguished those two subgroups at the subgeneric rank, whereas Bakhuizen van den Brink (1924) referred to them as sections. In contrast, Schumann (1890), Edlin (1935), and Hutchinson (1967) opted to recognize *Durio* s.str. and *Boschia* as distinct genera. We adopted this latter conception in our previous study of /Durioneae[1] (Nyffeler & Baum, 2000) and also follow it for the remainder of this paper.

Durio s.str. includes about 24 species almost entirely restricted to Borneo, Java, Sumatra, and the Malay peninsula. *Durio burmanicus* Soegeng, which is only known from a single herbarium specimen (Soegeng Reksodihardjo, 1965), occurs in Myanmar. *Boschia* con-

sists of about six species, which share the core distribution range with *Durio* s.str. One species, *Boschia mansonii* Gamble, occurs in Myanmar. The generic affinities of this latter species, however, are problematic. It combines the globular pollen locules typical of *Boschia* with the highly fused calyx segments typical of some species of *Durio* s.str., and a staminal tube with five terminal lobes resembling that found in *Cullenia*. Unfortunately, *Boschia mansonii* could not be obtained for this study and will not be discussed further.

In the present study we attempt to clarify phylogenetic relationships within *Durio* s.lat., to use these findings as a basis for developing a supraspecific classification system, and to elucidate the evolution of features related to pollination and dispersal biology. *Cullenia* was added to this investigation as outgroup based on results from a previous combined analysis of *ndh*F chloroplast sequences and sequences from the internal transcribed spacer (ITS) of nuclear ribosomal DNA, even though the latter marker provided limited information in support of this sistergroup relationship (Nyffeler & Baum, 2000).

Material and methods

Taxon sampling

Sixteen species of *Durio* s.lat. and two species of *Cullenia* were included in the present study. For 11 taxa, two or three accessions were sequenced in order to check for infraspecific variability. In total, 21 ITS sequences were generated (Table 1) and supplemented with 11 sequences from a previous investigation (Nyffeler & Baum, 2000). The chloroplast marker *ndh*F was not considered for this study because it did not provide enough variation.

DNA extraction, sequencing, and sequence analysis

Isolation of genomic DNA and sequencing of the ITS region was described in detail by Nyffeler & Baum (2000). The ITS data set was initially aligned using Clustal W 1.74 (Thompson et al., 1994) with a gap opening cost of 15 and a gap extension cost of 2, and then manually adjusted. Gaps of variable lengths due to runs of one type of nucleotide, which either could represent real information or artifacts of the sequencing process, were not considered (Schwarzbach & Ricklefs, 1998). Informative gaps were coded in a separate matrix as additional binary or multi-state characters (Baum et al., 1994). All sequences were submitted to GenBank (accession numbers AF233304-AF233310, AF233320, AF287700-AF287720), and the aligned data matrix and consensus trees are available from TreeBase (http://www.herbaria.harvard.edu/treebase).

Phylogenetic analyses

All phylogenetic analyses were conducted with PAUP* version 4.0b4a and 4.0b6 (Swofford, 2000, 2001). The program TreeView (Page 1996) was used for tree visualization.

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[1]A forward slash / is here used as clademark to indicate that the name corresponds to a phylogenetically defined clade. For more information see Baum et al. (1998a) and Nyffeler & Baum (2000).

Table 1. Accessions of taxa sequenced for the present study. Herbarium acronyms after Holmgren et al. (1990); MARDI = Malaysian Agricultural Research and Development Institute, Kuala Lumpur.

Taxon	Source and voucher information	Origin	GenBank accession number
Boschia			
B. acutifolia Mast.	Nyffeler 503 (A, SAR)	Sarawak, Gunung Gading N. P.	AF287700
B. grandiflora Mast.	MARDI	Peninsular Malaysia	AF233320
B. griffithii Mast. I	Church et al. 681 (A)	Kalimantan, Bukit Baka N.P.	AF233309
B. griffithii Mast. II	Nyffeler 479 (A)	Singapore, Bukit Timah	AF233310
Durio-1			
D. affinis Becc.	Ahmad et al. BRUN16577 (A)	Brunei, Sungai Liang	AF287705
D. beccarianus Kosterm. & Soegeng I	Church et al. 2010 (A)	Kalimantan, Serawai	AF287706
D. beccarianus Kosterm. & Soegeng II	Church et al. 1481 (A)	Kalimantan, Serawai	AF287707
D. oblongus Mast. I	Nyffeler 481 (A, SAR)	Sarawak, near Kuching	AF233307
D. oblongus Mast. II	Nyffeler 488 (A, SAR)	Sarawak, Semengoh Arboretum	AF287703
D. singaporensis Ridl. I	Nyffeler 473 (A)	Singapore B.G.	AF287701
D. singaporensis Ridl. II	MARDI	Peninsular Malaysia	AF287702
D. testudinum Becc. I	Nyffeler 491 (A, SAR)	Sarawak, Semengoh Arboretum	AF287704
D. testudinum Becc. II	Kocyan 97/6 (A)	Sabah, Tenom, Taman Pertanian	AF233308
Durio-2			
D. carinatus Mast.	Nyffeler 509 (A, SAR)	Sarawak, near Lundu	AF287708
D. dulcis Becc. I	Ambri & Arifin W656 (A)	Kalimantan, Wanariset	AF287713
D. dulcis Becc. II	Kocyan 97/5 (A)	Sabah, Tenom, Taman Pertanian	AF287715
D. dulcis Becc. III	Bogor BG, IV.I.199	Kalimantan, Jaro	AF287714
D. cf. *graveolens* Becc. I	Nyffeler 500 (A, SAR)	Sarawak, Semengoh Arboretum	AF287720
D. graveolens Becc. II	Cheng FRI21578 (K: MW5964)	Peninsular Malaysia, Kepong	AF287719
D. graveolens Becc. III	Nyffeler 469 (A)	Singapore B.G.	AF287718
D. kutejensis (Hassk.) Becc. I	Kocyan 97/1 (A)	Sabah, Tenom, Taman Pertanian	AF287717
D. kutejensis (Hassk.) Becc. II	Bogor BG, IV.I.45	Borneo, Lahi	AF287716
D. lanceolatus Mast.	Nyffeler 485 (A, SAR)	Sarawak, Semengoh Arboretum	AF287709
D. lowianus King	MARDI	Peninsular Malaysia	AF287711
D. oxleyanus Griff. I	Kocyan 97/3 (A)	Sabah, Tenom, Taman Pertanian	AF233306
D. oxleyanus Griff. II	Nyffeler 490 (A, SAR)	Sarawak, Semengoh Arboretum	AF287712
D. zibethinus L. I	Kocyan 97/2 (A)	Sabah, Tenom, Taman Pertanian	AF287710
D. zibethinus L. II	Bogor BG, 990.IX.32	Sulawesi, Luwu	AF233304
D. zibethinus L. III	Nyffeler 467 (A)	Singapore B.G.	AF233305
Cullenia (outgroup taxa)			
C. ceylanica (Gardn.) K.Schum. I	Ashton 2922 (A)	Sri Lanka, Ratnapura	AF233316
C. ceylanica (Gardn.) K.Schum. II	Ashton 2910 (A)	Sri Lanka, Kakutara	AF233317
C. rosayroana Kosterm.	Ashton 2905 (A)	Sri Lanka, Galle	AF233318

Maximum parsimony analyses and clade support: All maximum parsimony searches were run with 1000 random addition replicates, tree-bisection-reconnection (TBR) branch swapping, holding one tree per step, and keeping all most-parsimonious (MP) trees. First, a baseline search was conducted using equal weighting of characters and excluding coded gap characters. Additional analyses were performed as follows: (1) coded gap characters included and weighted equally to substitutions, (2) transversions weighted twice as much as transitions, and (3) transversions weighted three times as much as transitions.

The relative support for different clades was estimated using bootstrap (Felsenstein 1985, Hillis & Bull 1993) and decay analysis (Bremer, 1988; Donoghue et al., 1992). Bootstrap values were determined from 1000 bootstrap replicates subject to full heuristic searches with simple taxon addition. Decay indices of three or more steps were estimated with converse constraint heuristic searches based on 100 random sequence addition replicates (Baum et al., 1994). For well supported clades we evaluated whether the MP trees that include them are significantly better supported than trees that lack those clades (Whitlock & Baum, 1999; Lee, 2000). This was achieved by comparing the pool of MP trees from a converse constraint search with the unconstrained MP trees using a Wilcoxon signed-rank test (Templeton, 1983) as implemented in PAUP*. For each clade, the *P* value reported is the highest obtained across the pair-wise comparisons. A clade may be considered "strongly supported" if *P* < 0.1 (Lee, 2000).

Maximum likelihood and clock analysis: Four different models of sequence evolution (JC, F81, HKY, and GTR), all with rate heterogeneity following a discrete approximation to a gamma (Γ) distribution, were applied to maximum likelihood (ML) searches (Swofford et al., 1996). Previous studies have found that incorporating rate heterogeneity in analyses of ITS sequence data considerably improved the ability of trees to explain the data (e.g., Yang et al., 1994; Sullivan et al., 1996; Baum et al., 1998b). All heuristic searches used simple taxon addition and conducted TBR branch swapping to completion.

The analysis based on the JC model was carried out in a direct heuristic search with the rate heterogeneity parameter α estimated by ML. Due to computational limitations, analyses based on the three more complex models (F81, HKY, and GTR) were conducted using a successive approximation approach (Sullivan et al., 1996). For each model of molecular evolution, an initial analysis was completed with empirical base frequencies, substitution rate constant over sites (α = infinite), transition/transversion ratio of 2.0 (for HKY) or all substitution rates equal (for GTR). Based on the resulting topology for each model, base frequencies (for HKY and GTR), substitution model parameters, and the rate heterogeneity parameter α were estimated by ML. These parameter estimates were then fixed and new heuristic searches were conducted. This procedure was reiterated for each model until estimation of model parameters and tree topology ceased to change.

The relative fit of the different evolutionary models to the sequence data was evaluated with likelihood-ratio (*LR*) tests (twice the difference between the log-likelihood [-log*L*] values for the two models to be compared), and *P* values were determined based on a chi-square distribution (Huelsenbeck & Rannala, 1997). For each individual test, the number of degrees of freedom (df) was the difference in the free parameters. Ignoring parameters shared by all ML models (e.g., branch lengths), the parameters estimated by these four models are: JC = 1 (α); F81 = 4 (α, three base frequencies); HKY = 5 (α, ts/tv, three base frequencies); GTR = 9 (α, three base frequencies, five substitution rates). The optimal model of evolution for the data at hand is taken as the simplest model that is not significantly worse than the more parameter-rich model (Huelsenbeck & Rannala, 1997; but see Sanderson & Kim, 2000).

We used the Shimodaira-Hasegawa test (SH test; Shimodaira & Hasegawa, 1999) to evaluate whether the ML tree under the preferred model has a significantly higher likelihood score than the MP trees. This test was run with 1000 RELL bootstrap replicates using PAUP* 4.0b6 (Swofford, 2001).

After selecting the appropriate model of sequence evolution we sought to evaluate whether, under that particular model, clock-like sequence evolution can be rejected. Characters with gapped sites were excluded because, in our experience, this often reduces inferred rate inequalities. We then compared the log-likelihood of the data given the ML tree with and without assuming a clock. In the clock model we rooted the tree between *Cullenia* and the remaining taxa and reestimated all parameters by likelihood. A likelihood-ratio test (as above, but with 30 degrees of freedom based on the difference in the number of branches free to vary in each case) was used to compare the log-likelihood scores of the two models.

Currently, there is no reliable paleontological information specific to the study group, that would allow us to calibrate the molecular phylogeny. Therefore, we used the estimated rate of ITS sequence evolution from a previous study of /Bombacoideae, /Malvaceae (Baum et al., 1998b). That taxon, like /Durioneae, is composed of large, long-lived trees that appear to show a comparatively slow rate of molecular evolution (see Alverson et al., 1999: fig. 1). Baum et al. (1998b) suggested, based on palynological data, that the radiation into the genera of /Bombacoideae occurred 36–58 million years ago (mya). Given an HKY+ Γ model and a molecular clock, the depth of the nodes separating the four bombacoid genera (*Adansonia* L., *Bombax* L., *Pachira* Aubl., *Pseudobombax* Dugand) was approximately 0.1 substitutions/site (range 0.086–0.113). Dividing this average by 58 and 36 million years, respectively, suggested an approximate range of evolutionary rates of 0.0017–0.0028 substitutions/site/my. Estimates of divergence times in the present study group were, therefore, calculated based on these substitution rates.

Morphological analysis

Interspecific data on flower and fruit characters were mainly compiled from the most recent monographs of *Cullenia* (Robyns 1970) and *Durio* (Kostermans, 1958). Some additional information was obtained from herbarium specimens studied at Harvard University (A, GH), the Singapore Botanic Gardens (SING), and the Department of Forestry, Kuching (SAR).

Results

ITS sequence data

The aligned ITS data matrix comprised 710 bp. The aligned length for ITS1 was 302 bp (individual sequences 288–296 bp); for 5.8S rDNA it was 164 bp (163–164 bp); and for ITS2 it was 245 bp (228–240 bp). Overall, 173 positions (24.4 %) were variable, of which 122 (17.2 %) were parsimony informative. The aligned matrix included 27 indels which were 1 or 2 bp in length, two indels of 3 bp, and one of 6 bp. Of the 27 indels, 20 (74.1 %) were parsimony informative and were coded as additional characters, yielding a total matrix of 730 characters.

In our previous study of /Durioneae we recognized a wide range of different GC contents in ITS sequences and identified this as a potential cause of inaccurate phylogenetic inference (Nyffeler & Baum, 2000). Variation in GC content in this taxonomically more restricted data set of exemplars from *Durio* s.lat. and *Cullenia* was comparatively small, ranging from 67.2 % in *Durio singaporensis* I to 69.8 % in *Durio dulcis* III (including sequences of 5.8S rDNA). We believe that base composition is not a confounding problem for the present molecular analysis of ITS sequences (chi-square=8.616, df=93, P=1.0; test of base frequency homogeneity as implemented in PAUP* 4.0b4a).

Maximum parsimony and clade support

The baseline analysis, excluding the coded gap characters, yielded four MP trees (L=252, consistency index [CI]=0.80, consistency index excluding uninformative characters [CI']=0.75, retention index [RI]=0.93). The strict consensus tree from this analysis is given in Fig. 1. Inclusion of the coded indels in the parsimony analysis yielded 27 MP trees (L=282, CI=0.80, CI'=0.75, RI=0.93) whose strict consensus was congruent with the baseline analysis but slightly less resolved. Branches that were not supported in the latter analysis are marked with an asterisk in Fig. 1. The analyses with upweighted transversions, either two or three times, yielded one MP tree which was identical to one of the four trees from the baseline analysis.

Many previously recognized clades were strongly supported: *Cullenia* (bootstrap [BS] = 100 %; decay index [DI] = 15, P=0.003); *Boschia* (BS=100%, DI=15, P=0.003), and *Durio* s.str. (BS=91%, DI=5, P=0.302). Furthermore, as suggested by our previous
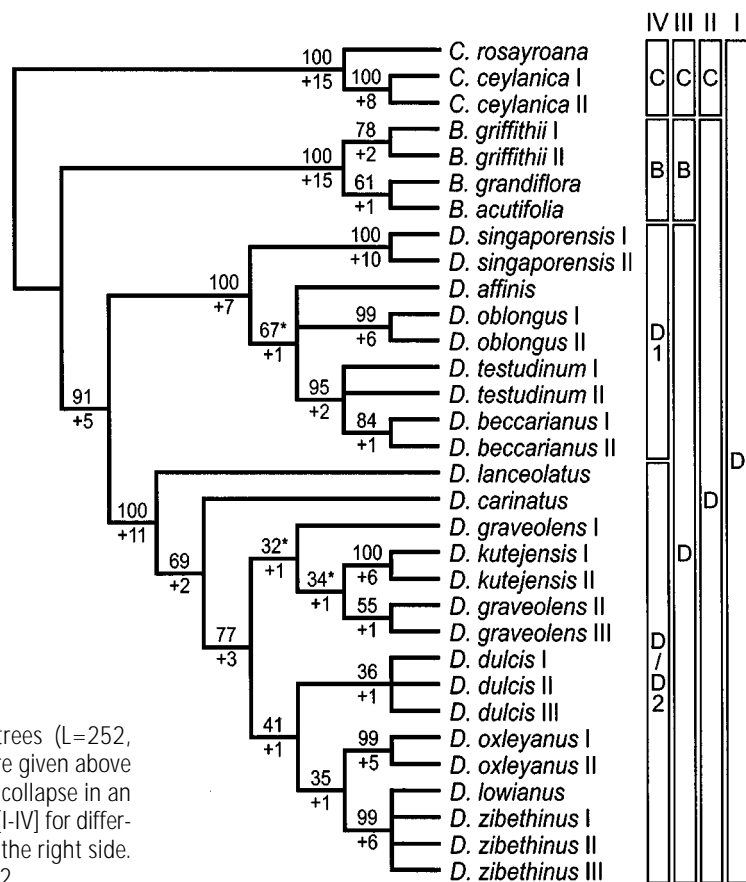

Fig. 1. Strict consensus of four most-parsimonious trees (L=252, CI=0.80, RI=0.93). Bootstrap values and decay indices are given above and below the branches. Clades marked with an asterisk collapse in an analysis with coded gaps included. Levels of inclusiveness [I-IV] for different circumscriptions of the genus *Durio* (D) are given on the right side. B=*Boschia*, C=*Cullenia*, D=*Durio*, D1=Durio-1, D2=Durio-2.

study (Nyffeler & Baum, 2000), *Durio* s.str. comprises two well supported subgroups: Durio-1 (BS=100%, DI=7, *P*=0.039) and Durio-2 (BS=100%, DI=11, *P*=0.013).

In contrast to the robust resolution at this broad scale, interspecific relationships within Durio-1 and Durio-2 are generally weakly supported, with bootstrap values less than 70 %. Exceptions are (1) the clade comprising *D. beccarianus* and *D. testudinum*[2] in Durio-1 (BS=95%, DI=2, *P*=0.4142), (2) the clade composed of all Durio-2 except *D. carinatus* and *D. lanceolatus* (BS=77, DI =3, *P*=0.3173), and (3) the clade consisting of *D. lowianus* and *D. zibethinus* (BS=99%, DI=6, *P*=0.0833).

Those species for which several samples were included in the analysis, with the exception of *D. graveolens* and *D. testudinum*, emerged as monophyletic (exclusive) groups, although sometimes with fairly weak support (e.g., *D. dulcis*: BS=36%, DI=1, *P*=0.7055). The three samples of *D. graveolens* formed a paraphyletic group with *D. kutejensis* nested within them. However, the identity of *D. graveolens* I from Sarawak, which takes a position as sister to the other two exemplars of *D. graveolens* (both from the Malay peninsula) + *D. kutejensis*, is not completely certain. Furthermore, the non-exclusivity of *D. graveolens* is only weakly supported (BS=34%, DI=1, *P*=0.6547). Likewise, while exclusivity of *D. testudinum* is not supported (Fig. 1), neither is it contradicted by these data. Thus, there is currently no molecular evidence suggesting a lack of exclusivity for any of the sampled species.

Maximum likelihood and clock analysis

Log-likelihood scores and likelihood-ratio values are listed in Table 2. The GTR model provided a significantly better explanation of the data than the other models considered. As the most parameter-rich model explored, it is unclear if GTR would be favored relative to yet more complex models. However, the monotonic decline in *P*-values (Table 2) suggested that GTR may well be close to optimal.

Phylogenetic analyses using all four models (JC, F81, HKY, and GTR) found the same optimal topology (Fig.

2A), which was identical to one of the four MP trees derived from the baseline analysis, but different from the tree yielded by the upweighted transversion MP analyses. Under the preferred GTR model, the ML tree did not explain the molecular data significantly better than the three other MP trees (SH test: *P*=0.52–0.71). Therefore, evolution of floral and fruit characters was investigated based on the four MP trees.

With gapped sites excluded, the ML tree had a log-likelihood value of -2365.42839 without assuming a clock, and a log-likelihood value of -2385.14287 when assuming a clock. A likelihood-ratio test suggested that this difference is not significant (*P*=0.112; df =30), which justified the use of the molecular-clock tree for estimating divergence times of the major lineages of the study group (Fig. 2B).

Based on the range of evolutionary rates of 0.0017–0.0028 substitutions/site/my as determined for ITS in /Bombacoideae (Baum et al. 1998b), the divergence of the major extant lineages (*Cullenia*, *Boschia*, and *Durio*) are estimated to have occurred 19.5–32.2 mya, whereas the split between Durio-1 and Durio-2 is dated to about 15.8–26.0 mya (Fig. 2B). It should be highlighted that these estimates are crude at best because of a number of confounding factors: (1) the possibility that the calibration from /Bombacoideae is incorrect (see Baum et al. 1998b); (2) the rate of evolution of /Bombacoideae and /Durioneae is unlikely to be identical; (3) the substitution model used for /Bombacoideae is different from the one applied to /Durioneae (HKY+Γ versus GTR+Γ); (4) there could be deviations from clock-like evolution in /Durioneae that are insufficient to be detected in a likelihood-ratio test, and; (5) there is stochastic error in branch-length estimates even under clock-like evolution. It should be noted that, without a clock, the ML tree (Fig. 2A) implies a slight increase in the substitution rate within *Durio* s.str. relative to *Boschia* and *Cullenia*. Correspondingly, a likelihood estimate assuming a clock for a tree where *Cullenia* and *Boschia* are sister-groups yields a slightly more favorable log-likelihood value of -2384.20252 than for the tree rooted using the outgroup criterion (Nyffeler & Baum, 2000). However, all time estimates based on this alternatively rooted tree are within seven percent of those reported in Fig. 2B.

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[2]*Durio testudinarum* is the original spelling as given by Beccari (1889). The species epithet is derived from Lat. *testudo, testudinis* (for tortoise or turtle) and the author states that it refers to "delle tartarughe" (Italian, plural: of the tortoises). According to Art. 32.5 of the ICBN (Greuter et al. 2000), the Latin termination of the epithet is changed here to 'testudinum' (third declension, genitive, plural; Stearn 1992) [Eggli, in litt.; Stroo, in litt.].

Table 2. Comparison of different models of molecular evolution and their fit with the present sequence data (* *P* < 0.01 ; ** *P* < 0.001).

Model of sequence evolution	- log*L*	2*LR* (Δdf)
JC+Γ	2593.54566	
F81+Γ	2546.81879	93.45374 (3)**
HKY+Γ	2501.84937	89.93884 (1)**
GTR+Γ	2494.30737	15.084 (4)*

Discussion

Relationships within Durio s.lat., levels of inclusiveness, and phylogenetic definitions of clade names

The present study confirmed the traditional view that *Durio* s.lat. consists of two basic and strongly supported clades (i.e., *Boschia* and *Durio* s.str.) which are clearly distinguishable and unambiguously characterized by major differences in anther architecture (Fig. 3, Kostermans, 1958; Nyffeler & Baum, 2000). In addition, the present study revealed that *Durio* s.str. contains two well supported subclades (Fig. 1, Durio-1 and Durio-2) that have not previously been formally recognized. However, the key to species in the taxonomic revision of *Durio* s.lat. by Kostermans (1958) highlights a major subdivision based on whether the filaments are almost free or largely fused. With the exception of the misplacement of *D. carinatus*, the two subgroups identified by Kostermans (1958) correspond to Durio-1 and Durio-2 as rec-

ognized here. Durio-1 comprises taxa with largely free calyx segments and filaments that are fused into a distinct staminal tube; Durio-2 consists of taxa with a cup-shaped, distinctly connate calyx and filaments or phalanges of filaments fused up to, at most, one third of their total length (Fig. 3).

Based on the phylogenetic trees presented here and in a previous study (Nyffeler & Baum, 2000), it appears that the generic name *Durio* has been applied to monophyletic groups at three different levels of inclusiveness (Fig. 1): (level I) *Durio* s.lat. + *Cullenia* as in Bakhuizen van den Brink (1924) and Mabberley (1997), (level II) *Durio* s.lat. as in Kostermans (1958), and (level III) *Durio* s.str. as in Schumann (1890) and Hutchinson (1967). Given the clear subdivision of *Durio* s.str. into two distinct and well supported subclades, a fourth equally viable scheme could be proposed in which the usage of the generic name *Durio* is restricted to what is here called Durio-2 (Fig. 1, level IV). This clade contains the type species of *Durio*, *D. zibethinus*.
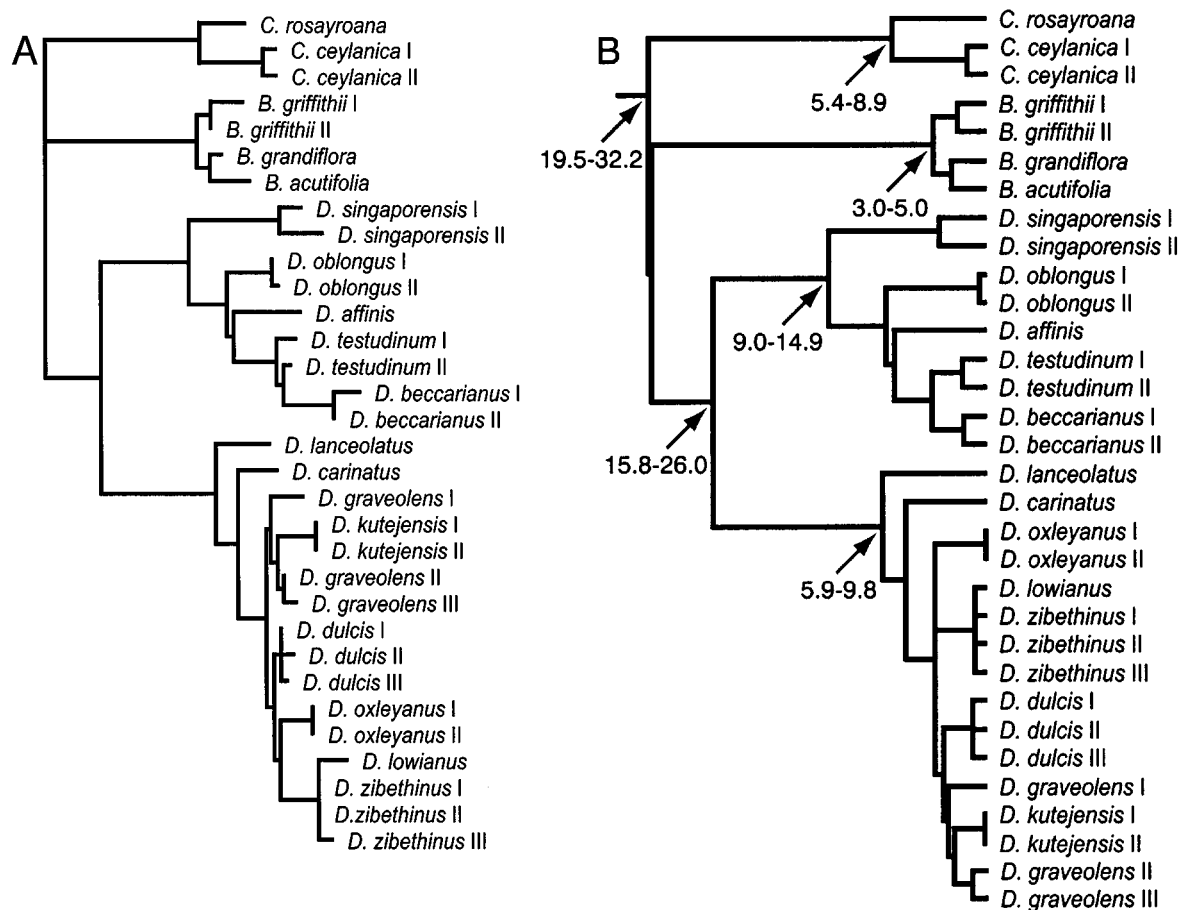

Fig. 2. Maximum likelihood trees based on the GTR+Γ model of sequence evolution. A. Phylogram without assuming a molecular clock (-log*L*=2494.30737; analysis based on a data set including all sites). B. Phylogram assuming a molecular clock (-log*L*=2385.14287; analysis based on a data set with gapped sites excluded). Estimates of absolute age for a number of major clades are added to the figure as based on a substitution rate of 0.0028–0.0017 substitutions/site/my.
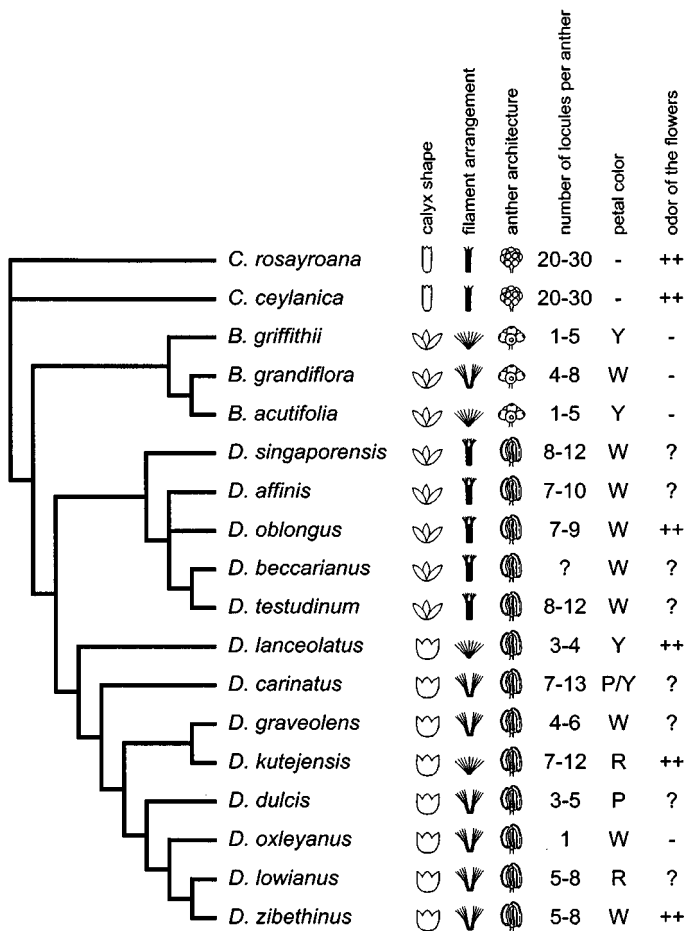
Fig. 3. Distribution of floral characters relative to the strict consensus of the four most-parsimonious trees. Calyx shape (narrowly tubular; cup-shaped, lobes largely free; cup-shaped, lobes connate), filament arrangement (staminal tube with five terminal lobes; staminal tube with filaments fused for more than half of their length; distinct phalanges fused for less than half of their length; filaments largely free), anther architecture (large clusters of small pollen locules opening with a circumscissile slit; small clusters of pollen locules opening with an apical pore; small clusters of elongate pollen locules opening with longitudinal slits), number of locules per anther, petal color (P: pink; R: red; W: white; Y: yellow), odor of the flowers (-: no odor; +: slightly odorous; ++: strongly odorous).

The binomial structure of species names, as governed by the current rules of the International Code of Botanical Nomenclature (ICBN, Greuter et al., 2000), has the consequence that they are affected by changes of the circumscription of genera. Therefore, in order to assure nomenclatural stability, it is essential to carefully select the level in the hierarchy of relationships to be recognized at the generic rank. With the hierarchical relationships among the taxa of the study group worked out, four classification schemes are possible (Fig. 1, levels I–IV). How should one decide among these options? Various criteria have been proposed as guidelines to assign the generic rank to taxa (e.g., Stuessy, 1990 and references therein), of which we would highlight six: (1) monophyly, (2) consistency with historical and established usage, (3) avoidance of new names at other levels in the Linnean hierarchy, (4) the ability to assign herbarium specimens to genus, (5) the ability to assign sterile plant material in the field to genus, and (6) the desire to emphasize groups that are evolutionarily and ecologically distinct. Of all these criteria, the only one that rests on an objective property of living organisms is monophyly. However, in the present case all four generic circumscriptions

would meet this basic requirement and, therefore, the ranking decision has to be made using one of the other, more subjective criteria. Since three of the four schemes have been used by prominent authorities at one time or another, criterion (2) only serves to argue against the narrowest conception of *Durio* as being equivalent to Durio-2. Criterion (3) is compatible with the three previously proposed generic classification schemes, whereas the narrowest conception (level IV) would require one to publish a new generic name for Durio-1. There is no current generic synonym available for this clade: *Lahia* Hassk., typified by *D. kutejensis*, falls into Durio-2. Criterion (4) is compatible with all four schemes, whereas criterion (5) favours the two broadest conceptions of *Durio*. Criterion (6), in contrast, argues for the narrowest possible circumscription of *Durio* since it provides a distinct name for each of the four major clades of the *Durio* s.lat. + *Cullenia* alliance.

In light of the fact that different considerations argue for a more or less inclusive circumscription of *Durio*, there is no single, satisfying generic classification fitting all major criteria discussed above. Our decision, therefore, is to propose a classification system consis-

Table 3. Phylogenetic classification system of the *Durio* s.lat. + *Cullenia* alliance with reference to the phylogeny given in Figure 1. Traditional generic names and some diagnostic characters are listed for each of the major clades.

Level–clade (Fig. 1)	Phylogenetic clade name	Genus name	Diagnostic characters (potential synapomorphies)
I–D	/Dubocu	–	Anthers deviating from the bithecate, tetrasporangiate condition; seeds with enclosing arils (lost in a few species).
II–C	/Cullenia	*Cullenia*	Calyx narrowly tubular; petals wanting; staminal tube with 5 terminal lobes; clusters of small pollen locules opening with circumscissile slits.
II–D	/Durbos	–	Calyx cup-shaped, petals present; filaments not with 5 terminal lobes; pollen-locules opening with apical pores or longitudinal slits.
III–B	/Boschia	*Boschia*	Pollen-locules opening with apical pores.
III–D	/Durio	*Durio*	Pollen-locules opening with longitudinal slits.
IV–D1	/Tubulidurio	–	Calyx segments largely free; filaments fused for more than half of their length.
IV–D2	/Palatadurio	–	Calyx fused with short terminal segments; filaments free, in distinct phalanges, or fused for up to one third of their length.

tent with the application of the names *Boschia*, *Cullenia*, and *Durio* to level III in Fig. 1, but to structure it within the framework of phylogenetic nomenclature (for a draft of the forthcoming Phylocode see http://www.phylocode.org), which allows us to provide formal, unranked names for other important clades. In order to accommodate the fact that sterile specimens often can be assigned only to the *Durio* + *Boschia* + *Cullenia* clade, we name that clade /Dubocu (comprising the first two letters of each nested clade). Likewise, in recognition of the fact that *Cullenia* is distinct from the remainder of /Dubocu in floral structure and distribution, we give the clade composed of *Durio* + *Boschia* the name /Durbos (comprising the first three letters of each nested clade). Finally, in order to allow researchers to talk usefully about the two distinct clades within *Durio*, we name the Durio-1 clade /Tubulidurio (in reference to the fused staminal tube) and name the Durio-2 clade /Palatadurio (in reference to the palatable fruits). Some diagnostic features of the individual clades are given in Table 3 and phylogenetic definitions, or circumscriptions (Stuessy, 2001), for all seven named clades are provided in Appendix 1.

Pollination and the evolution of floral characters

The /Dubocu clade is characterized by its highly modified anther architecture (van Heel 1966, Nyffeler & Baum, 2000). In contrast, the three other genera of /Durioneae, *Neesia, Coelostegia*, and *Kostermansia*, have anthers conforming to the bithecate, tetrasporangiate architecture typical of most angiosperms (van Heel 1966, Nyffeler & Baum 2000). It was hypothesized (Nyffeler

& Baum, 2000) that the shift away from this typical anther bauplan happened at the base of /Dubocu and that it might have been caused by a change from insect pollination, most likely involving beetles, to primarily vertebrate pollination. Hence, as suggested by the occurrence of vertebrate pollination in each of the four major clades of /Dubocu, this hypothesis predicts that this pollination system is plesiomorphic for the study group.

Vertebrate pollination has been confirmed in /Cullenia (Ganesh & Davidar, 1997; Ganesh & Devy, 2000), /Boschia (*B. grandiflora*; Yumoto, 2000), /Palatadurio (*Durio zibethinus* [e.g., Gould, 1977; Soepadmo & Eow, 1977], *D. graveolens* [Start & Marshall, 1976], *D. kutejensis* [van der Pijl, 1936; Yumoto, 2000]), and /Tubulidurio (*D. oblongus*; Yumoto, 2000). However, there is considerable variation among these taxa in the identity of the pollinating vertebrates, which may include bats, birds, and non-flying mammals, and in the pollination mechanisms. For example, /Cullenia is quite different from the other clades, being apetalous and rewarding pollinators with a nectar-soaked, detachable calyx (Ganesh & Devy, 2000). Thus, while the evidence is consistent with vertebrate pollination being plesiomorphic in /Dubocu, it is unclear what kind of vertebrates were the original pollinators. Furthermore, there is one documented case of reversal to pollination by insects, namely bees in *Boschia griffithii* (Momose et al., 1998).

Within /Dubocu there is extensive variation in flower morphology (Fig. 3). The traditional interpretation of this diversity would be that it reflects adaptation to different pollinators in different lineages. Using inferences based on pollination syndromes (Faegri & van der Pijl,

1979) one might predict that /Cullenia is primarily pollinated by bats and non-flying mammals, that /Boschia is primarily bee-pollinated, and that /Durio is primarily bat-pollinated with some possibility of bird- (e.g., *D. lowianus*) or bee-pollination (e.g., *D. oxleyanus*) in /Palatadurio. However, before we can use these inferences as a means to reconstruct the history of shifts in pollination systems we need to evaluate whether pollinators can be inferred reliably from floral biology.

Yumoto (2000) conducted careful field studies of the pollination biology of *B. grandiflora*, *D. oblongus*, and *D. kutejensis*. These species represent the three major clades of /Durbos and have flowers that differ greatly in color (white or red), size (5–12 cm diam.), nectar volume (10.6 µl to 9.1 ml), and time of anthesis (11.30 to 16.30 h). Based on a consideration of floral biology one might have predicted that *B. grandiflora* is pollinated by bees with the other two being bat-pollinated (in the case of *D. kutejensis* bird pollination is suggested by the red petals, whereas bat-pollination is suggested by the intense floral odor and late afternoon anthesis). However, based on direct observations and day-night bagging experiments, Yumoto (2000) found that all three species were pollinated by birds (spiderhunters), with some additional pollination in *D. kutejensis* by bats and bees.

There are several ways to interpret the discrepancy between predicted pollinators and those observed by Yumoto (2000). The first possibility is that all the species are functioning as generalists. However, if this were so we would expect less floral variation than is seen within /Durbos (Fig. 3). Therefore, we prefer two alternative explanations. First, that floral biology has been shaped in evolutionary time by one kind of pollinator but that, in 1996, when Yumoto conducted his research, spiderhunters were acting as opportunistic pollinators due to a lack of legitimate pollinators. In this regard, the reduced abundance of bats due to human disturbance is pertinent (Tuttle, 1990; Yumoto, 2000). Second, the species studied by Yumoto (2000) could be in the midst of shifting between pollinators, for example from bat to bird pollination in *D. kutejensis* and *D. oblongus*, and from bee to bird pollination in *B. grandiflora*. Under this latter interpretation some floral features (e.g., the red coloration in *D. kutejensis*, or the midday anthesis in *D. oblongus*) would be interpreted as recently evolved, adaptive traits. It will not be easy to distinguish between these two explanations but, in either case, the results obtained by Yumoto (2000) are compatible with the claim that the diversity of flowers within /Durbos reflects current and historical variation in pollination systems. At the same time, however, it is clear that instead of depending on inferences based on floral syndromes, additional field data are needed before we can arrive at a better understanding of floral evolution in the durians.

Seed dispersal and the evolution of fruit character

Most species of /Dubocu have large (generally 10–20 cm), globular or ellipsoid, spiny, capsular fruits (Fig. 4) with up to five seeds per locule. *Boschia acutifolia* and *B. griffithii* are different in having distinctly smaller fruits, two or three locules, and only one or two seeds per locule (Kostermans, 1958). The armature of the fruits varies from long, slender, and rather soft spines (up to 2 cm long in *Durio dulcis*) to broadly pyramidal, sharp projections (up to 1 cm in *Durio zibethinus*, 2–3 mm in *Boschia griffithii*). Given the size and weight of the fruits, it is not unexpected that they generally are found on strong, thick branches or the upper part of the main trunk (Kostermans, 1958). Exceptions are *Boschia acutifolia* and *B. griffithii* with their comparatively small fruits emerging from the axils of leaves on rather slender branches, and *Durio beccarianus* and *D. testudinum* of /Tubulidurio, which have their fruits attached to gnarls at the base of the trunk, generally less than 30 cm from the ground (Kostermans, 1958).

Since arils play an important role in the dispersal biology of /Dubocu (cf. 'Durian Theory'; Corner, 1949), we sought to map aril characters, including texture, color and odor, onto the molecular phylogeny to look for possible character correlations in the timing of fruit dehiscence and other important features of dispersal biology (Fig. 4). Although there is a lack of information for many species, two different dispersal syndromes appear recognizable. Species with arils reported to be regularly consumed by humans (Fig. 4, data from Soegeng Reksodihardjo, 1962; Brown, 1997) are dispersed primarily by various mammals (Corner 1988). These fruits tend to open only after falling to the ground and have fleshy, yellow or cream arils, which often develop a strong odor. The alternative syndrome consists of fruits that open to expose the seeds while still attached to the tree, and with the arils thin and rather leathery, generally red or orange, and odorless. This latter syndrome probably reflects adaptation to bird dispersal. Some species, however, cannot easily be assigned to one or the other category. For example, *Durio graveolens* has fleshy arils eaten by humans, and presumably by other mammals, but otherwise shares features that we ascribe to a bird-dispersal syndrome.

Based on outgroup comparison (using information from *Coelostegia*, *Kostermansia*, and *Neesia*) the plesiomorphic condition in /Dubocu are fruits that open on the tree (Nyffeler & Baum, 2000). Given this assumption and the inferred phylogeny, the most parsimonious reconstruction with equal weighting of gains and losses would suggest that delayed fruit dehiscence for dispersal by terrestrial mammals has either evolved twice with one reversal in *Durio graveolens*, or three times independently. Field-based investigations are needed to provide more detailed comparative data allowing validata-
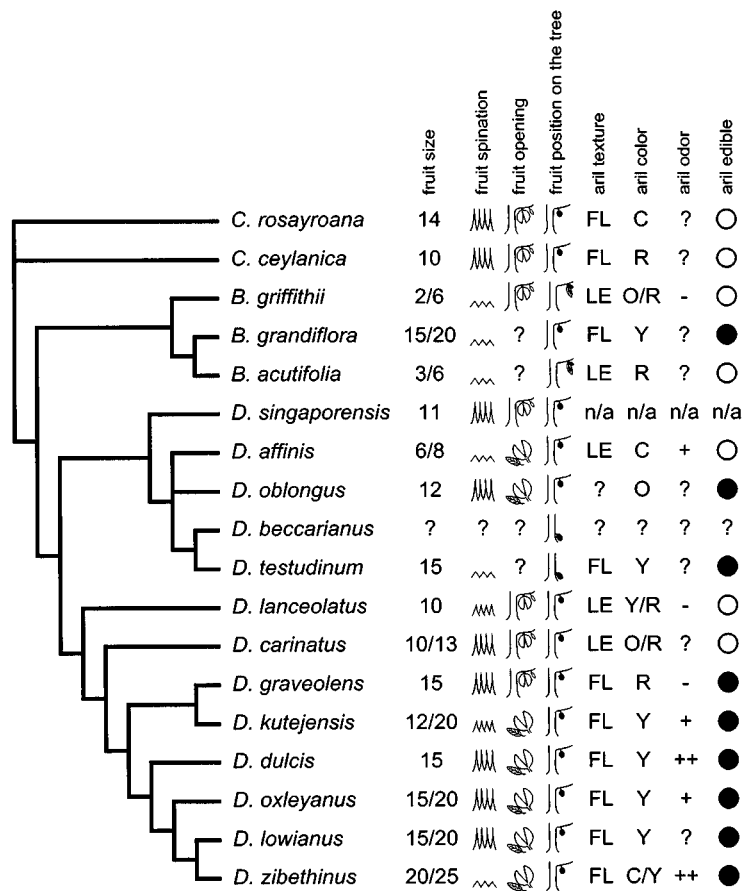
	fruit size	fruit spination	fruit opening	fruit position on the tree	aril texture	aril color	aril odor	aril edible
C. rosayroana	14	⫶⫶	⟩⊘	⟩⟨	FL	C	?	○
C. ceylanica	10	⫶⫶	⟩⊘	⟩⟨	FL	R	?	○
B. griffithii	2/6	⩘	⟩⊘	⟩⟨	LE	O/R	-	○
B. grandiflora	15/20	⩘	?	⟩⟨	FL	Y	?	●
B. acutifolia	3/6	⩘	?	⟩⟨	LE	R	?	○
D. singaporensis	11	⫶⫶	⟩⊘	⟩⟨	n/a	n/a	n/a	n/a
D. affinis	6/8	⩘	⟩⤸	⟩⟨	LE	C	+	○
D. oblongus	12	⫶⫶	⟩⤸	⟩⟨	?	O	?	●
D. beccarianus	?	?	?	⟩⟨	?	?	?	?
D. testudinum	15	⩘	?	⟩⟨	FL	Y	?	●
D. lanceolatus	10	⩘	⟩⊘	⟩⟨	LE	Y/R	-	○
D. carinatus	10/13	⫶⫶	⟩⊘	⟩⟨	LE	O/R	?	○
D. graveolens	15	⫶⫶	⟩⊘	⟩⟨	FL	R	-	●
D. kutejensis	12/20	⩘	⟩⤸	⟩⟨	FL	Y	+	●
D. dulcis	15	⫶⫶	⟩⤸	⟩⟨	FL	Y	++	●
D. oxleyanus	15/20	⫶⫶	⟩⤸	⟩⟨	FL	Y	+	●
D. lowianus	15/20	⫶⫶	⟩⤸	⟩⟨	FL	Y	?	●
D. zibethinus	20/25	⩘	⟩⤸	⟩⟨	FL	C/Y	++	●

Fig. 4. Distribution of fruit characters relative to the strict consensus of the four most-parsimonious trees. Fruit size (cm; diameter/length), fruit spination (slender, rather soft; intermediate; broadly pyramidal, sharp), fruit opening (on the tree; after falling to the ground), fruit position on the tree (on strong branches and the upper part of the trunk; in the axils of rather slender branches; at the base of the trunk), aril texture (FL: fleshy; LE: leathery, dry), aril color (C: cream; O: orange; R: red; Y: yellow), aril odor (-: no odor; +: slightly odorous; ++: strongly odorous), aril edible (open circles: not eaten by humans; filled circles: eaten by humans).

tion of our hypothesis of two major dispersal syndromes in /Dubocu.

Center of diversity and origin of /Durioneae

The species of /Durbos mainly occur on Borneo, Java, Sumatra, and the Malay peninsula. About 16 of the 21 species of /Durbos present on Borneo are endemic, whereas only five of 12 species reported for the Malay peninsula exclusively occur there (Kostermans, 1958; Brown, 1997). Just a few species of /Durbos, in particular the widely cultivated *Durio zibethinus*, are found in the Philippines, Thailand, and Myanmar. *Neesia*, *Coelostegia* and *Kostermansia* show a similar concentration in Malesia. The latter two genera are sister to /Dubocu while *Neesia* is sister to all other /Durioneae (Nyffeler & Baum, 2000). In contrast, /Cullenia is distinct in being restricted to Sri Lanka and SE India. This distribution pattern implies an origin and radiation of /Durioneae in Southeast Asia (Mendoza, 1941; Brown, 1997).

/Durioneae have a fairly rich palynological record (Muller, 1981; Nilsson & Robyns, 1986), which suggests that this clade originate on the Gondwana landmass, and that it arrived in Asia via rafting on the Indian subcontinent. There are a number of reports for

Durio type pollen from different localities in India as early as the upper Paleocene and lower Eocene (Kar, 1985; Morley, 2000). At that time, India was still an island and not yet attached to the Asian landmass, which happened around 54–45 mya (Dewey et al., 1989; Rowley, 1996). The earliest *Durio* type pollen reported for localities in Southeast Asia are from the middle Eocene, after the Indian Plate collided with Asia. This biogeographic scenario has been proposed for a number of different plant groups that are currently diverse in tropical Asia, most notably Dipterocarpaceae (Ashton & Gunatilleke, 1987; Morley, 2000). Krutzsch (1989), however, proposed an alternative interpretation of the palynological data and suggested that /Durioneae originated in Laurasia.

The extant distribution of the sister-groups of /Durioneae (i.e., the tribe Helictereae; cf. Nyffeler & Baum, 2000) does not provide support or contradict the hypothesis of an Indian rafting. *Reevesia* Lindl. and *Ungeria* Schott & Endl. are restricted to Austral-Asia (*Reevesia*, Asia; *Ungeria*, Norfolk Islands), but there are reports of old pollen deposits of *Reevesia* in Europe (Muller, 1981). *Helicteres* L. is widely distributed in the tropics and subtropics of Asia and America. Finally, *Mansonia* J. R. Drumm. and *Triplochiton* K. Schum.

currently occur in Africa and India, with fossil pollen tentatively assigned to *Mansonia* also reported for Europe (Muller, 1981).

Under the assumption of an Indian origin of /Durioneae, the phylogenetic evidence suggests two different scenarios. One possibility is that the radiation of /Durioneae occurred on India, with /Cullenia representing a relict of that ancestral range. Based on this hypothesis one would have to invoke three independent migrations to Malesia (*Neesia*, *Coelostegia* + *Kostermansia*, and /Durbos) and three corresponding extinctions in India and adjacent areas. Although this is an unparsimonious scenario, it cannot be rejected out of hand on account of the extensive migrations and extinctions that are reported to have occurred after the collision of the Indian subcontinent with Asia (Morley, 2000). The alternative hypothesis is that rafting occurred on India but that the group did not radiate until after migrating to Malesia. In that case one would have to invoke one extinction in India and one dispersal of /Cullenia back to India.

Knowledge of the temporal framework of the radiation of /Durioneae could help decide between the two biogeographic scenarios. Specifically, the hypothesis of a radiation in Asia would be weakened if the radiation of /Dubocu predated the collision of India with Asia 55–45 mya. However, the molecular clock analysis conducted here implies that the radiation of /Dubocu occurred no more than about 32 mya. Therefore, our data are compatible with either an Indian or Malesian center of radiation. Further palaeontological evidence is needed before a more convincing picture of the biogeographic history of the study group can be drawn.

Acknowledgements

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Appendix 1. Phylogenetic definitions of clades of the *Durio* s.lat + *Cullenia* alliance. We provide here stem-based and, in one case, node-based definitions for a number of well supported clades. Taxon membership can be inferred from Fig. 1. The type species of *Boschia, B. excelsa* Korth., was not sampled in the present study. It is thought to be very closely related to *B. grandiflora* and is therefore represented here by this latter exemplar. A forward slash (/) is used as a clademark to differentiate between traditional taxon names in accordance with the current ICBN (Greuter et al., 2000) and phylogenetic clade names based on the forthcoming Phylocode (http://www.phylocode.org). /Durioneae was defined in an earlier paper by Nyffeler & Baum (2000).

/Dubocu: Stem-based definition: the most inclusive clade containing the type specimens of *Boschia excelsa, Cullenia ceylanica, Durio oblongus,* and *Durio zibethinus,* but not the type specimens of *Coelostegia griffithii* Benth., *Kostermansia malayana* Soegeng, or *Neesia altissima* (Bl.) Bl.

/Durbos: Stem-based definition: the most inclusive clade containing the type specimens of *Boschia excelsa, Durio oblongus,* and *Durio zibethinus,* but not the type specimens of *Coelostegia griffithii, Cullenia ceylanica, Kostermansia malayana,* and *Neesia altissima.*

/Boschia: Stem-based definition: the most inclusive clade comprising the type specimen of *Boschia excelsa,* but not the type specimens of *Cullenia ceylanica, Durio oblongus,* and *Durio zibethinus.*

/Cullenia: Stem-based definition: the most inclusive clade comprising the type specimen of *Cullenia ceylanica,* but not the type specimens of *Boschia excelsa, Durio oblongus,* and *Durio zibethinus.*

/Durio: Node-based definition: the least inclusive clade comprising the type specimens of *Durio oblongus* and *Durio zibethinus.*

/Palatadurio: Stem-based definition: the most inclusive clade comprising the type specimen of *Durio zibethinus,* but not the type specimens of *Durio oblongus, Boschia excelsa,* or *Cullenia ceylanica.*

/Tubulidurio: Stem-based definition: the most inclusive clade comprising the type specimen of *Durio oblongus,* but not the type specimens of *Durio zibethinus, Boschia excelsa,* or *Cullenia ceylanica.*